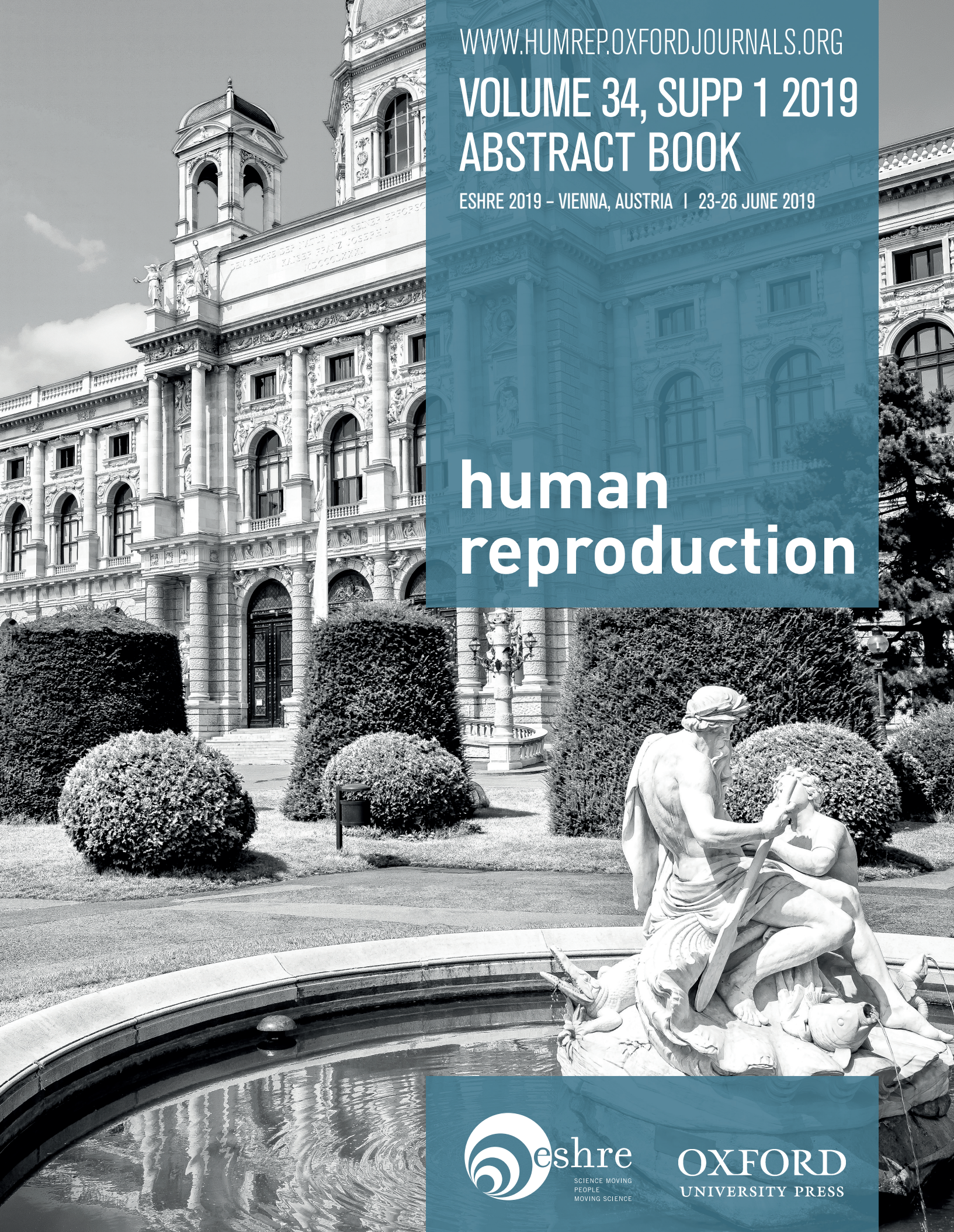

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VOLUME 34, SUPP 1 2019
ABSTRACT BOOK

ESHRE 2019 – VIENNA, AUSTRIA | 23-26 JUNE 2019

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Abstracts of the 35th Annual Meeting of the European Society of Human Reproduction and Embryology

Vienna

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24 to 26 June 2019

Abstracts

35th Annual Meeting of the
European Society of
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OXFORD UNIVERSITY PRESS

Published for the
European Society of Human Reproduction and Embryology
by Oxford University Press,
Oxford, UK

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ORAL PRESENTATIONS

Monday, 24 June 2019

(*continued overleaf*)

Tuesday, 25 June 2019

Wednesday, 26 June 2019

- **INVITED SESSIONS**
- **SELECTED ORAL COMMUNICATION SESSIONS**

Human Reproduction, Vol.34, No.Suppl, pp. i1–i511, 2019

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ESHRE 2019 / Oral presentations

INVITED SESSION

SESSION 01: KEYNOTE SESSION

Monday 24 June 2019 Mozart 08:30 - 09:30

O-001 Human Reproduction keynote lecture - Pre- and early pregnancy diet is associated with fertility and health in pregnancy

O-002 Gene editing - past, present and future

B. Davies[1]

[1]*University of Oxford, Wellcome Centre for Human Genetics, Oxford, United Kingdom*

Abstract text Over the past two decades, enzymes capable of cleaving chromosomal DNA at specific sequences have been developed and refined, with, most recently, the CRISPR/Cas family of enzymes drawing considerable interest due to their high efficiency and ease of use. The discovery of these enzymes and their successful application in mammalian cells has led to the emergence of the new technique of gene editing, whereby specific DNA sequences can be mutated, removed or inserted at will. This exciting technology is already transforming biomedical preclinical research and significant progress is being made in its therapeutic application for disease correction in somatic tissues and stem cells. These gene editing tools have been shown to function efficiently in the early embryo, and a number of proof-of-concept studies have demonstrated that the technology can be used for the correction of genetic mutations in the human embryo. Considerable ethical concerns surround the use of these technologies for germline editing and the need for an informed societal debate on the implications of these new technologies is acute. However, there exists a large knowledge gap in the understanding of scientists, clinicians, patient groups and the general public concerning the feasibility and associated risks of the new technology.

Drawing on my own experience with gene editing in mouse embryos, this session will outline how the different tools work and present different techniques for their application in the preimplantation embryo. Importantly, I will also introduce and present examples of the disadvantageous aspects of gene editing technology which currently limit the safety and feasibility of germline editing. Lastly, I will also discuss new developments in the field which are tackling some of the unpredictable consequences of gene editing techniques.

SELECTED ORAL COMMUNICATIONS

SESSION 02: NOVEL APPROACHES FOR PREDICTING EMBRYO VIABILITY

Monday 24 June 2019 Mozart 10:00 - 11:30

O-003 Mapping the follicular fluid bio-molecular profile: Dynamic interactions set the algorithm for oocyte maturation, embryo development and successful outcomes in IVF cycles

N. Chimote[1], **B. Chimote**[2]

[1]*Vaunshdhara Fertility Centre, Embryology & Reproductive Endocrinology, Nagpur, India ;*

[2]*Vaunshdhara Fertility Centre, Embryology- Reproductive Endocrinology, Nagpur, India*

Study question: To evaluate bio-molecular components in follicular-fluid and assess if they share dynamic correlationships to influence embryo development and pregnancy outcomes in IVF cycles

Summary answer: Evaluated biomolecular components in follicular-fluid correlate with individual embryonic parameters and also interact dynamically to set the algorithm for successful outcomes in IVF cycles

What is known already: The molecular mechanisms controlling endometrial-receptivity have remained an enigma because apart from the ovarian steroids estrogen and progesterone, cyclical remodeling of endometrium is also regulated at paracrine level by myriad growth-factors, cytokines and proteases. Hence, no factor has yet univocally been established as a predictive-marker. We envisaged that 'follicle-maturation' is the rate-determining step that sets off a cascade mechanism triggering oocyte-maturation, fertilization, embryo development, implantation, pregnancy. Therefore, we attempted the holistic approach of profiling representative biomarkers in the follicular-fluid microenvironment to not just obtain thresholds of individual biomarkers but also to assess if they share systematic correlations predictive of pregnancy-outcome.

Study design, size, duration: Prospective study of n=1150 (Power of study >85%) women (mean age 30.22±4.25 years, BMI 23.97±4.53, W/H ratio 0.88±0.06) undergoing antagonist stimulation protocol with r-FSH for IVF (September 2015-December 2018). On day of ovum pick-up, original aspirate per follicle was pooled per patient. Biomolecules like AMH, E_2, DHEAS, IGF-1, PAI-1, G-CSF and GM-CSF were evaluated in pooled FF by radioimmunoassay/ELISA as applicable, using diagnostic kits. Each protein-biomolecule was expressed as a ratio of total protein content.

Participants/materials, setting, methods: Elderly women (age>35 years), women with polycystic-ovaries, endometriosis were excluded. Embryo-transfer (ET) was done either at Day3-cleavage stage or day5 blastocyst stage. Micronized progesterone was provided as luteal-phase support. All cycles were divided into low, high groups to study correlation of each FF-biomarker with individual embryonic parameters. All cycles were later divided into pregnant and non-pregnant groups to study biomarker inter-relations and their correlation with pregnancy. Clinical pregnancy, live birth rate were main outcome measures.

Main results and the role of chance: FF-AMH correlated with oocyte-quality (Pearsonr=0.48); lower-levels increased the odds of top-quality oocytes. FF-PAI-1 correlated with oocyte-maturity (Pearsonr=0.51), lower-levels increased the odds of obtaining MII-oocytes. FF-DHEAS correlated with fertilization (Pearsonr=0.39); higher-levels increased the odds of fertilization. FF-IGF-1 correlated with embryo-quality (Pearsonr=0.40); higher-levels increased the odds of top-quality embryos. FF-GCSF and FF-GMCSF correlated with embryo quality (Pearsonr= 0.36 and 0.45).

Thresholds of FF-biomarkers for clinical pregnancy/live-births were: FF-AMH (<1.8 ng/mg protein;ROC_{AUC}:85%); FF-PAI-1 (<504.4 ng/mg protein;ROC_{AUC}:76%); FF-DHEAS (>1850ng/ml;ROC_{AUC}:69%); FF-E_2 (>160000 pg/ml;ROC_{AUC}:69%); FF-IGF-1 (>60ng/mg protein;ROC_{AUC}:78%).

FF-AMH showed strong positive correlation with FF-PAI-1 (Pearsonr=0.66) whereas these both inversely correlated with FF-E_2 (Pearsonr= -0.43; Pearsonr= -0.046) and FF-IGF-1 (Pearsonr= -0.27; ns). FF-DHEAS and FF-IGF-1 shared strong positive correlation with FF-E_2 (Pearsonr= 0.43 and Pearsonr=0.58). FF-IGF-1 directly correlated with FF-GCSF (Pearsonr=0.31) and FF-GMCSF (Pearsonr=0.36).

FF-AMH and FF-PAI-1 inversely correlated with serum E_2 on day7 (Pearsonr= -0.62; Pearsonr= -0.42) and day14 (Pearsonr= -0.66; Pearson r= -0.50) as well as serum progesterone on day7 (Pearsonr= -0.51; Pearsonr= -0.68) and day14 (Pearsonr= -0.63; Pearsonr= -0.80) post embryo-transfer. The likelihood of live-birth was highest when all biomarkers maintained their algorithmic thresholds; major deviations from threshold values of any parameter increased the odds of no-pregnancy, biochemical pregnancy, early pregnancy loss or miscarriage.

Limitations, reasons for caution: Several yet unknown factors are presumably involved in the implantation process. This study represents just a few biomarkers present in follicular- fluid, their individual significance, their interrelationships and eventually their co-relationship with pregnancy outcome. Multi-centric systematic studies are needed to corroborate these findings and account for the probable confounding factors.

Wider implications of the findings: Follicular-fluid is a metabolite reservoir, a medium for exchange of biologically active components responsible for follicular/oocyte growth, fertilization and embryonic development. Our results suggest that the dynamic interactions (probably initiated by AMH) within this micro-environment set off an algorithmic cascade for embryo-development, further triggering a receptive endometrium conducive for pregnancy.

Trial registration number: Not applicable

O-004 Artificial intelligence (AI) technology can predict human embryo viability across multiple laboratories with varying demographics with high accuracy and reproducibility.

M. VerMilyea[1,2], A. Miller[1], A. Picou[1], M. Lane[3,4], G. Adaniya[5], B. Bopp[6], D. Morbeck[7], E. Behnke[8], L. Click[8], R. Matthews[9], A. Lim[10], J. Hall[11], A. Murphy[11], D. Perugini[11], M. Perugini[11]

[1] *Ovation Fertility, Laboratory, Austin, U.S.A. ;*
[2] *Texas Fertility Center, Clinic, Austin, U.S.A. ;*
[3] *Monash IVF Group, Laboratory, Richmond, Australia ;*
[4] *Repromed, Laboratory, Adelaide, Australia ;*
[5] *Ovation Fertility, Laboratory, Indianapolis, U.S.A. ;*
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[10] *Alpha Fertility Centre, Laboratory, Petaling Jaya, Malaysia ;*
[11] *Life Whisperer, Company, Adelaide, Australia*

Study question: Can artificial intelligence (AI) and computer vision provide improvement to embryo viability prediction using static 2D images of Day 5 embryos from multiple laboratories?

Summary answer: The development of a general AI model produced 32% improvement in accuracy regarding embryo viability assessment compared to traditional embryologist morphology assessment.

What is known already: Recent studies have shown that artificial intelligence (deep learning) and computer vision can increase the efficacy of embryo selection and prediction of clinical pregnancy using images of human embryos. This automated, non-invasive approach to embryo selection can be used as a cohort ranking tool whereby embryos with certain morphological features are ranked in order of their likelihood to result in a positive pregnancy with a fetal heartbeat. A validation study of the current AI model using ~5000 images of Day 5 blastocysts resulted in an accuracy of 67.7% in identifying embryo viability by positive fetal heartbeat across two blind datasets.

Study design, size, duration: Approximately 20,000 static 2D images of Day 5 blastocysts with related pregnancy, pre-implantation genetic testing for aneuploidy (PGT-A) outcomes, demographic and clinic geographical location information have been collected. Images were divided into three groups: training, validation and blind test sets. An AI model was trained, validated and tested on 2217 embryo images from Day 5 blastocysts followed by a further blind set of 286 images from a separate clinic and demographic.

Participants/materials, setting, methods: 7,847 separate traditional phase-contrast microscope images from patients undergoing fertility treatment at 12 IVF laboratories in four countries were used to train and develop the Life Whisperer's embryo viability assessment model (LW General Model v1). Images of Day 5 blastocysts which had a blastocoel cavity and were subsequently transferred individually were used for this study. This study was determined exempt from IRB review by Sterling IRB, USA (#6467).

Main results and the role of chance: 5282 images from Monash IVF Group (Repromed, Adelaide, SA, Australia) were split into training (~74%), validation (~7%) and two blind validation (~19%) datasets. A blind validation data set is used to conduct an unbiased accuracy assessment to ensure the model is generalizable to all embryo images. The accuracy in identifying viable embryos is calculated as a percentage of the number of viable embryos (i.e. blastocysts that resulted in a successful pregnancy) identified by the AI model divided by the total number of viable embryos in the dataset. The same process applies to non-viable embryos.

The total mean accuracy of the embryo viability assessment model when applied to both blind validation datasets was 74.1% for identifying viable embryos, 65.3% non-viable embryos and 67.7% total accuracy across both viable and non-viable embryos. When comparing the accuracy in identifying viable/non-viable embryos for the model versus world-leading embryologists, the AI model correctly identified embryo viability 66.7% compared to 51.0% by embryologists.

After subsequent training on a mixed demographic dataset, a blind test of 74 images from three independent laboratories (Ovation Fertility Austin, San Antonio and Indianapolis), resulted in the model correctly identifying viable embryos at 84.6%, non-viable at 57.1% and total accuracy at 71.6%.

Limitations, reasons for caution: While the analysis of the blind validation datasets is small, our results provide evidence that AI can reduce the variability of morphological embryo selection across multiple laboratories and embryologists. Additional training on larger datasets is currently ongoing to further improve the generalizability of the AI model.

Wider implications of the findings: Embryo selection is heavily based on morphological/morphokentetic assessment and other invasive technologies including embryo biopsy for embryo ploidy classification. For the first time, this data represents how an AI model can be applied across a multi-centered, mixed demographic dataset resulting in ~70% overall accuracy for viable and non-viable embryo identification.

Trial registration number:
Not Applicable

O-005 The presence of filopodia in human blastocysts is associated with the probability of clinical pregnancy with fetal heart.

J. Eastick[1], S. Cooke[2], C. Venetis[3], M. Chapman[4]

[1] *IVFAustralia, Embryology, New Lambton Heights, Australia ;*
[2] *IVFAustralia, Scientific Director, Greenwich, Australia ;*
[3] *IVFAustralia, Clinical, Greenwich, Australia ;*
[4] *IVFAustralia, Clinical, Greemwich, Australia*

Study question: The aim was to determine if the presence of filopodia in human blastocysts is associated with probability of clinical pregnancy with fetal heart after transfer.

Summary answer: The presence of filopodia in human blastocysts is associated with the probability of clinical pregnancy with fetal heart after transfer.

What is known already: Filopodia have been described as "string like" structures that connect the inner cell mass to the trophectoderm cells and have been hypothesised to be a communication network. In human embryos, their presence past the expansion stage has been suggested to be negatively

associated with the implantation potential of blastocysts. Using time-lapse image analysis, the filopodia can now be seen and accurately categorised, and artificial intelligence has been shown to be an accurate method of predicting clinical pregnancy with fetal heart beat (CPFH).

Study design, size, duration: This was a two-centre case-control study performed between February 2018 and December 2018. The study involved 200 single blastocyst transfers from 181 women undergoing both IVF and ICSI utilising fresh oocytes. 100 of these resulted in a CPFH (cases) while 100 did not (controls). All embryos had been cultured in Embryoscope+ post-insemination and until day 5.

Participants/materials, setting, methods:
Inseminated zygotes were placed into Embryoscope+ for culture until day 5. The presence of filopodia was examined after the transfer for each blastocyst, by an assessor blind to the outcome of the transfer who reviewed the time-lapse video. AI software (Ivy) was also applied to the entire fertilised cohort to identify the blastocyst for transfer with the highest CPFH probability. Generalized estimating equation was used for analysis to control for the clustered nature of data.

Main results and the role of chance:
A blastocyst which resulted in CPFH after transfer, compared to the controls, had a higher chance of having a filopodia (Odds ratio-OR: 3.34, 95% CI:1.28-8.74). Furthermore, the filopodia seemed to be present for longer (12.2hrs vs 10.6hrs; P=0.035) and in the ultrastructure of the filopodia, more vesicles were seen travelling along the filopodia (4.6 vs 3.7; P=0.006) in blastocysts resulting in CPFH as compared to those that did not. The filopodia presence, appearance length and ultrastructure vesicles has not been reported to be linked to positive CPFH previously.

Limitations, reasons for caution:
The Embryoscope+ only allows 11 focal planes to view the embryos thus the possibility exists for underrepresentation of the filopodia. Results whilst interesting, should be confirmed in larger prospective studies.

Wider implications of the findings:
With modern time-lapse incubators allowing viewing and timing of blastocyst ultrastructure in multiple focal planes, its logical that new methods of assessing embryos are expected. The study of filopodia represents an ultrastructure marker that can aid in the identification of blastocysts with the highest potential to produce a clinical pregnancy.

Trial registration number:
not applicable

O-006 Time of morulation and trophectoderm quality are associated with live birth after euploid blastocyst transfer: a multicenter study

L.F. Rienzi[1], D. Cimadomo[1], A. Delgado[2], M.G. Minasi[3], G. Fabozzi[1], R. Del Gallego[2], M. Stoppa[1], J. Bellver[2], A. Giancani[1], M. Esbert[4], A. Capalbo[5], J. Remohì[2], E. Greco[3], F.M. Ubaldi[1], M. Meseguer[2]

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[2]*IVI-RMA, IVI-Valencia, Valencia, Spain*
[3]*European Hospital, Center for reproductive medicine, Rome, Italy*
[4]*IVI-RMA, IVI-Barcelona, Barcelona, Spain*
[5]*Igenomix, Igenomix Italia, Marostica, Italy*

Study question: Does the morphodynamic characterization of human euploid blastocysts' preimplantation development increase the prognostic value upon their reproductive competence?

Summary answer: Time of morulation (tM) and trophectoderm (TE) quality after blastulation (tB) increase the prediction upon euploid blastocysts' chance to result in a live birth (LB).

What is known already: To date, the definition of a normal chromosomal constitution through comprehensive-chromosome-testing represents a powerful predictive parameter upon blastocyst's reproductive potential. However, more than 40-50% of euploid blastocysts fail to implant. The assessment of preimplantation embryo development via time-lapse has been broadly investigated to find a possible correlation between morphokinetic parameters and the clinical outcomes after IVF. Although, some conflicting data and limited evidence have been shown. The predictive value of morphokinetics during IVF cycles including preimplantation-genetic-testing of aneuploidies (PGT-A) has been instead limitedly investigated, and therefore needs further assessment.

Study design, size, duration: In Phase1, 511 first single embryo transfers (SETs) of vitrified-warmed euploid blastocysts (N=147 center1; N=364 center2; training set) from 1069 PGT-A cycles between January-2016 and September-2017 were retrospectively recruited. A predictive model of LB was defined. In Phase2, this model was tested in a validation set including 319 consecutive SETs from 546 PGT-A cycle performed between September-2017 and June-2018 in 3 IVF centers. The ongoing pregnancy rate (OPR) was defined as primary outcome.

Participants/materials, setting, methods: Only cycles conducted through continuous media in time-lapse incubator were included. In Phase1, all timings of development up to tSB (starting-blastulation) were compared among implanted and non-implanted euploid blastocysts. Static assessment of TE and inner cell mass (ICM) at tB was also performed. Logistic regressions outlined the parameters associated with LB. The model was applied to the validation set in Phase2 and its predictivity estimated through Receiver operating characteristic (ROC) curve analysis.

Main results and the role of chance: The average LB rate after euploid SET in Phase1 was 40%, consistent at both centers. The euploid blastocysts resulting in a LB at both centers showed a concordant significantly faster development than non-implanted/miscarried ones for tPB2, t4, t5, t8, s3, cc3, tM and tSB. Similarly, a high-quality ICM and the TE at tB were concordant as positively associated with a LB. However, the multivariate logistic regression outlined only tM and TE quality as putative predictors. Therefore, we defined 80hr as the cut-off tM, corresponding to the 50th percentile of prediction of a LB after vitrified-warmed SET in the training set. A model was then created based on TE quality (high or low) and tM (<80hr or ≥80hr), which showed a significant AUC of 0.65 from the ROC curve analysis. The predictive model was validated on an independent dataset composed of 319 euploid SETs from 3 different IVF centers. The euploid blastocysts characterized by a high-quality TE at tB and a tM <80hr resulted in an OPR of 61.2% (N=41/67), while those with low-quality TE at tB and a tM≥80hr resulted in an OPR of 30.0% (N=15/50; p<0.01). However, the ROC curve showed a poorly clinically-significant AUC of 0.59.

Limitations, reasons for caution: This model is limited to euploid blastocysts produced from a population of patients indicated to PGT-A and cultured in continuous media with oxygen control in time-lapse incubators. Moreover, time-lapse timings beyond tSB were not accounted due to different blastocyst biopsy approaches adopted, namely with and without zona-opening in day3.

Wider implications of the findings: The reproducibility of predictive models based on time-lapse parameters is highly dependent on culture conditions, that must be consistent. Moreover, these data by shedding light on the time of morulation, incite future investigations of this crucial phase of preimplantation development. A stage entailing massive morphological, cellular and molecular changes.

Trial registration number: None.

O-007 Dynamic and cytological analyses of pronuclei and centrosome positions in relation to the first cleavage plane in human embryos

M. Nakaoka[1], K. Yumoto[1], T. Shimura[1], Y. Mio[1]

[1]*Mio Fertility Clinic, Reproductive Centre, Yonago, Japan*

Study question: How are the pronuclear axis and the positions of centrosomes related to determination of the first cleavage plane?

Summary answer: The pronuclear axis is involved in determining the first cleavage plane and is related to the positions of centrosomes, which subsequently become mitotic spindle poles.

What is known already: Several studies have investigated how the site of the second polar body affects plane formation during the first cleavage, with suggestions that the second polar body position defines the first cleavage plane in human embryos. However, these ideas are controversial. Recently, time-lapse monitoring has been used to monitor and analyze early-stage embryonic development in a clinical setting. By using time-lapse monitoring, we previously obtained data which suggested that both the axis and locations of male and female pronuclei are involved in determining the first embryonic cleavage plane, and thereby affect further development.

Study design, size, duration: From January to December 2015, we conducted time-lapse imaging (EmbryoScope®) of 1062 cultured oocytes from infertile couples after intracytoplasmic sperm injection (ICSI). Of these oocytes, 798 were normally fertilized, showing male and female pronuclei (2PN), and we analyzed 607 embryos that formed the first cleavage plane and developed to the two-cell stage. Furthermore, seven frozen/thawed 2PN zygotes, donated by patients who had completed treatment and given informed consent, were used for immunofluorescence analysis.

Participants/materials, setting, methods: A straight line connecting the centers of the pronuclei was defined as the 2PN axis. ICSI oocytes were cultured in an EmbryoScope®, with images acquired every 15 minutes for 40 hours. Immunofluorescence analysis of centrosomes and nuclear membranes of 2PN zygotes was performed using a rabbit polyclonal anti-pericentrin antibody and a goat polyclonal anti-lamin B antibody. Images were obtained using an FV1000 laser scanning confocal microscope (Olympus).

Main results and the role of chance: We obtained suitable images from 476 out of 607 analyzed embryos. Of these, 427 (89.7%) formed a cleavage furrow parallel to the 2PN axis at the first cleavage, while the remaining 49 formed a cleavage furrow perpendicular to the 2PN axis. In the 2PN zygotes, we identified centrosomes and nuclear *membranes* using anti-pericentrin and anti-lamin B antibodies, respectively. We confirmed the presence of two pericentrin signals in all seven 2PN zygotes. Six of the zygotes showed two pericentrin signals aligned around the interface between the male and female pronuclei, and the angle between the line connecting the two centrosomes and 2PN axis ranged from 90 to 110 degrees. The remaining zygote showed two pericentrin signals; one was located around the interface between the male and female pronuclei while the other was distinctly distant from the interface of the two pronuclei. In addition, the angle between the line connecting the two centrosomes and the 2PN axis of this zygote was about 170 degrees. This irregular positioning of centrosomes might be the reason for some embryos forming a cleavage furrow perpendicular to the 2PN axis.

Limitations, reasons for caution: Although the sample size for immunofluorescence analysis in this study was small, it was extremely difficult to collect the human zygotes for ethical reasons. Further studies, including analysis of centrosome dynamics by live-cell imaging, may help define the mechanisms that underlie determination of the first cleavage plane in human embryos.

Wider implications of the findings: Our findings suggest that the 2PN axis strongly influences the positions of the centrosomes, which become mitotic spindle poles and define the first cleavage plane. Furthermore, aberrant centrosome positioning in relation to the 2PN axis might cause some embryos to form the first cleavage furrow perpendicular to the 2PN axis.

Trial registration number: not applicable

O-008 First polar body fragmentation is not a marker of in vitro oocyte aging and does not affect embryo quality nor cumulative livebirth rates

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Study question: Is first polar body (IPB) fragmentation a consequence of an increased time between oocyte retrieval and injection and does it compromise ICSI outcome?

Summary answer: IPB fragmentation is unrelated with in vitro oocyte aging and its occurrence does not affect fertilisation, embryo quality or cumulative livebirth rates.

What is known already: The IPB is a byproduct of oocyte meiotic division that degenerates up to 24h following its formation. Traditionally, the presence of a fragmented IPB is considered a morphological indicator of postovulatory in vitro aging, deterioration and impaired developmental potential, with previous studies suggesting that it may be associated with hindered embryo development and decrease both implantation and pregnancy rates. However, these results derive from studies with small sample sizes and do not ascertain whether IPB fragmentation is a reliable time-dependent marker of overmaturity of the oocyte at the time of ICSI.

Study design, size, duration: Retrospective cohort analysis of 1175 ICSI cycles performed between 2007 and 2017. Only metaphase II oocytes at the time of denudation were considered and categorized according to whether they presented an intact or fragmented IPB at the time of injection. We evaluated the effect of increasing time between oocyte retrieval and ICSI on the incidence of IPB fragmentation, as well as the prognostic value of IPB fragmentation on fertilisation, embryo quality and cumulative livebirth rates.

Participants/materials, setting, methods: Multivariable multilevel mixed-effects regression was performed to account for relevant potential confounding factors and the clustering of oocytes derived from the same treatment cycle. Adjusted odds-ratios (aORs) with 95% confidence intervals (CIs) were calculated for each variable included in the regression models. Timing between oocyte retrieval and injection was exclusively dependent on laboratory workload and the best quality embryos were selected for transfer regardless of IPB morphology.

Main results and the role of chance: IPB fragmentation was identified in 47.9% of the oocytes, with up to 85% of the cycles having at least one oocyte with this feature. The mean time elapsed between oocyte retrieval and injection was 5.6h ± 0.8h and the occurrence of IPB fragmentation was unrelated to this time interval (aOR 1.001, 95% CI 0.999-1.002). Female age, body mass index, smoking habits, stimulation regimen or total number of oocytes retrieved were unrelated with the occurrence of IPB fragmentation. However, multivariable regression model showed that higher dosages of exogenous FSH (aOR per each 100 IU 1.014, 95% CI 1.003-1.522) was associated with a higher risk of IPB fragmentation, as well as the use of recombinant FSH (aOR 1.242, 95% CI 1.014-1.522). IPB fragmentation did not affect fertilisation rates (aOR 0.918, 95% CI 0.824-1.022) nor did it affect embryo quality (aOR 0.992, 95% CI 0.786-1.251). Most importantly, our results did not support an association between the presence of IPB fragmentation and clinical pregnancy (aOR 0.919, 95% CI 0.551-1.533), miscarriage (aOR 1.230, 95% CI 0.516-2.930), livebirth (aOR 0.906, 95% CI 0.553-1,483) or cumulative livebirth (aOR 0.958, 95% CI 0.685-1.568) rates, which were 38.2%, 17.4%, 31.1% and 32.6%, respectively.

Limitations, reasons for caution: This study is limited by its retrospective nature and the restriction of our patient population to women who are entitled to treatment reimbursement according to national legislation (<40 years old). For that reason, a possible effect of more advanced maternal ages in the occurrence of IPB fragmentation cannot be excluded.

Wider implications of the findings: Being the largest study evaluating the clinical relevance of IPB fragmentation, our analysis provides evidence that this morphological feature is not a reflection of in vitro aging nor is it a relevant predictive marker of embryo implantation potential, thus providing reassurance when rescheduling injection timings according to laboratory workload.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 03: BIOMARKERS AND SIGNALS IN ENDOCRINOLOGY

Monday 24 June 2019 Haydn 1 10:00–11:30

O-009 Antimullerian Hormone as a driving force of PCOS, independently from insulin-resistance

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Study question: In women with Polycystic Ovarian Syndrome (PCOS), do high serum Anti-Müllerian Hormone (AMH) levels discriminate a phenotype that would be more LH- than insulin-dependent?

Summary answer: Among PCOS women with the highest serum AMH levels, high serum LH levels and other ovarian PCOS features are more common while insulin-resistance is less.

What is known already: The presence of high serum LH levels in PCOS women is a common feature that theoretically contributes to worsen ovarian hyperandrogenism by stimulating theca cells. The serum AMH level is closely related to the follicle number per ovary (FNPO) at ultrasound. Therefore, serum AMH level and FNPO are both elevated in PCOS. Recent experimental data in mice argue in favor to a stimulating effect of AMH on the activity of GnRH neurons, which could explain the positive relationship between serum LH and AMH levels that is observed in PCOS women but there is very little evidence to support this hypothesis.

Study design, size, duration: This is a retrospective monocentric cross-sectional study included 639 patients with PCOS according to Rotterdam Criteria and 137 control women that were recruited from January 2009 to January 2016 in an academic institution.

Participants/materials, setting, methods: Clinical (menstrual disorder, hyperandrogenism, BMI, waist circumference), hormonal (gonadotropins, AMH, androgens, SHBG), metabolic (fasting insulin) and ultrasound (ovarian area, FNPO) data were recorded consecutively and included in a database. Discriminant analysis (DA) was used to identify and to rank in order of importance the different variables potentially involved in serum AMH levels in PCOS patients, compared to control women. Only the first (Q1) and fourth (Q4) quartiles of each population were used for the DAs.

Main results and the role of chance: In controls, only age, FNPO and obviously AMH were significantly different between Q1 and Q4. In PCOS patients, the frequencies of oligo-anovulation and hyperandrogenism were greater in the Q4 than in the Q1 group. As well, serum levels of total Testosterone, Androstenedione, 17 hydroxy-progesterone and LH ranked significantly higher, as did the FNPOs and ovarian areas. In contrast, serum FSH levels, BMIs, waist circumferences and plasma insulin (I) levels ranked significantly lower and obesity was less frequent in Q4 than in Q1. The SHBG levels ranked similarly between the 2 groups.

In PCOS, the distribution of phenotypes A, C and D was different between the two groups ($p < 0.0001$). Mild phenotypes (C and D) were more common in Q1 while phenotype A was more frequent in Q4.

In the controls, after stepwise DA, FNPO and age discriminated independently and significantly Q4 from Q1 ($p<0.0001$ each), with R^2 at 0.62 and 0.27, respectively. In PCOS patients, the FNPO, LH, FSH, phenotype and BMI variables discriminated Q4 from Q1, independently and significantly, with R^2 at 0.371, 0.304, 0.166, 0.108, and 0.075, respectively ($p < 0.0001$ for all). In contrast, T and A were not included in any significant model.

Limitations, reasons for caution: Our controls did not represent the general population since they were recruited in an ART centre. As previously published, we used in-house thresholds to define the follicle excess with FNPO and/or serum AMH level. Women with PCOS were relatively young, which could have minimized the effect of age.

Wider implications of the findings: Our data in humans corroborate experimental data in mice and support the hypothesis that in some patients, high AMH levels, through stimulation of LH secretion, drive PCOS. In such an AMH-dependant PCOS phenotype, hyperandrogenism and oligo-anovulation would be prominent features while the role of insulin-resistance would be of less importance.

Trial registration number: non applicable

O-010 Anti-Müllerian hormone levels in adolescence in relation to long-term follow up for presence of polycystic ovary syndrome

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Study question: Can serum anti-Müllerian hormone (AMH) levels and/or polycystic ovary syndrome (PCOS) associated features in adolescence predict the presence of PCOS in adulthood?

Summary answer: Our results confirm that AMH measured in adolescence correlate with PCOS features, however AMH as prognostic marker does not contribute to diagnosing PCOS in adulthood.

What is known already: PCOS associated features as oligo-amenorrhea, hyperandrogenism and polycystic ovarian morphology correlate with high AMH. However, diagnosing PCOS in adolescence is difficult and there is a strong call for a marker that can predict the occurrence of PCOS in adulthood. Whether AMH measured in adolescence can predict PCOS in adulthood is still unknown.

Study design, size, duration: This long-term follow-up study is based on a unique adolescent study on menstrual irregularities performed between 1990-1997 by the VU University Medical Center, Amsterdam. By the use of stored material we assayed 271 adolescent blood samples for AMH. In addition, we contacted this study group more than two decades after the initial study for a questionnaire on their current menstrual cycle pattern and presence of PCOS features in adulthood.

Participants/materials, setting, methods: In 271 adolescents with a mean age of 15.2 years various data on menstrual cycle information, physical information, ovarian morphology and hormonal measurements could be combined with AMH serum levels. In 160 of the 271 (59%) participants we collected information in adulthood about their menstrual cycle pattern and presence of PCOS (features) by a questionnaire.

Main results and the role of chance: AMH levels at adolescence were significantly higher in girls with oligo-amenorrhea compared with regular cycling girls: 5.5 versus 3.1 µg/L, $P < 0.001$. Adolescents with PCOM had significantly higher AMH levels with a mean AMH of 5.2 µg/L, compared to 3.1 in the non-PCOM group ($P < 0.001$). AMH levels correlated with the presence of hyperandrogenism.

In the follow-up study, the mean age was 39.6 years. 22.5% of these women reported oligomenorrhea and 11.9% were identified as having PCOS. Women with PCOS in adulthood had a mean adolescent AMH of 4.7 compared with 3.6 µg/L in the non-PCOS group ($P = 0.051$). Women with oligomenorrhea in adulthood had a significantly higher adolescent AMH of 4.5 compared with 3.5 µg/L in adults with regular cycles ($P = 0.025$). ROC curve analysis of AMH at adolescence showed poor area under the curve for PCOS and oligomenorrhea in adulthood: 0.638 and 0.623, respectively.

Of the adolescent cohort with an oligo-amenorrhea, 23.1% had adult PCOS; compared to 6.5% in adolescents with a regular cycle. Also, 40.4% of the oligomenorrheic adolescents were classified as adult oligomenorrhea. Given adolescent oligomenorrhea, using high AMH as factor to predict adult PCOS or adult oligo-amenorrhea was of no value.

Limitations, reasons for caution: The adult PCOS diagnosis is based on a self-reported questionnaire, without physical examination and/or ovarian ultrasound which may have caused that we have missed some women with PCOS.

Wider implications of the findings: This is the first very long-term follow-up study investigating AMH and PCOS features in adolescence as marker for adult PCOS. Our results show that adolescent AMH can distinguish between PCOS features, however it does not contribute as prognostic marker for PCOS. We do not recommend routine use in clinical practice.

Trial registration number: Dutch Trial Registry, NTR5871

O-011 Optimizing follicle-stimulating hormone concentration during human IVM

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Study question: What is the optimal concentration of follicle-stimulating hormone (FSH) during human *in vitro* maturation (IVM) in terms of oocyte maturation rate?

Summary answer: The FSH concentration can be reduced to 40 mIU/mL without reducing the rate of oocyte maturation.

What is known already: Exogenous FSH is commonly used for ovarian stimulation *in vivo* as well as for oocyte maturation *in vitro*. Nonetheless, there is increasing evidence suggesting that elevated FSH levels can impair oocyte developmental capacity. To our knowledge, no study has defined the optimal FSH concentration for human IVM. Hence, the great number of immature oocytes recovered from the surplus medulla tissue of non-stimulated patients receiving ovarian tissue cryopreservation for fertility preservation will potentially be able to benefit patients and improve reproductive outcome. Additionally, patients that cannot receive ovarian stimulation may also benefit from optimized conditions for human IVM.

Study design, size, duration: This is a control-versus-treatment study. Immature oocytes were recovered from surplus tissue of 22 patients (age 14-40 years) who had one ovary excised for fertility preservation by ovarian tissue cryopreservation. Most oocytes derived from follicles with diameters below 3mm. Immature oocytes with similar diameters and that did not show signs of degeneration, such as darkened cytoplasm, or misshapen zona pellucida, were selected and homogeneously distributed into culture media with different concentrations of FSH.

Participants/materials, setting, methods: A total of 566 immature oocytes were divided into three categories according to their cumulus mass: cumulus-oocyte complexes (COCs) with large cumulus mass (L-COCs), small cumulus mass (S-COCs) and naked oocytes (N-Oocytes), and then submitted to 48h IVM in the presence of increasing concentrations of recombinant FSH (Rekovelle, Ferring, Copenhagen, Denmark): 20 mIU/mL, 40 mIU/mL, 70 mIU/mL or 250 mIU/mL. As outcome parameters, oocyte nuclear maturation and diameter were recorded.

Main results and the role of chance: On average, 26 oocytes were recovered per ovary (range 8-73), being 42% L-COCs (N=239), 39 % S-COCs (N=218), and 19% N-Oocytes (N=109). After IVM, mean oocyte diameters were similar ($P > 0.05$) among all treatments (range 113.3-114.1 µm, zona pellucida not included). Including all oocyte categories (L-COCs, S-COCs, and N-Oocytes), the three treatments with FSH above 20 mIU/mL i.e., 40, 70, and 250 mIU/mL, significantly increased oocyte nuclear maturation (22% MII vs. 35% MII, respectively) ($P < 0.03$). Regardless of the FSH concentration, oocyte maturation increased when increasing cumulus cell mass (18% N-Oocytes, 28% S-COCs, and 35% L-COCs) ($P < 0.01$) and oocyte diameter ($P < 0.0001$). Thus, considering only oocytes with cumulus cells (S-COCs and L-COCs) (N=457), there was an increase in oocyte maturation of almost 13% when FSH exceeded 20 mIU/mL (from 24.7% to 37.4% MII) ($P < 0.03$), resulting in an average of 8 MII oocytes per ovary. There were no significant differences in oocyte maturation when FSH concentration increased from 40 to 250 mIU/mL.

Limitations, reasons for caution: Since Danish legislation does not consider IVM as clinical practice, the obtained MII oocytes could not be fertilized *in vitro*. Therefore, only oocyte nuclear maturation, and no cytoplasmic maturation and fertilizing capacity, was assessed. Hence, further experiments are needed to elucidate the developmental capacity of these oocytes.

Wider implications of the findings: Similar maturation rates were achieved using 40 mIU/mL FSH to those described in non-stimulated patients with 75 mIU/mL FSH. Reducing FSH concentration could diminish its detrimental effect on oocytes. Using oocytes from small antral follicles (1-5 mm) can improve the fertility preservation of patients by complementing ovarian cortical tissue freezing.

Trial registration number: Not applicable

O-012 NK3R antagonist ameliorates metabolic status and reverses obesity in mice

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Study question: Does NK3R antagonist (NK3Ra) play a role in weight loss and improving glycolipid metabolism?

Summary answer: NK3Ra can promote fat conversion and metabolic efficiency, improve insulin resistance and glucose tolerance, and thus improve the overall metabolism of the body.

What is known already: NKB binding with its receptor NK3R induces kisspeptin release and further regulates the pulsed release of GnRH in the hypothalamus. NK3Ra can ameliorate endocrine disorders by decreasing LH and androgenlevel. Recent studies have shown that lipid metabolism can affect the NKB-NK3R signaling pathway, thereby affecting reproductive endocrine function and adolescent sexual maturation. But the role of NK3R in metabolic regulation is unclear.

Study design, size, duration: A prospective, randomized mouse experimentation aiming to identify the effect of NK3Ra on the improvement of metabolic disorders were conducted. Fifty female Balb/C mice were randomly divided into 5 groups of equeal size for high-fat diet, constant light, high-fat diet + constant light, NK3Ra + high-fat diet + constant light and controls. All mice were sacrificed 14 weeks after induction of the metabolic disorder model.

Participants/materials, setting, methods: The mice were weighed regularly and micro-CTwas conducted before sacrificed. Glucose and insulin tolerance tests were conductedand serum levels of glycolipid correlated indexe was detected by chemical colorimetry. The adipose tissues were observed and UCP1 expression was detected using immunohistochemistry. The marker molecules level of adipose synthesis and deposition were detected using RT-qPCR. The Luminex detecting technique was used to determine expression differences of inflammatory markers. Data were analyzed using GraphPad Prism 6.

Main results and the role of chance: We found that the weight of mice in NK3Ra group was significantly decreased, and the volume of subcutaneous fat and visceral fat was significantly reduced, indicating that NK3Ra can promote the decomposition and utilization of body fat. The serum levels of total cholesterol, triglyceride and low density lipoprotein in NK3Ra group decreased, while the levels of high density lipoproteinand apolipoprotein increased, which proved that NK3Ra could improve fat transport and metabolic efficiency in mice. NK3Ra significantly reduced the levels of IL-1 and MCP-2 in serum, indicating that NK3Ra can improve the level of inflammatory reaction caused by metabolic disorders to a certain extent, thus breaking the vicious circle of mutual intensification between metabolic disorders and inflammation. NK3Ra can trigger the beiging of white adipocytes, accordingly, the expression of UCP1 increases. Fatty acid binding protein 4, peroxisome proliferator-activated receptor gamma, resistin and leptin were significantly decreased in the adipose tissue of mice in NK3Ra group, indicating that NK3Ra could inhibit adipose synthesis and deposition. In addition, NK3Ra can also improve insulin resistance and glucose tolerance in mice.

Limitations, reasons for caution: These findings are based on a small sample size of mice. The results need to be confirmed by human clinical trials.

Wider implications of the findings: NK3Ra are known to be improve the endocrine state by reducing LH pulse frequency and androgen level, combined with the results of this study, we conclude that NK3Ra may offer therapeutic benefit in the treatment of diseases with reproductive endocrinedisorders and glycolipid metabolism problems, such as PCOS.

Trial registration number: not applicable

O-013 Anti-Müllerian Hormone (AMH) as a quantitative and qualitative marker of euploid blastocysts

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Study question: Is AMH an effective tool to predict the percentage of euploid embryos and blastocyst formation irrespective of the age of the patient?

Summary answer: Higher AMH levels are associated with a higher rate of euploid embryos and increased blastocyst formation on day 5.

What is known already: AMH is an established marker of the ovarian reserve 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 and it has been demonstrated that high AMH levels, e.g. due to the Polycystic Ovary Syndrome, are at increased risk of poor blastocyst development. Reduced ovarian reserve might be *per se* associated with decreased oocyte developmental competence leading to increased aneuploidy rates in embryos independent of the age of the patient.

Study design, size, duration: A retrospective analysis was performed between March 2017 and August 2018 including couples planned for Preimplantation Genetic Testing for Aneuploidies (PGT-A). Patients were split into two groups and were analyzed individually; (i) the fresh group comprised of couples who underwent PGT-A with only fresh oocytes (n=516) and (ii) the vitrified group (n=184) in which vitrified oocytes were accumulated from 1.97 (±1.26)previous ovarian stimulation cycles, as a strategy to increase the number of potential euploid embryos.

Participants/materials, setting, methods: Vitrification and warming were performed with the Cryotop method (Kitazato, Biopharma). Trophectoderm biopsy samples were subjected to Next Generation Sequencing (NGS) to screen the cells. AMH serum levels (ng/ml) were determined using a commercial fully automated assays Elecsys® (Roche) and values >5 were excluded. Blastulation rate was defined as the number of fertilized embryos capable of cavitating on day 5.

Main results and the role of chance: Linear regression analysis was conducted to verify the predictability of AMH values and the percentage of euploid embryos and blastulation rate on day 5. A Poisson regression model was used to correlate AMH levels with the number of euploid embryos according to the number of embryos biopsied. In the fresh group, average maternal age was 35.8 years (± 5.95), AMH 1.95ng/ml (±1.27), 54% (±33%) blastulation rate on Day 5, 46% (±35%) euploid rate. Higher AMH values were found to have a statistically significant effect on the percentage of euploid embryos (p=0.001) and blastocyst formation on day 5 (p<0.001) as well as for the number of euploid embryos (p<0.001). In the vitrified group, average maternal age was 38.55 (± 5.35), AMH 1.2ng/ml (±1.06), 8.43 (±5.57) MII oocytes warmed, 86% (±21%) survival rate, 34% (±33%) blastulation rate on day 5, 31% (±39%) euploid rate. As in the fresh group, higher AMH values were found to have a statistically significant effect on the percentage of euploid embryos (p=0.009) as well as for the number of euploid embryos (p=0.003). However, no significant differences were found between higher AMH levels and blastocyst formation (p=0.249).

Limitations, reasons for caution: Retrospective design of the study.

Wider implications of the findings: The independent relationship between AMH and the percentage of euploid embryos suggests that AMH is not only a quantitative but also a qualitative biomarker of oocyte-embryo competence. As the effect of AMH on blastocyst formation is lost after oocyte vitrification, the use of oocyte accumulation should be further evaluated.

Trial registration number: N/A

O-014 Time span for recovery and degree of change of ovarian reserve markers after discontinuation of long-term use of combined oral contraceptives

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Study question: How early does the ovarian reserve markers Anti-Müllerian-hormone (AMH) and antral follicle count (AFC) normalize after discontinuation of long-term use of combined oral contraceptives (COC)?

Summary answer: After discontinuation of COC the ovarian reserve markers increased with 59% (AMH) and 48% (AFC), and normalization occurred within two months.

What is known already: Combined oral contraception is the most preferred contraceptive method in the Western world. Cross-sectional studies reported 20-40% lower levels of AMH and AFC during COC-use. Women use COC for years to decades while postponing childbearing; many women therefore wish to address their ovarian reserve during contraceptive usage. The recovery of AMH/AFC after discontinuation has not been investigated systematically for long-term-users of COC and it is not fully elucidated when a recovery occurs.

Study design, size, duration: The study was conducted as a prospective cohort investigating the ovarian reserve markers during COC-use and serially in a 3-month period after discontinuation. The study population comprised 74 women from the general population with a history of long-term use of COC and who wished to withdraw COC for three months. The women were included in the study between 2016 to 2018. In total, 69 women completed the 3-month follow-up period.

Participants/materials, setting, methods: Inclusion criteria: COC-use for ≥3 consecutive years, age between 24-41 years. Intervention: Six serial examinations including investigation of the ovarian reserve markers (AMH, AFC and ovarian volume) and analyses of reproductive hormones on the following time-points: During COC-use, 5-7 and 14 days after discontinuation, and for 3 consecutive menstrual cycles (cycle day 2-5). AMH was measured with the Roche Elecsys-assay. Statistical analyses included linear regression and mixed models for repeated measurements adjusted for relevant co-variates.

Main results and the role of chance: Mean age was 29.5 years (SD3.7) and mean duration of COC-use was 11.6 (SD3.7) years. Nearly 80% regained spontaneous menstrual cycle within 35 days after the COC-caused withdrawal bleeding after COC-discontinuation. Six women met the Rotterdam criteria for PCOS by the end of the follow-up period. Mean values of AMH increased significantly from 15.9 pmol/L, (SD10.4) (2.2 ng/ml) to 26.3 (SD19.7) (3.7 ng/ml), p<0.0001, and AFC increased from 19.1 (SD10.5) to 26.8 (SD14.2), p<0.0001, during the three months follow-up after discontinuation of the COC. This corresponded to an increase in AMH and AFC of 59% and 48%, respectively. There was a significant increase in AMH and AFC from baseline until second menstrual cycle, but not from the second to third. Longer duration of COC-use and higher age were significantly associated with lower absolute change in AFC (p=0.02 and p=0.01, respectively), whereas women that fulfilled Rotterdam criteria for PCOS was associated with higher increments (p=0.002). Age and PCOS-status were significantly associated with AMH increments in a likewise manner (p=0.03). We believe these results were not caused by chance due to the low p-values in combination with a reasonable number of participants and within-individual comparisons and a priori statistical power calculations.

Limitations, reasons for caution: We have used an improved and validated AMH assay and hereby reduced earlier reported assay variability. The prospective design and repeated measurement within the same individual decrease risk of bias. The number of individuals is, however, limited in order to assess whether the findings also account for subgroups of individuals.

Wider implications of the findings: This study highly contributes to the understanding of the ovarian reserve markers during COC-usage, and since AMH and AFC is often included in the testing of the ovarian reserve, the results from this study can directly contribute as a guide to testing of women in terms of their ovarian reserve.

Trial registration number: Clinical trials: NCT02785809 Regional Ethical Committee: H-16021266

SELECTED ORAL COMMUNICATIONS

SESSION 04: FERTILITY OUTCOMES AND THE MALE

Monday 24 June 2019 Haydn 3 10:00–11:30

O-015 Stakeholder and patients' perspectives for success, risks and cost implications on 319,105 IVF/ICSI and 30669 IUI cycles suggest IUI provides favourable first line treatment option

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Study question: What is the efficacy of IUI and IVF treatments with regards to success, risks and costs?
Summary answer: IUI should be the first line treatment choice providing significantly better overall benefits than IVF when pregnancies, risks and cost values are factored in.
What is known already: Most success and risks factors are known individually. However, for the first time an integrated analysis of success and risks from an extremely large database generated independently of researchers removes selection biases. Risk factors relating to multiple births, fetal reductions, terminations and OHSS have not previously been addressed on a large scale. Previous cost-effective analyses have avoided factoring in the risks of treatment procedures, while cost effectiveness studies artificially favour IVF, having incorrectly been compared against substandard abnormal IUI practices. IVF clinics have innovative ways of presenting inflated success rates which can be misleading to stakeholders and patients.
Study design, size, duration: A retrospective observational study was performed, based on 319,105 IVF/ICSI and 30669 IUI cycles registered on the HFEA database from 2012-2016. Additional data on associated risks was gained under the Freedom of Information (FOI). Direct costing analyses used updated Bank of England figures for multiple birth costs, while cost effective studies was based on each techniques ability to deliver one live birth against prevailing tariffs. Data submission to the HFEA is on a mandatory requirement.
Participants/materials, setting, methods: Normal IUI and IVF practice data was obtained from the large UK regulated HFEA database for 2012-2016, combined with FOI requested for the associated risks. The data was collected entirely by HFEA staff removing any selection biases. Direct cost studies incorporated the COMBS (2005) model whereas indirect cost relied upon incremental cost-effective ratios (ICER). Statistics z-analyses on trends was considered statistically significant showing $p < 0.05$.
Main results and the role of chance: From 2012-2016 there was a significantly increased 10.4-fold ($p<0.05$) practice of IVF over IUI while IVF: IUI twins was 38.4-fold and 7.6-fold triplets. Multiple births from IVF appear to be managed by fetal reductions and terminations. For the period 2012-16 the there was a 11.5%/cycle PR (pregnancy rate) yielding 8.6% twins and 0.93% triplets. The IVF/ICSI revealed 27%/cycle LBR yielding 13.7% twins and 0.25% triplets and minimal quadruplets. OHSS per birth and cycle treatment was 0.9% and 0.25% respectively. Fetal reduction was 0.2% of all births, and 0.81% termination of all births. Multiple birth as a proportion of all births was significantly higher following IVF than following IUI [IVF: 13.88% (13.65-14.11); IUI: 9.59% (8.62-10.56). RR: 1.45 (1.31-1.60) $p<0.05$]. Approximately 50% contribution to IUI multiple births came from private IVF clinics that also produced relatively high IUI success rates. New costing analyses are represented by 1 IUI success made up of 91.2%: 7.9%:0.85% singleton, twin and triplet respectively whereas for IVF/ICSI this is 86.2%:13.6%:0.13%. The UK IVF industry is worth over £300 million but provides a risk cost burden of over £500 million. IUI is cheaper than IVF/ICSI with baseline success rates, extending to £35,000-55,000/LB saving with 15-17%/cycle IUI.
Limitations, reasons for caution: Further costs due to OHSS, fetal reduction, termination, low birth weight IVF singletons, embryo freezing, and embryo culture costs and add on procedure remain undisclosed. Stimulation methodology is not revealed. More desirable was numbers of women, mean numbers of IUI and IVF cycles undertaken, both unavailable from the UK HFEA.
Wider implications of the findings: IUI is better than IVF for patients and stakeholders after factoring in success, risks and cost effectiveness. IVF risk burden nationally exceeds the total value of the IVF industry and these burdens have not previously been disclosed. IVF success is inherently interlinked to NICE initiated decline of UK IUI practices.
Trial registration number: Not applicable

O-016 Prediction of successful ICSI cycles by Oxidation-reduction potential (ORP) and sperm DNA fragmentation (SDF) analysis. A Prospective study

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Study question: Does the clinical utilization of SDF and ORP tests helps in accurate prediction of fertilization in ICSI cycles?
Summary answer: SDF and ORP testing could be used as a valuable diagnostic tool in IVF clinics to predict fertilization but not blastulation in ICSI cycles.
What is known already: Seminal oxidative stress (OS) and sperm DNA fragmentation (SDF) are two advanced sperm function tests that are increasingly used in the evaluation of infertile men. OS has recently been identified as a major mediator in the various causes of male infertility. It is well established in the literature that a high percentage of SDF due to various factors including OS has adverse effects on ART outcomes.
Study design, size, duration: This prospective pilot study evaluated a total of 50 patients undergoing ICSI treatment for male factor infertility (n=50). The study was carried out from June 2017 to December 2018 and was approved by Biomedical Ethics Committee of the University of the Western Cape, Cape Town, South Africa. All participants signed written informed consent form.
Participants/materials, setting, methods: The flourometric TUNEL assay (Promega Corporation, Madison, USA) and the MiOXSYS system (Aytu Bioscience, Englewood, CO) were used to measure SDF and the oxidation-reduction potential (ORP), respectively. The data generated was then correlated with the fertilization and blastulation rates. Statistical analysis including ROC curve analysis was performed. The study included patients using autologous fresh gametes only and excluded all day 3 embryo transfers and advanced maternal age.
Main results and the role of chance: ROC curve analysis to predict fertilization and blastulation rates used published cut off values of 36% TUNEL-positive cells for SDF and 1.36 mV/10^6 sperm/ml, respectively for ORP. For SDF ($P<0,0001$ for fertilization and $P=0,0897$ for blastulation), the ROC curve analysis resulted in a sensitivity of 67,6% and 89,7%, specificity 84,6% and 50.0%, positive predictive value 92,6% and 89,7% and negative predictive value 47,8% and 50,0% for fertilization and blastulation, respectively. [APA1] The areas under the curve [AUC] were 0,830 and 0,699 for fertilization and blastulation rate, respectively. For ORP ($P<0.001$), the analysis showed a sensitivity of 100.0%, a specificity of 62.9%, a positive predictive value of 55.6% and a negative predictive value of 95.0% with an AUC of 0.834 for fertilization. However, no significance was seen for blastulation ($P=0.8146$).
Limitations, reasons for caution: The results observed in this study should be confirmed using a larger number of samples.
Wider implications of the findings: The study demonstrated strong predictive capabilities of SDF and seminal ORP measurement in the clinical setting. This information is useful in providing the clinician with a valuable diagnostic tool for prediction of fertilization in ICSI cycles.
Trial registration number: Not Applicable.

O-017 Impact of paternal age, ejaculatory abstinence length and semen quality on the outcomes of intracytoplasmic sperm injection (ICSI) in an egg-sharing donation program

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Study question: Can paternal age, ejaculatory abstinence length (EA) and semen quality influence ICSI outcomes in recipients' cycles from the same donor in an egg-sharing donation program?

Summary answer: Increasing paternal age and EA, and poor semen parameters negatively impact ICSI outcomes, from fertilization rate to pregnancy, in recipients' cycles from the same donor.

What is known already: The impact of male partner characteristics on IVF is often ignored, even though male-factor infertility is known to play a role in 50% of the cases. Indeed, the male factor infertility is equally important to establish the success of assisted reproduction cycles. However, few studies have focused on the influence of male factors on IVF outcomes, with conflicting results, probably due to confound variables introduced in the analysis, when it comes to autologous cycles. Considering this, the use of an egg-sharing donation program may be extremely useful to study the impact of partner characteristics and semen parameters on ICSI outcomes.

Study design, size, duration: Data analyzed in this historical cohort study were obtained via chart review of 268 vitrified oocyte donor ICSI cycles, and 321 oocyte recipients undergoing 427 oocyte recipient ICSI cycles, participating in an egg-sharing donation program, between January/2015 and May/2017. For that sample size, computed achieved post-hoc power was 95.7%. Oocyte donors were between the age of 19 and 35 years, and recipients were between the age of 26 and 59 years.

Participants/materials, setting, methods: This study was performed in a private university–affiliated IVF center. General Mixed Models fit by restricted maximum likelihood, generated using covariates as fixed effects and egg-donors and egg-recipients as random effects, with unstructured covariance structure, were used to investigate the impact of paternal age, EA and semen quality on recipients' ICSI outcomes. The results are expressed as regression coefficient (B), standard error (SE), exponentiation of B (ExpB), 95% confidence interval (CI), and p-value.

Main results and the role of chance: Fertilization rate was negatively affected by paternal age (B: -0.276, p: 0.001) and positively affected by sperm count (B: 0.075, $p < 0.001$). High-quality embryos rate on day 3 was negatively correlated with paternal age (B: -0.040, p: 0.021) and EA (B: -0.003, p: 0.028). Normal embryo development (cleavage speed) rate on day 3 was negatively affected by paternal age (B: -2.750, p: 0.001) and EA (B: -0.300, p: 0.036), and positively affected by the percentage of progressive sperm motility (PSM, B: 0.017, p: 0.024). Blastocyst development rate was negatively influenced by paternal age (B: -0.070, p: 0.043) and EA (B: -0.589, p: 0.016), and positively influenced by sperm count (B: 2.155, p: 0.015) and total motile sperm count (TMSC, B: 1.057, p: 0.038). Paternal age was negatively correlated with high-quality blastocysts rate (B: -44.058, p: 0.031). Implantation rate was negatively affected by paternal age (B: -0.060, $p < 0.001$) and EA (B: -0.012, $p < 0.001$), and positively affected by sperm count (B: 0.025, $p < 0.001$), PSM (B: 0.183, $p < 0.001$) and TMSC (B: 0.008, p: 0.009). Paternal age was associated with reduced odds of pregnancy (ExpB: 0.664, p: 0.033).

Limitations, reasons for caution: In Brazil, egg donation may not be conducted for profitable purposes, so oocytes are donated from patients undergoing IVF treatments. Thus, the oocytes included in this study originated from infertile couples. Nevertheless, the evaluation of paternal characteristics on oocytes from infertile couples is a realistic situation in assisted reproduction.

Wider implications of the findings: Further tracking of the impact of paternal characteristics on ICSI outcomes should be encouraged. Despite paternal age is uncontrollable, and there are only so many things that can be done concerning semen quality, shortening of EA length could be used as a strategy to optimize ICSI outcomes.

Trial registration number: None.

O-018 Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes

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Study question: Does the duration of donor sperm storage affect the chances of success among women undergoing medically assisted reproduction?

Summary answer: Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes.

What is known already: Cryopreservation of donor semen is a widely used technique in artificial insemination by donor (AID) and in vitro fertilization (IVF) for azoospermia and fertility preservation. Although cryopreservation has negative effects on the plasma membrane of sperm, no data have been reported on the effects of long-term storage of sperm on clinical outcomes to date.

Study design, size, duration: This study included 119,558 specimens retrieved using a clinical information database of young adult men who were qualified sperm donors at the Hunan Province Human Sperm Bank of China, from January 1 2001, to December 31 2016. Clinical information included semen parameters before and after freezing, the date of the semen analysis and clinical outcomes after semen use.

Participants/materials, setting, methods: Three groups of semen categorized by their lengths of crystorage were analyzed: 0.5–5, 6–10, and 11–15 years. Between-group differences were tested using the Chi-square test. Sperm motility before and after cryostorage was compared using the paired sample *t*-test and the Wilcoxon signed-ranks (paired) test.

Main results and the role of chance: The sperm's frozen-thaw survival rate decreased from 85.72% to 73.98% after 15 years of cryopreservation ($P < 0.01$). The clinical pregnancy rate of women undergoing AID was 23.09%, 22.36% and 22.32%, the clinical abortion rate was 10.06%, 10.02% and 12.00% and the the live birth rate was 82.17%, 80.21% and 80.00% in the groups with 0.5–5, 6–10 and 11–15 storage years, respectively. The clinical pregnancy rate of women undergoing IVF was 64.29%, 64.94% and 53.48% in the groups with 0.5–5, 6–10 and 11–15 storage years, respectively. The clinical abortion rate was 12.26%, 11.38% and 17.39% and the live birth rate was 81.63%, 79.11% and 73.91%, in the groups with 0.5–5, 6–10 and 11–15 years, respectively. The clinical pregnancy, abortion and live birth rates were not significantly different between donor sperm with 6–10 and 11–15 storage years and sperm with the shortest storage length (0.5–5 storage years).

Limitations, reasons for caution: The study's limitations include lack of data on congenital abnormalities based on pregnancy outcomes and lack of a clear medical diagnosis; Our findings are not based on the general population of men, as the sperm donors in the study had better sperm quality than most men.

Wider implications of the findings: Although long-term cryostorage might not affect clinical outcomes, sperm banks should provide sperm in their order of cryopreservation.

Trial registration number: not applicable

O-019 Patients with childhood cancer who underwent thoracic or abdominal irradiation have poor gonadal function in adulthood

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Study question: Which therapy used to treat childhood cancers has the worst effect on the reproductive function?

Summary answer: Patients treated with chemotherapy plus non-cranial radiotherapy have worse testicular function than patients treated with cranial irradiation plus chemotherapy or chemotherapy alone.

What is known already: Altered sperm parameters, hypogonadism and infertility are potential long-term sequelae in adult males treated for childhood cancer. Cranial irradiation with doses >22 Gy has been associated with pituitary dysfunction, while it has been shown that testicular irradiation and treatment with high doses of alkylating agents are responsible for gonadal dysfunction.

Low inhibin B, high FSH and total (left + right) testicular volume (TV) ≤24 mL are considered predictive factors for azoospermia in childhood cancer survivors. The age of treatment does not seem to influence reproductive outcomes.

Study design, size, duration: Seventy-nine patients who underwent annual hematological follow-up visit in 2018 at the Pediatric Oncohematology Clinic of the "Gaspare Rodolico" University Hospital, University of Catania, were enrolled in this cross-sectional study.

Participants/materials, setting, methods: Patients had been treated with chemotherapy alone, chemotherapy plus radiotherapy, and/or bone marrow transplantation and were declared disease-free for at least 5 years. Patients who underwent testicular irradiation or orchiectomy for testicular neoplasia, and who were in testosterone replacement therapy at the time of the visit were excluded. All patients underwent scrotal ultrasound; 71 patients underwent blood sampling for LH, FSH and total testosterone (TT) measurement; 21 patients did also sperm analysis.

Main results and the role of chance: At the time of enrollment, patients' mean age was 23 years (range 16-38). At the time of diagnosis, patients' mean age was 6 years (range 1-16). Main diagnoses were: acute lymphoblastic leukemia (n. 53), non-Hodgkin's lymphoma (n. 10), Hodgkin's lymphoma (n. 5), hepatoblastoma (n. 3), acute myeloid leukemia (n. 2), nephroblastoma (n. 2). 50 patients had been treated with chemotherapy alone; 25 with chemotherapy plus radiotherapy; 4 patients underwent bone marrow transplantation. Overall, 33% of patients showed total TV <24 ml, 31% had TT <3.5 ng/mL, 48% had decreased sperm count (half of them were azoospermic, the other half had oligozoospermia). As expected, patients who underwent bone marrow transplantation showed the worst gonadal function. Patients who underwent chemotherapy plus cranial irradiation showed no statistically significant differences in gonadotropin and TT levels, TV, and sperm count compared to patients who underwent chemotherapy alone. Patients treated with chemotherapy and thoracic or abdominal irradiation had higher FSH levels ($p<0.001$) and lower TV ($p=0.001$) than patients treated with chemotherapy alone. Regarding the age of treatment, patients treated at an age ≥10 years showed higher FSH levels ($p=0.027$), however data became not statistically significant when we excluded patients who underwent thoracic or abdominal irradiation.

Limitations, reasons for caution: These are preliminary data. The number of patients is small, nevertheless enrollment is continuing. We have not been able to find in the old medical records the exact dosages of some drugs and radiotherapy administered. Many patients did not want to undergo sperm analysis.

Wider implications of the findings: Our data are in agreement with other recent studies showing that, even at a dosage of 24 Gy, cranial irradiation doesn't affect reproductive outcomes. On the contrary, thoracic and abdominal irradiation have a strong negative effect on testicular germinal function. Better shielding of the gonads is, therefore, needed during irradiation.

Trial registration number: Not applicable

O-020 ICSI using surgically retrieved testicular sperm of non-azoospermic men with high sperm DNA fragmentation index and blastocyst ploidy: a safe approach

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Study question: Is blastocyst euploidy affected by the use of surgically retrieved spermatozoa from non-azoospermic men with high sperm DNA fragmentation (SDF) for intracytoplasmic sperm injection (ICSI)?

Summary answer: The probability of having euploid blastocysts is not affected by the use of testicular sperm from non-azoospermic men with elevated SDF for ICSI.

What is known already: Recent studies have reported that the use of testicular sperm in preference over ejaculated sperm for ICSI have a positive effect on the chances of pregnancy, in particular, among non-azoospermic men with high SDF in their ejaculate. However, concerns were raised regarding a possible detrimental effect of using testicular sperm from these men due to the reportedly higher aneuploidy rates in spermatozoa harvested from the seminiferous tubules of azoospermic individuals. Yet, data concerning trophectoderm biopsy analysis using comprehensive chromosome testing that allow the assessment of the whole karyotype of embryos resulting from surgically retrieved spermatozoa is lacking.

Study design, size, duration: Single-center retrospective analysis of 940 trophectoderm biopsies from 362 infertile couples subjected to ICSI with own ejaculated (280 patients; 764 embryos) or surgically retrieved sperm (82 patients; 176 embryos) between 2016-2017. Preimplantation genetic testing for aneuploidy (PGT-A) was indicated because of advanced maternal age, severe male factor, recurrent miscarriage/implantation failure, and concerns about the ploidy status of embryos. SDF analysis was carried out using the sperm chromatin dispersion test in all eligible non-azoospermic men.

Participants/materials, setting, methods: Biopsied trophectoderm cells were analyzed by next-generation sequencing analysis (NGS). Logistic regression was applied to the dataset. The dependent variable was blastocyst genetic status (euploid/aneuploid) whereas the independent variables were female age, male age, origin of sperm for ICSI (testicular/ejaculated), sperm DNA fragmentation index (DFI) as well as the reason for using surgically retrieved sperm (obstructive azoospermia [OA], non-obstructive azoospermia [NOA], and elevated (>30%) DFI [high DFI]). Computations were performed using JMP 13.

Main results and the role of chance: The mean female and male ages were 38.9±3.6 and 42.4±6.7 years, and the mean number of biopsied blastocysts per patient was 2.6. Overall, the percentage of euploid embryos in our cohort was 36.4% whereas the mean number of euploid blastocysts per patient was 0.73. The fitted model selected female age as the only relevant predictor ($p<0.0001$), but the origin of sperm and the reason for using testicular sperm had a nested effect on blastocyst euploid within female age. Male age and DFI results did not affect blastocyst euploidy. The logistic model generated the probability that a mature oocyte become a euploid blastocyst once injected with either ejaculated or surgically retrieved sperm, adjusted by female age. There was a significant ($p<0.0001$) decrease in the probability of a blastocyst being euploid with every year of female age, irrespective of the type of sperm used for ICSI. The use of testicular sperm from men with high DFI had no significant effect on blastocyst euploidy when compared to ejaculated sperm and testicular sperm from OA men. In contrast, use of testicular sperm from NOA men had an overall adverse effect on blastocyst euploid ($p=0.03$), but the effect varied according to female age.

Limitations, reasons for caution: The impact of other covariates on embryo genetic status could not be assessed from the dataset. Other limitations are the study's retrospective nature and the difference in the number of embryos subjected to PGT-A according to sperm origin and the reason for using testicular sperm for ICSI.

Wider implications of the findings: Embryos resulting from ICSI using either testicular sperm of non-azoospermic men with high DFI or ejaculated sperm have similar chances to be euploid, depending only on female age. Our data corroborate the safe utilization of surgically retrieved sperm from non-azoospermic men with high SDF for ICSI.

Trial registration number: NA

SELECTED ORAL COMMUNICATIONS

SESSION 05: ITS ALL ABOUT THE ENDOMETRIUM

Monday 24 June 2019 Haydn 2 10:00–11:30

O-021 Artificial cycle for frozen embryo transfer is associated with increased miscarriage rate compared to natural/stimulated cycle : a large multicenter cohort study (14421 cycles)

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Study question: Does the endometrial preparation protocol (artificial cycle vs natural cycle vs stimulated cycle) affect reproductive outcomes of Frozen / thawed embryo transfer (FET) ?
Summary answer: In FET, artificial cycles were significantly associated with a higher miscarriage rate and a lower live birth rate compared to stimulated or natural cycles.
What is known already: FET represent a growing part of embryo transfers. Three protocols are used for the endometrial preparation: the natural cycle (NC), the stimulated cycle (SC) with gonadotrophin stimulation and, the more widely used, the artificial cycle (AC) with sequential estrogen/progesterone treatment. To date, there is no consensus on the optimal endometrial preparation regarding outcomes. However, some studies have reported a higher miscarriage rate with the artificial cycle compared to the natural or stimulated cycles. But no significative difference were found on ongoing pregnancy rate or live birth rate (LBR). Furthermore, no studies have compared the three protocols in a large population.
Study design, size, duration: A multicentric retrospective study was conducted in nine french reproductive units, using the same software to record medical files, between 1st January 2012 and 31th December 2016. The primary outcome was miscarriage rate at 10 weeks of gestation. A sample size calculation was made to detect an increase of 5% in the miscarriage rate (21 to 26%) with risk alpha 0.5 and power 0.8 : 1126 pregnancies were needed in each group, 3378 in total.
Participants/materials, setting, methods: The data were collected by automatic extraction using the same protocol. All the consecutives autologous FET cycles were included : 14421 cycles (AC 8139, NC 3126 and SC 3156) corresponding to 3844 pregnancies (HCG > 100 IU/L) (AC 2214, NC 812 and SC 818). An online questionnaire was conducted for each center to describe its routine practice for FET, particularly the reason of choice of a protocol over another one.
Main results and the role of chance: The AC represented 56.5% of the FET cycles. The mean age (SD) was 33.5 (4.3). The mean number of embryo replaced was 1.5 (0.5). The groups were comparable except for history of dysovulation p=0.01 and prior delivery p=0.03, significantly higher in AC. Overall, the miscarriage rate was 31.5%, AC 36.5%, NC 25.6%, SC 23.6%. Univariate analysis, showed a significant association of miscarriage with age > 38y, history of miscarriage, dysovulation and duration of freezing > 6 months. After adjustment (multivariate regression), the miscarriage rate remained significantly higher in the artificial vs the natural cycle OR 1.63 (95% CI) [1.35-1.97]; p < 0,0001 and in the artificial vs the stimulated cycle OR 1.87 [1.55-2.26]; p < 0,0001. Overall the LBR per transfer was 17.8 %, AC 16.9%, NC 18.8%, SC 19.3%, p < 0,003. There was no significative difference between the natural and the stimulated cycle. The biochemical pregnancy rate (HCG 10-100 UI/L) was comparable between the three protocols, 10.7% per transfer.
Limitations, reasons for caution: This study is limited by its retrospective design, which included selection bias. Routine practice within centers were heterogeneous. In AC, however, luteal phase support and timing of embryo replacement were similar. The univariate analysis showed no difference between centers. Moreover, a large number of parameters were included in the analysis.
Wider implications of the findings: Our study shows a significant decrease in LBR when using artificial cycle for endometrial preparation before FET. These results suggest either a larger use of natural or stimulated cycle, or an improvement of artificial cycle by individualizing hormonal substitution to patients in order to avoid the excess of pregnancy losses.
Trial registration number: not appplicable

O-022 Microbiota in Endometrial Fluid and Vaginal Secretions in Infertile Women with a History of Repeated Implantation Failure

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Study question: Is specific microorganisms or microbiota in the endometrial fluid (EF) and vaginal secretions (VS) associated with etiology and pathogenesis of repeated implantation failure (RIF)?
Summary answer: Some unusual local microorganism and dysbiotic flora were found in EF and VS of infertile women with a history of RIF.
What is known already: Despite that the uterine cavity and vagina are closely located, their bacterial communities are different in the same individuals and seems not to be hormonally regulated during the acquisition of endometrial receptivity. While Lactobacillus-dominated microbiota (LDM, defined as >90% Lactobacillus species) in the EF of the receptive phase was reported to be associated with favorable reproductive outcome, non-LDM (<90% Lactobacillus species) was found to decrease implantation, clinical pregnancy, ongoing pregnancy, and live birth rates. The local microbial environment in the female reproductive tract, however, remains largely unknown in infertile patients with a history of RIF.
Study design, size, duration: A preliminary analysis of a registered ongoing prospective case-control study. From September 2017 to October 2018, the paired EF and VS samples were obtained from twenty-eight infertile women with a history of RIF (defined as three or more serial negative serum pregnancy tests following transfer of five or more morphologically good blastocysts) and eighteen infertile women undergoing the first in vitro fertilization attempt (the control group) under a given informed consent.
Participants/materials, setting, methods: Sampling was performed carefully avoiding contamination on day 6 to 8 after luteinizing hormone surge in the natural cycle, human chorionic gonadotropin trigger in the oocyte pick up cycle, or on day 5 following initiation of luteal support in the hormone replacement cycle. Extracted genomic DNA was pyrosequenced for V4 region of 16S ribosomal RNA using next-generation sequencer. The sequences were clustered to operational taxonomic units (OTU) and assigned to bacterial taxonomy using QIIME.
Main results and the role of chance: The OTU sequences in the EF and VS microbiota were similar ($p = 0.52$ and 0.65, respectively) between the RIF (mean 29,283 and 38,188, range 2,824–43,657 and 18,232–43,936, respectively) vs control group (mean 22,745 and 36,973, range 1,065–38,877 and 28,898–42,210, respectively). Shannon-index and Chao1 richness (alpha-diversity) of EF microbiota in the RIF (mean ± SE, 0.893 ± 0.567) was also comparable ($p = 0.60$) to the control group (mean ± SE, 1.431 ± 0.931), whereas the unweighted UniFrac distance (beta-diversity) in the EF (but not VS) microbiota showed a significant difference ($p = 0.0089$) between the two groups. LDM rate in the EF and VS was similar ($p = 0.12$) between the RIF (64.3% and 67.9%, respectively) and control group (38.9% and 44.4%, respectively) as well as the detection rate of the bacterial vaginosis-associated genera such as Gardnerella, Atopobium, and Prevotella (39.3%, 7.1%, and 17.9% in the RIF and 27.7%, 16.7%, and 44.4% in the control group, respectively, p =

0.107). Meanwhile, Burkholderia was detected in 25% of EF in the RIF group, whereas it was undetectable in any of EF in the control group (p = 0.032, relative risk 4.53, 95% CI 0.006-1.68)

Limitations, reasons for caution: The limitation of this study is that the design is not a randomized controlled trial, although it is prospective. A current potential bias is the small sample size.

Wider implications of the findings: The beta-diversity in EF microbiota was significantly different between infertile patients in the RIF group and control group. Burkholderia was detected in a quarter of EF in the RIF group, but not in any of the control group, suggesting a potential involvement of this pathogen in RIF.

Trial registration number: UMIN-CTR 000029449

O-023 Endometrial scratching in women with one failed IVF/ICSI cycle: results of a randomized controlled trial (SCRaTCH trial)

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Study question: Does mid-luteal endometrial scratching prior to the stimulation cycle increase pregnancy rates in women with one failed IVF/ICSI cycle?

Summary answer: Based on a preliminary analysis, mid-luteal scratching using an endometrial biopsy catheter may not significantly improve pregnancy rates in women with one failed IVF/ICSI cycle.

What is known already: Previous trials on endometrial scratching have been reported to have methodological limitations and high risk of bias making it unclear whether this procedure improves pregnancy rates. Even the recent large, properly designed PIP trial suffers from the fact that a very heterogeneous study population was included and the timing of scratching was not standardized (scratching was permitted at any time during a ~30 day time frame before start of stimulation). An RCT in a more homogeneous population which standardizes timing and method of scratching may provide additional information on the effectiveness of scratching.

Study design, size, duration: A multicenter, non-blinded, randomized controlled trial was conducted between January 2016 and July 2018 in the Netherlands. Women were allocated 1:1 to endometrial scratching or no procedure, using a web-based system that ensured allocation concealment. Using an expected difference in live birth rate (LBR) after the IVF/ICSI cycle following randomization of 9% (39% vs. 30%, respectively), the sample size was set at 900 participants (80% power and two-sided alpha of 0.05).

Participants/materials, setting, methods: Participants with a failed first IVF/ICSI cycle (≥1 embryo transfer) were eligible. Endometrial scratching was performed using an endometrial biopsy catheter in the mid-luteal phase prior to ovarian stimulation. The primary outcome was LBR from the fresh embryo transfer post-randomization; secondary outcomes included cumulative pregnancy outcomes. This abstract reports preliminary complete case results for the secondary outcomes biochemical and clinical pregnancy rate (BPR; CPR), as the 12-month follow-up period and data collection are still ongoing.

Main results and the role of chance: A total of 942 women were included in the trial (471 scratch/471 control). Baseline characteristics, duration and cause of infertility were comparable between the two groups. The participation rate was 89% (942/1060 eligibles). The BPR was 34.3% (137/399) in the scratch and 30.9% (119/385) in the control group (RR 1.11 [95%CI 0.91-1.36]). The CPR was 26.9% (105/390) in the scratch and 26.1% (99/380) in the control group (RR 1.03 [95%CI 0.82-1.31]). Ongoing pregnancy rate (OPR) and LBR cannot be reported reliably as follow-up and data collection are ongoing, but the completed data will be available at ESHRE. Important strengths include the fairly homogeneous study population and the standardization of the timing and method of scratching. The 12-month follow up period is unique as it offers the possibility to study longer-term effects of endometrial scratching on cumulative pregnancy rates which will also be presented at ESHRE. The high participation rate in this trial is probably due to the fact that endometrial scratching is not offered as part of clinical care in the Netherlands. This improves the inference for the effect of endometrial scratching for daily practice.

Limitations, reasons for caution: The presented results are based on preliminary complete case analysis which is due to ongoing follow-up. Also, despite strict inclusion criteria, some heterogeneity in the study population still exists, for example in the number of embryo transfers prior to randomization.

Wider implications of the findings: Preliminary analysis of this second largest RCT with strict inclusion criteria and standardized scratching method showed a small but non-significant difference in BPR, but no difference in CPR. Follow-up will show if endometrial scratching results in a clinically important difference for LBR or cumulative pregnancy outcomes (12-month follow-up).

Trial registration number: This trial was prospectively registered in the Dutch Trial Register (Nederlands Trial Register) under number 'NTR 5342'.

O-024 Endometrial preparation for frozen-thawed embryo transfer (FET) in an artificial cycle: Transdermal versus vaginal estrogen

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Study question: Is there any difference in endometrial thickness and patient satisfaction between transdermal and vaginal estrogen for endometrial preparation for FET in an artificial cycle?

Summary answer: Transdermal estrogen significantly increases endometrial thickness after 10±1 days of treatment and improves patient satisfaction compared to the vaginal route, with comparable pregnancy outcomes.

What is known already: To date, there is no consensus on the optimal hormonal preparation regimen for artificial FET, despite its widespread use in everyday practice. Several studies have compared oral, vaginal and transdermal estrogen for endometrial preparation before FET, and have shown comparable pregnancy outcomes. A recent prospective randomized trial compared transdermal and oral estrogen, and showed significantly better endometrial thickness with transdermal estrogen on day 10±1, but better patient satisfaction with oral estrogen. However, to our knowledge, no study has compared endometrial thickness and patient satisfaction between the transdermal and vaginal route in artificial FET.

Study design, size, duration: We performed a prospective monocentric cohort study between January and December 2017 at the Angers university hospital. All artificial FET cycles were included (n=318). The two administration routes were explained to the patients during the consultation with the physician or the head nurse before initiating treatment, and the choice between the two was left to the patient. 119 (37.3%) chose transdermal and 199 (62.6%) chose vaginal estrogen, and all signed an informed consent before inclusion.

Participants/materials, setting, methods: All patients who had an artificial FET using transdermal or vaginal estradiol and filled the satisfaction survey after treatment were included. The main objective was to compare the endometrial thickness between the two routes after 10±1 days of treatment. Secondary outcomes were the patient satisfaction on transfer day (score over 10), the side effects, the serum hormone levels, the duration of treatment and the cycle outcome.

Main results and the role of chance: Patients' characteristics were comparable between the two groups. The endometrial thickness at 10±1 days was significantly higher in the transdermal group compared to the vaginal (9.9 vs 9.3 mm, p=0.03, respectively.) The serum estradiol level was significantly lower in the transdermal group (268 vs 1332 pg/ml, p<0.001, respectively), but the progesterone levels were comparable between the transdermal and vaginal groups (0.5 vs 0.6 ng/ml, p=0.61, respectively). The mean duration of treatment was significantly shorter in the transdermal group (13.6 vs 15.5 days, 0>0.001), and significantly fewer patients required ultrasound reassessment for endometrial thickness compared to the vaginal group (10.1% vs 30.6%, p<0.001, respectively). The overall patient satisfaction score was significantly higher in the transdermal group (8.2 vs 7.4, p=0.04), with significantly more patients reporting no side effects compared to the vaginal group (42.4% vs 22.8%, p=0.03, respectively). 86% of patients (36/42) who received the two types of preparation in two successive cycles reported favoring the transdermal route. Finally, live birth rates were comparable between the transdermal and the vaginal route (18% vs 19%, p=0.1, respectively).
Limitations, reasons for caution: The main limitation of our study is the lack of randomization and the monocentric design. Moreover, the patient satisfaction survey answers could have been influenced by the outcomes (positive or negative) of previous FET cycles using one route or the other.
Wider implications of the findings: Transdermal estrogen for endometrial preparation in artificial FET cycles was associated with better endometrial thickness, shorter treatment duration, fewer side effects, and higher patient satisfaction compared to the vaginal route. Therefore, it could be the preferred administration route for artificial FET. Larger randomized trials are needed to confirm our findings.
Trial registration number: Clinical Trial NCT03518528.

O-025 Development and validation of an original test to evaluate human endometrial receptivity and embryo implantation

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Study question: Could we predict endometrial receptivity and embryo implantation by RT-qPCR expression analysis of genes involved in endometrial receptivity and maternal-fetal dialogue?
Summary answer: Adhesio RT, diagnostic tool, can predict receptivity and embryo implantation by RT-qPCR using an original panel of genes involved in endometrial receptivity and maternal-fetal dialogue.
What is known already: Implantation failure caused by suboptimal endometrial receptivity is a main issue. An endometrium is receptive to an embryo in a spatially and temporally restricted period called the implantation window which is usually more or less delayed in recurrent implantation failures. Therefore, it appears essential to identify inadequate endometrial receptivity to offer personalized care management. Molecular diagnostic tools currently available to characterize this process are very limited. In this study, a new diagnostic tool for endometrial receptivity and embryo implantation based on the transcriptomic signature is presented for the first time.
Study design, size, duration: As a result of a single site study at ovo clinic from December 2016 to March 2019, the development and clinical validation of a new test, Adhesio RT, allowed us to analyze 215 biopsies of which 50 endometrial biopsy samples and 35 autologous endometrial co-culture samples were analyzed by using microarray technology and 130 biopsies from IVF-patients with a known pregnancy outcome were used for clinical validation.
Participants/materials, setting, methods: 50 biopsies were performed in natural cycle during the optimal theoretical implantation window LH+7 to LH+11 (35 with successful clinical pregnancy 15 with implantation failure). Similarly, a total of 29 co-culture biopsies were performed on autologous-endometrial co-culture (14 endometrial cells cultured in absence of embryo, 5 in presence of good-quality embryo successfully transferred, 10 with good quality embryo but with implantation failures). Samples were analyzed using microarrays and selected biomarkers were assessed using RT-qPCR.
Main results and the role of chance: Adhesio RT included 10 new selected genes by using a new approach that incorporates two specific transcriptomic signatures obtained by different bioinformatic and statistical technologies applied to microarray analyses: A first specific transcriptomic signature of 1717 genes specifically modulated associated to biopsies from patients with successful clinical pregnancy *versus* biopsies from patients with implantation failure. Gene ontology analyzes revealed that cell division, cellular proliferation, cell adhesion and mitotic cycle are the most over-represented biological terms in this group of genes. A second specific transcriptomic signature of 60 genes associated to endometrial co-culture successfully transferred was obtained using class prediction approach. Gene expression was validated by RT–qPCR. Clinical validation was performed on 130 biopsies from IVF-patients with a known pregnancy outcome.
Limitations, reasons for caution: Successful implantation is a complex process requiring a receptive endometrium, a viable embryo and synchronized dialogue between maternal and embryonic tissues. Adhesio RT only focuses on receptive endometrium and dialogue between maternal and embryonic tissues.
Wider implications of the findings: Evaluation of receptivity and embryo implantation of an endometrium with this new molecular signature can predict IVF success and may help in the management of endometrial preparation for embryo transfer and optimizes chances of successful pregnancy for many couples.
Trial registration number: Not applicable.

O-026 Uterine immune profiling for patients with unexplained recurrent miscarriage.

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Study question: Is an endometrial immune evaluation able to identify uterine immune mechanisms generating unexplained recurrent miscarriages (RM)?
Summary answer: Local immune deregulation was detected in 83% of the cases. Personalized treatment adapted to the patient's endometrial immune profile may help patients with RM.
What is known already: We previously documented immune deregulation in infertile patients with repeated history of embryo implantation failures. Endometrial remodeling events begin before implantation and are a vital process for pregnancy, preparing future maternal immune tolerance and regulating the placentation process. During "the implantation window", an influx of immune cells occurs and nearly completely switches local immunity from an adaptive (Th1) to an innate (Th2) type. This transient immune switch, together with adequate uNK-cell activation, appears fundamental in enabling local maternal tolerance and fetus survival. An over-activation can generate embryo rejection, while an under-activation can disturb embryo adhesion and local angiogenesis.
Study design, size, duration: Between 2014 and 2018, 180 patients with a history of unexplained RM underwent endometrial immune profiling. If an immune deregulation was diagnosed (over or low-endometrial immune activation), treatment was adapted accordingly. A three-month follow-up was conducted to evaluate subsequent live birth rate if a pregnancy occurred during that period with or without medical assistance.
Participants/materials, setting, methods: Recurrent miscarriage was defined as at least three miscarriages during the first trimester despite a negative checkup (auto-immunity, thrombophilia, caryotype). An endometrial biopsy was performed under substituted cycle in the luteal phase. We quantified uNK by immunohistochemistry using anti-$CD56^+$IgG and mRNA of IL-15 (uNK cells activation/maturation state), IL-18 (Th-1/Th-2 cytokines balance) and TWEAK/Fn-14 (immuno-regulation) by Real-Time PCR.
Main results and the role of chance: Among the 180 patients, 83% (149/180) had deregulation of their endometrial immune environment at the time of the evaluation. An over-activation was diagnosed in 45%, an under-activation was diagnosed in 23.9% and concomitent under and over activation

(a Th-1 deviation of the endometrial environment with immatures NK cells) was diagnosed in 13.9%. Depending on the immune profile, personalized treatment was suggested to counteract the identified mechanisms. In case of pregnancy in the three months following the evaluation birth rate was only 16% if no deregulation had been observed. In patients with treated deregulation birth rate was 49 % (51.8%, 48% et 40% respectively in the over-under or mix deregulated group).

Limitations, reasons for caution: Only a randomized control study among deregulated RM patients may confirm these results.

Wider implications of the findings: When deregulation is diagnosed on endometrial immune profiling in patients with unexplained RM (83%), the effective understanding of local endometrial immune deregulations may be useful to provide treatment that reduces the risk of a new miscarriage.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 06: REPRODUCTIVE EPIDEMIOLOGY, HEALTH ECONOMICS AND ACCESS TO CARE

Monday 24 June 2019 Haydn 4 10:00–11:30

O-027 Travelling from France for CBRC: an internet survey as a first step to measure this phenomenon

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Study question: The objective was to explore some essential features of use of CBRC by French patients in order to design a larger survey quantifying CBRC.

Summary answer: CBRC in France is characterized by strong information and social networks (whatever the socio-demographic profiles, ART used, or destinations) that make a larger survey possible.

What is known already: CBRC is described as a worldwide, growing and multifaceted phenomenon, concerning people with diverse sociodemographic characteristics and reasons for crossing borders. France is one of the top four European countries whose residents seek CBRC elsewhere. However, the scale of CRBC cannot be estimated from existing studies because they are mainly qualitative or based on non-representative samples. A preliminary statistical work proposed a cutting-edge methodological study design to quantify the CBRC population based on the benchmark-multiplier method. However, the information channels and social networks mobilized by CBRC patients first need to be tested.

Study design, size, duration: A three-month online cross-sectional survey was conducted in 2018 to explore CBRC. It was approved by the Ined Data Protection Officer (2017-CIL-0013). The survey was diffused by specialized associations and related professional networks. Inclusion criteria were: planning to travel or having already travelled to another country for CRBC, and being resident in France. This questionnaire (designed with Voozanoo® software) included questions on ART techniques planned or already used, destination countries, experience sharing and information sources.

Participants/materials, setting, methods: The internet survey was completed by 419 respondents. Analysis was based on the 348 (83%) respondents who met the inclusion criteria and fully completed the questionnaire. Descriptive statistics were obtained using SAS® software to analyse respondents' profiles and journeys, destinations and networks used.

Main results and the role of chance: Based on the internet survey, CBRC patients from France showed diverse sociodemographic profiles (single women, same-sex and heterosexual couples). They used ART in many different countries in Europe but also in the USA and Canada. However, Belgium and Spain were the main destinations for French residents. An important finding was the complexity of CBRC pathways, as a single respondent could successively have used different ART techniques in different countries. All respondents used Internet as the main CBRC information source, together with specialized associations and French medical doctors. Moreover, three out of four respondents knew at least one person using ART abroad. It will therefore be possible to use these important interpersonal and social networks to broadcast the large planned survey and to include a large sample. A large proportion of CBRC patients shared and intended to share their CBRC project with family (3/4), friends (3/4) and colleagues (1/2). This demonstrates that in France CBRC is no longer a highly sensitive and taboo issue. This is the time to launch a large survey with a good prospect of acceptability.

Limitations, reasons for caution: It is not possible to ensure the representativeness of the internet sample. Some profiles and techniques may be underrepresented, especially single men, male couples and surrogacy. For the larger survey, the questionnaire will be broadcast through more associations and clinics for better coverage of the CBRC population.

Wider implications of the findings: Based on these findings, an ambitious research project on CBRC from France is now planned to produce a first estimate of the size of the CBRC population. More broadly, this will allow further use of the cutting-edge benchmark-multiplier method for studies on CBRC from other European countries.

Trial registration number: Not applicable.

O-028 Why do patients return for more treatment? A 10-year analysis from a single IVF centre from inception

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Study question: To investigate the return rate for further assisted conception treatment after initial treatment, the time lapsed before returning and the factors that most significantly influence the return rate.

Summary answer: Statistically significant factors included age, previous cycle failure, failed fertilisation (reduced return), higher eggs numbers, availability of supportive app and frozen embryos (increased).

What is known already: Drop-out from assisted conception treatment is common. Patients must overcome disappointment, physical and psychological burden and often financial obstacles in order to return for further treatment. Studies have analysed medical factors associated with early dropout 1,2 but there has been little focus on those who do return for further treatment.

1. Gameiro S et al, Why do patients discontinue fertility treatment? A systematic review of reasons and predictors of discontinuation in fertility treatment. *Hum Reprod Update* 2012;18: 652-669.

2. Troude P et al. Medical factors associated with early IVF discontinuation. *Reprod Biomed Online* 2014;28: 321-329.

Study design, size, duration: Single centre retrospective database study, with 6034 cycles of ovulation induction, intrauterine insemination, IVF/ ICSI and frozen embryo transfer undertaken by 3114 patients from 2008-2018. Age, nationality, primary diagnosis, number of previous treatment cycles, number of previous pregnancies, couple category, funding source, availability of MediEmo supportive App and postcode/ CCG were recorded. Cycle specific characteristics of treatment type, stimulation protocol, number of eggs collected/fertilised, number of embryos transferred, and cycle success/ failure were also obtained.

Participants/materials, setting, methods: Analysis was performed using multi-level mixed effects modelling to account for the repeated nature of the data, with multiple cycles for some patients. Linear regression models were used for time elapsed between cycles. NHS and self-funded patients were modelled separately to identify key characteristics. The end point for those with a failed cycle (no live birth) is whether a new cycle begins within one year and, for those who had successful cycle, within 3 years.

Main results and the role of chance: Over half of the patients who had one treatment cycle return for further treatment cycles (n=1571, 58.0%). Of the cycles analysed, 4147 (68.7%) were self-funded and 1887 (31.3%) were NHS-funded. Following a failed cycle, the average time to return for further treatment was 5 months. Following a successful cycle, the average time to return was 2.5 years.

Patients who did not succeed in having a live birth were more likely to return if they had a higher number of eggs collected [OR 4.39; p<0.001], frozen embryo(s) [OR 9.39; p<0.001], were single women [OR 2.32; p<0.001], same sex couples [OR 1.83; p<0.001] and had used the MediEmo App [OR 2.46; p<0.001]. They were less likely to return for further treatment with increasing age [for each year increase OR 0.958; p<0.001], increased number of previous cycles [OR 0.924; p=0.049], those who already had a frozen embryo transfer [vs IVF: OR 1.31; p=0.03] or had previous failed fertilisation [OR 0.254; p<0.001]. Success group modelling results are imminently awaited.

Limitations, reasons for caution: This study is limited by its retrospective nature and is based on a single centre's data over time within the UK.

Wider implications of the findings: These results should interest fertility centres and compliment the available literature on characteristics of patients who drop out from assisted conception treatment.

Trial registration number: Not Applicable

O-029 The coaching program; 'Smarter Pregnancy' is the first effective mHealth intervention to adopt healthy nutrition and lifestyle behaviours in subfertile couples: a randomised controlled trial

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Study question: What is the effectiveness of 24 weeks coaching of the mHealth program 'Smarter Pregnancy' to adopt healthy nutrition and lifestyles in couples undergoing IVF/ICSI treatment?

Summary answer: The mHealth coaching program 'Smarter Pregnancy' significantly enhances the adoption of healthy nutrition and lifestyle behaviours among subfertile couples undergoing IVF/ICSI treatment.

What is known already: Periconceptional healthy nutrition and lifestyle improve reproduction, pregnancy outcome and long-term health in mothers and offspring. While the amount of nutrition and lifestyle programs on the mobile phone (mHealth) is overwhelming, evidence on effectiveness is often lacking. At the Erasmus MC in Rotterdam, we have developed and launched in 2012 the online, web-based coaching program 'Smarter Pregnancy' [www.smarterpregnancy.co.uk] for the mobile phone to adopt healthy nutrition and lifestyle behaviours. In a previous survey that included more than 2,000 couples, we already showed a high compliance (65%) and effectiveness to adopt healthy behaviours (30-70%) and increase the chance of pregnancy (~20%).

Study design, size, duration: Multicentre randomized controlled trial conducted between July 2014 and March 2017 in six IVF clinics throughout the Netherlands. Couples undergoing IVF or ICSI treatment were randomly assigned to the intervention group (308 women, 106 men) or the control group (318 women, 116 men).

Participants/materials, setting, methods: After baseline screening on vegetable, fruit and folic acid supplement intake (nutrition) and smoking and alcohol use (lifestyle), the intervention group received tailored coaching on the identified unhealthy behaviours comprising of feedback, seasonal recipes, tips, and incentives by email or text message. The control group received a condensed 'light' version of the program. Primary outcome was the difference in improvement of adopting healthy behaviours after 24 weeks of coaching, expressed as a risk score.

Main results and the role of chance: Compared with controls, women (β=0.78, 95%CI 0.46 to 1.09) and men (β=0.83, 95%CI 0.42 to 1.28) in the intervention group showed a significantly larger improvement in adopting healthy behaviours. Women in the intervention group also showed a significantly larger improvement of healthy lifestyle behaviours (β=0.11, 95%CI 0.02 to 0.20).

Limitations, reasons for caution: The power of the study is not enough to evaluate the clinical effectiveness, such as the chance of pregnancy. Although folate blood levels are correlated with the reported nutrition behaviours, the completion of self-administered questionnaires by the participants may have induced desirability- and recall bias.

Wider implications of the findings: The enhanced adoption of healthy nutrition and lifestyle behaviours may have clinical implications, such as an increased chance of pregnancy, that warrants further research.

Trial registration number: Dutch Trial Register: NTR4150, http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4150

O-030 Fertility treatments in early onset female cancer survivors – A Finnish register-based study on 8,929 survivors

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Study question: Do female early onset cancer survivors receive more fertility treatments compared to female siblings without a history of cancer?

Summary answer: Female early onset cancer survivors have an increased use of assisted reproductive technology (ART) from 2003 onwards compared to siblings.

What is known already: The population of early onset cancer survivors has been expanding over the past four decades, with 5-year survival rates reaching 80% in Western Europe. As survival rates increase, so does the risk for chronic health conditions, including gonadal dysfunction among female cancer survivors. Many studies have shown reduced pregnancy rates in female cancer survivors compared to the general population. There are, however, only a few studies focusing on fertility treatments among female cancer survivors. In one of them, survivors were as likely as their siblings to seek medical help for their infertility, but less likely to receive fertility treatments.

Study design, size, duration: In this retrospective, register-based study, data from Finnish registers on cancer, birth and prescribed medications were merged to identify 8,929 survivors (diagnosed with cancer between 1953 and 2012 at the age of 0-39 years) and 9,495 siblings without previous deliveries.

Participants/materials, setting, methods: Fertility drug purchases in cancer survivors and siblings in 1993-2012, at the age of 16-41 years, were identified from the Reimbursement Register on Prescribed Medicines. A Poisson regression model was used to estimate incidence rate ratios (IRR) for the use of fertility drugs (sub-classified into ovulation inductions and ARTs) between survivors and siblings, adjusting for attained age and calendar time. Heterogeneity in the IRR between time periods and age intervals was also estimated.

Main results and the role of chance: Fertility treatments were more common in cancer survivors compared to siblings, as 6.1% of survivors compared to 3.8% of siblings had bought fertility drugs (IRR 1.43, 95% confidence interval [CI] 1.25-1.65, p<0.001). A sub-classification of fertility treatments into ovulation inductions and ARTs, showed increased use of ART (IRR 2.41, 95 % CI 1.97-2.96, p<0.001), whereas the use of ovulation induction was similar in survivors and siblings. Analyses by calendar time periods showed the IRR for the use of ART to be significantly higher in the most recent decade, from 2003 onwards, compared to 1993-1997.

Limitations, reasons for caution: In this study, information on the indication for fertility treatment was unavailable and only fertility treatments with autologous oocytes could be included. Limited duration of follow-up was available for women diagnosed with cancer in the most recent calendar time period.

Wider implications of the findings: Female early onset cancer survivors have an increased risk for subfertility, which should be acknowledged by clinicians. However, our results mirror a more active approach among clinicians towards offering fertility treatments to cancer survivors during the most recent years.
Trial registration number: The National Institute for Health and Welfare (Dnr THL/1/5.05.00/2014) and the Social Insurance Institution (Dnr KELA/69/522/2014)

O-031 Comparison of reproductive outcomes of female couples undergoing Reception of Oocytes from PArtner (ROPA) versus classic IVF-ICSI

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Study question: Is live birth after Reception of Oocytes from PArtner (ROPA) comparable to classic *in vitro* fertilization-intracytoplasmic sperm injection (IVF-ICSI) in lesbian couples?
Summary answer: ROPA presents a higher rate of live birth rates compared to classic IVF-ICSI.
What is known already: This study focuses on IVF and ROPA in lesbian couples. In IVF, a woman is both the provider of the oocytes and the recipient of the embryos, while in ROPA the process is shared between the partners: one of them undergoes ovarian stimulation and ovum pick-up, whilst the other undergoes endometrial preparation and carries the pregnancy. Although ROPA is increasing popular among lesbian couples, no clear understanding of its outcomes is present in the literature, making it difficult for clinicians to counsel properly these couples.
Study design, size, duration: This is a retrospective matched cohort study of lesbian couples in one large fertility center. The study included 70 couples that underwent for the first time ROPA, matched (1:2) with 140 patients of similar characteristics that underwent, also for the first time, classic IVF-ICSI, making a total of 210 couples. This study includes embryo transfers (ETs) performed between February 2012 and May 2018.
Participants/materials, setting, methods: ROPA and IVF couples were matched 1:2 by these variables: age of the woman providing the oocytes (±5 years), number of transferred embryos and day of ET. Laboratory and clinical outcomes are compared between groups. We provide descriptive statistics, univariable (Chi^2 test) and multivariable analyses (logistic regression) adjusted for: age of the woman providing the oocytes, BMI of the recipient, and number of previous treatments (intrauterine inseminations). A p-value <0.05 is considered statistically significant.
Main results and the role of chance: The mean age and BMI of women were: 33.9 years (SD 4.5) and 24.2 (SD 4.5) in the ROPA's oocyte provider; 34.3 years (SD 5.8) and 24.0 (SD 4.3) in the ROPA's recipient; 34.2 years (SD 3.9) and 23.8 (SD 3.9) in IVF/ICSI. 65.7% (n=92) of couples undergoing IVF/ICSI had underwent previous IUI treatments, against 24.3% (n=17) of the ROPA couples ($p<0.001$). Most ETs were performed on D2-3 (86.2%) with transfer of 2 embryos (80%). Regarding laboratory outcomes, ROPA led to 9.1 (SD 4.5) mature oocytes (MII) vs. 8.2 (4.5) in IVF/ICSI (p=0.16). Fertilization rate was 73.6% in ROPA vs. 76.2% in IVF/ICSI (p=0.37). Clinical outcomes in ROPA vs. IVF/ICSI were: biochemical pregnancy rate 68.6% vs.46.4% (p=0.002); clinical pregnancy rate 57.1% vs. 38.6% (p=0.011), ongoing pregnancy rate 55.7% vs. 35.7% (p=0.006), and live birth rate was 53% vs. 29.3% (p=0.001). After adjusting for potential confounders, we still observe a significant improvement in ROPA for biochemical (OR=2.71, 95%CI 1.33, 5.52; p=0.006), clinical (OR=2.4, 95%CI 1.19, 4.87; p=0.015), ongoing pregnancy (OR=2.39, 95%CI 1.18, 4.83; p=0.015), and live birth (OR=3.49, 95%CI 1.63, 7.49; p=0.001). Our results suggest that ROPA might be more efficient than classical IVF-ICSI in lesbian couples.
Limitations, reasons for caution: Study limitations include an "oocytes' age" significantly lower in ROPA, more IVF/ICSI patients going into this treatment after previous failed IUIs (worse prognosis), and ROPA recipients undergoing endometrial preparation but not ovarian stimulation (better uterine conditions). Adverse events during pregnancy and perinatally were not evaluated in this study.
Wider implications of the findings: ROPA offers improved treatment participation for lesbian couples, and it might improve reproductive outcomes through the possibility of selecting between 2 oocyte providers and 2 gestational mothers. As oocyte donation pregnancies present higher hypertensive disorders, a careful evaluation of risks and benefits is recommended before this treatment.
Trial registration number: not applicable

O-032 Assisted reproductive technology treatment and risk of breast cancer

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Study question: Is the risk of breast cancer (BC) increased after assisted reproductive technology (ART) treatment?
Summary answer: An increased risk of BC after ART treatment was apparent among women initiating ART treatment when aged 40+ years.
What is known already: The majority of BC cases are sensitive to estrogen. Ovarian stimulation in ART treatment has been suggested to increase the risk of BC by influencing endogenous estrogen levels. The level of ovarian response to hormone stimulation is dependent on age, and thus, age is an important factor in the choice of ART treatment protocol. Nulliparity is a risk factor for BC. Previous studies on ART treatment and BC have varied in their findings. Several previous studies suffer from lack of power and short follow-up time. The latency time for development of BC has been estimated at 10+ years.
Study design, size, duration: The Danish National ART-Couple II (DANAC II) cohort includes all women treated with ART at Danish fertility clinics in 1994-2015. Each woman in ART treatment was age-matched with ten women from the background population without a history of ART treatment. The women were followed until first cancer diagnosis, death, migration or end of study December 31st 2015. The cohort consisted of 58,534 women treated with ART and 567,178 women without a history of ART treatment.
Participants/materials, setting, methods: Multivariable analyses were conducted using cox proportional hazards regression. Having a primary cancer diagnosis other than BC was incorporated as a competing risk. Adjustment for confounders included baseline nulliparity, educational level, partnership status, treatment year, endometriosis and PCOS and time-dependent adjustment for age. Stratified analyses were conducted to assess effect modification by age at treatment initiation. Also, the risk of being diagnosed with BC was observed over time in order to detect potential patterns.
Main results and the role of chance: During follow-up 3894 women were diagnosed with BC, 464 (0.8 %) among ART-treated women and 3430 (0.6 %) among untreated women. Overall, women undergoing ART treatment had a higher risk of BC than non-ART women (HR 1.10, 95% CI 1.07-1.13). Female cause of infertility was not associated with an increased risk of BC (HR 1.03, 95 % CI 1.00-1.06). Among women initiating ART treatment when aged 40+ years the risk of BC gradually increased during 12 years after ART treatment initiation compared to untreated women (12-year HR 1.53, 95% CI 1.24-1.90). Considering that a higher prevalence of nulliparity among ART treated women could explain this finding, ART treated and untreated women with a first birth at age 40+ were subsequently compared. The increased risk was also apparent when restricting analyses to women with a first birth after ART treatment at 40+ years of age compared with untreated women with a first birth at similar age (HR 1.65, 95 % CI 1.40-1.94).

Limitations, reasons for caution: Although hormone dosages and number of ART treatments are relevant risk factors for development of BC, the selection of individuals to be exposed to more treatments is not random. It depends on both achieved pregnancies and who chooses to terminate treatment, and such results would be difficult to interpret.
Wider implications of the findings: It is possible that ovarian stimulation increases the risk of BC among women who initiate ART treatment when aged 40+. An increased risk could be due to age-related vulnerability to hormone exposure or to higher doses of hormones during ART treatment.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 07: CHILDREN'S HEALTH OUTCOMES IN MAR
Monday 24 June 2019 Strauss 1+2 10:00–11:30

O-033 Health of 2 year-old singletons born after in vitro maturation of oocytes compared to peers born after controlled ovarian stimulation: results from a cohort study

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Study question: Does in vitro maturation (IVM) of immature oocytes affect the health of 2 year-old singletons born to mothers with polycystic ovaries (PCO)?
Summary answer: This study comprising 76 singletons born after IVM showed no significant differences in anthropometry compared with 111 peers born after conventional controlled ovarian stimulation (COS).
What is known already: The paucity of available data related to children's health following IVM is an important impediment to a more widespread use of this technology. Concerns that prolonged culture time might affect growth which may be linked to epigenetic alterations in IVM oocytes have not been confirmed.

Previous reports on the neonatal outcome after IVM are generally reassuring, but many studies have flaws that hamper the interpretation of outcomes. Moreover, only few studies reported health outcomes after IVM beyond infancy, and particularly data on growth and overall health in children born after IVM of immature oocytes from mothers with PCO are lacking.
Study design, size, duration: This single-center cohort study compared anthropometry and health outcomes in 76 singletons born after ICSI with transfer of in vitro matured oocytes (IVM group) with 111 singletons born after ICSI (COS group). All participants were born to mothers who were diagnosed with polycystic ovary syndrome (PCOS) phenotype A, C, D or polycystic ovary morphology (PCOM) and reached the age of 2 years between November 2012 and April 2018. Only singletons living in Belgium were eligible.
Participants/materials, setting, methods: Anthropometric parameters and health status data were collected at birth, 4 months and at 2 years in cohorts of singletons followed since birth. Singletons born after COS were randomly selected by a computer program for follow-up until young adulthood. Results were adjusted for neonatal (birth weight z-score, birth order), treatment (day and mode of transfer, number of embryos transferred) and parental (maternal smoking, age, BMI, anti-Mullerian hormone level, gestational diabetes, hypertensive disorder, paternal BMI) characteristics.
Main results and the role of chance: Mothers giving birth to a child conceived by IVM were younger than mothers in the control group, but their body mass index was comparable. The proportion of children born to mothers with PCO phenotype A was higher in the IVM group than in the COS group (48.7% versus 12.6%). Overall no differences were found for body weight, height and head circumference z-score between IVM and COS children at birth, at 4 months or at 2 years (all $P>0.05$). In addition, z-scores of waist and mid-upper arm circumference were comparable in IVM and COS children at 2 years of age. Adjustment for neonatal, treatment and parental characteristics did not change the outcome. Finally, although the hospital admission rate was higher among IVM children (34.2% versus 18%; $P=0.01$), the majority of the hospitalisations was for (minor) infectious pathology. The frequency of surgical interventions up to 2 years was not different between IVM children and peers born after ICSI ($P>0.05$). Comparing neonatal (birth weight), treatment (day of transfer, number of embryos transferred, mode of transfer) and maternal (age, BMI, AMH levels) characteristics between participating IVM and COS and non-participating IVM and COS children respectively, no differences were found.
Limitations, reasons for caution: Although our study describes the largest cohort to date of singletons born after IVM applied to mothers with a well-defined PCO phenotype, the current sample size only allowed to detect moderate differences in anthropometry. Also, follow-up of children born after IVM for other indications than PCO is highly recommended.
Wider implications of the findings: We did not observe adverse effects of IVM on health parameters in offspring up to 2 years of age when compared to COS without IVM, but future studies should focus on cardiovascular and metabolic outcomes in these children and adolescents given their mother's PCO status.
Trial registration number: not applicable

O-034 Perinatal outcomes from vitrified blastocysts following prolonged initial embryo culture

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Study question: To compare the perinatal outcomes of vitrified day-6 blastocysts with vitrified day-5 blastocysts
Summary answer: The perinatal outcomes were similar, with a higher mean birthweight centile than expected, with a tendency to the day-6 blastocysts being heavier.
What is known already: Compared with cleavage stage embryos, births after transfer of blastocyst embryos are associated with increased incidence of babies who are "large for gestational age" (LGA), possibly due to the prolonged embryo culture to day-5. Day-6 blastocysts, with their 20% longer culture, are frequently cryopreserved and transferred in a subsequent cycle. Comparative studies between day-5 and day-6 vitrified blastocysts show variable outcomes in terms of warming survival, implantation rates and subsequent birth outcomes.
Study design, size, duration: Retrospective analysis of frozen embryo transfer treatment cycles from blastocysts vitrified on either day-5 or day-6 between September 2010 and December 2016 in a single UK centre. The day-5 group comprised 754 cycles in 580 women. The day-6 group comprised 177 cycles in 138 women.
Participants/materials, setting, methods: All vitrified blastocysts were subsequently transferred as if they were day-5 blastocysts. Cycles where day-5 and day-6 blastocysts were transferred together and cycles involving donated oocytes were excluded. Definitions: small for gestational age (SGA) is birthweight $<10^{th}$ centile for expected gestation; LGA is birthweight $>90^{th}$ centile (UK birthweight charts)
Main results and the role of chance: The day-5 group comprised 754 cycles in 580 women. The day-6 group comprised 177 cycles in 138 women. There was no difference in BMI or circulating AMH but the day-5 group were younger at the time of vitrification (34.5 ± 4.2 years vs. 35.7 ± 3.6, $P<0.001$). The proportion of embryos surviving the warming procedure was higher in the day-5 group (88% vs. 83%, $P<0.01$). There was no difference in the live birth rate (39.1% vs. 39.6%, $P=0.908$). When only singleton births were analysed, (day-5, N = 254; day-6, N = 54) there was no difference in mean birthweight (3488 ± 578g vs. 3558 ± 594g, respectively, $P=0.417$), gestational age at delivery (272 ± 15 days vs. 273 ± 14, $P=0.781$), birth centile (60 ± 28 vs. 65 ± 24, $P=0.254$), proportion of SGA (5.1% vs. 1.9%, $P=0.306$) or LGA (16.9% vs. 18.5%, $P=0.778$). Nevertheless there was a tendency for singletons resulting from the day-6 blastocysts to be heavier in absolute terms and larger

for gestational age, despite the mothers actually being older at the time of vitrification and subsequent embryo transfer.
Limitations, reasons for caution: The study population dates from when we started vitrifying embryos to the recent past and current survival rates are considerably higher. There were only 54 singleton live births in the day-6 group and a larger dataset is required to confirm the above findings.
Wider implications of the findings: Day-5 singletons (cf. cleavage stage) are heavier, possibly attributable to the longer culture media exposure. However, the longer-term potential epigenetic/health effects remain to be evaluated. Day-6 singletons, having had an additional initial 20% culture media exposure, showed a tendency to be bigger still, which could have longer-term health implications.
Trial registration number: Not applicable

O-035 Obstetric and perinatal risks in 8,368 singletons and 1,167 twins conceived after fresh and frozen blastocyst transfers in the Nordic countries – a CoNARTaS collaboration

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Study question: Are obstetric and perinatal outcomes similar in children conceived after blastocyst (BT) and cleavage stage transfers (CT)?
Summary answer: Extended embryo culture to the blastocyst stage has the potential to compromise perinatal outcomes of the infants and to increase the risk of same-sex twins.
What is known already: Blastocyst transfer optimizes the selection of top-quality embryos and increases pregnancy and live birth rates per transfer. However, concerns have been raised as extended culture may increase obstetric complications and impair perinatal outcomes. Studies show higher risks of preterm birth (PTB) and large for gestational age (LGA) among infants conceived after BT compared with CT. Children conceived after BT are also prone to have a higher risk of monozygotic twins.
Study design, size, duration: Nordic registry-based cohort study including two cohorts: 69,751 singletons and 18,154 twins conceived after assisted reproductive technology (ART) in the Nordic countries. Herein 8,368 singletons conceived after BT and 61,383 singletons conceived after CT; Denmark including singletons born 1997-2014 (BT: n=1,152; CT: n=23,344), Norway 2010-2015 (BT: n=397; CT: n=6,686) and Sweden 2002-2015 (BT: n=6,819; CT: n=31,353). The twin cohort consisted of 1,167 children conceived after BT and 16,987 children conceived after CT.
Participants/materials, setting, methods: Data were obtained from the large Nordic cohort (Committee of Nordic ART and Safety - CoNARTaS) containing information from the national ART and Medical Birth Registries. Obstetric and perinatal outcomes, and risk of same-sex twinning after fresh and frozen BT and CT were compared using linear mixed model regression analyses to account for the correlation of outcomes amongst siblings. Adjustments were made for fertilization method (IVF/ICSI), sex, country, birth year, parity (first/later) and maternal age.
Main results and the role of chance: In the adjusted multiple regression analyses, singletons conceived after fresh BT had a higher risk of being LGA (adjusted odds ratio (aOR) 1.23 (95%CI 1.05; 1.44)) compared with fresh CT-singletons. Singletons conceived after frozen BT had a higher risk of PTB both when calculated based on the second-trimester ultrasonography (aOR 1.39 (95%CI 1.18; 1.65)) and from the day of embryo transfer (aOR 1.23 (95%CI 1.07; 1.40)) compared with frozen CT singletons. In singleton pregnancies a higher risk of placenta previa both after fresh (aOR 2.04 (95%CI 1.73; 2.41)) and frozen (aOR 1.68 (95%CI 1.16; 2.44)) BT compared with CT was observed. We found no excess risks of perinatal (aOR 1.08 (95%CI 0.65; 1.82)) nor neonatal deaths (aOR 1.20 (95%CI 0.74; 1.96)) comparing singletons conceived after BT and CT, fresh or frozen. Adjusted analyses showed a higher risk of twin births after single embryo BT compared with single embryo CT; for fresh cycles aOR was 1.79 (95%CI (1.48; 2.15) and for frozen cycles aOR was 1.30 (95%CI (1.05; 1.62), compared with single fresh or frozen CT. Furthermore, twins conceived after single embryo fresh BT had increased risk of same-sex twins (aOR 2.51 (95%CI 1.31; 4.82)) compared with single embryo fresh CT.
Limitations, reasons for caution: Retrospective cohort studies may have inadequate adjustment for potential confounding factors, however with this large controlled cohort we assume that residual confounding is limited. Though we were not able to adjust for years of infertility or type of cryopreservation in FET children (vitrification or slow freeze technique).
Wider implications of the findings: Blastocyst transfer is associated with a higher risk of PTB, LGA and placental complications. These results are important since an increasing number of all ART treatments are performed with BT.
Trial registration number: ISRCTN11780826

O-036 Growth of children born after frozen compared to fresh embryo transfer during the first five years

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Study question: Is the growth of term-born frozen embryo transfer (FET) children and fresh embryo transfer (fresh ET) children comparable until five years of age?
Summary answer: Childhood growth is similar between FET and fresh ET children after adjustments for age at measurement, maternal BMI and parity.
What is known already: Birth weights of FET children have been shown to be higher than fresh ET children's or even spontaneously conceived children's in multiple studies. Knowledge on the childhood growth patterns of these children is still very limited. There is some evidence of catch-down or catch-up growth for FET and fresh ET children, which might have implications on their long–term health.
Study design, size, duration: A prospective observational cohort of all term-born (gestational weeks 37-42) FET and fresh ET singletons born in Oulu University Hospital, Northern Finland, in 2006-2011, n=130 for FET and n=201 for fresh ET. After lost-to-follow-up and exclusion of subsequent births to the same mother, the group sizes were n=110 for FET and n=181 for fresh ET.
Participants/materials, setting, methods: Using unique personal ID codes given to every Finnish citizen/permanent resident, information on the infertility treatment, pregnancy and postnatal period were matched from the hospital database. Growth data were then obtained from municipal child health clinics. Mean weights, lengths/heights and head circumferences (HC) were compared between the groups at birth; 4, 8, 18 months; 3 and 5 years. Adjustments were made for exact age at measurement (gestational age at birth), maternal BMI and parity.
Main results and the role of chance: In our data there were no significant differences between FET and fresh ET children for birth weight (3537 and 3525 grams, respectively), length (50.3 and 50.3 centimeters) or HC (35.0 and 35.0 centimeters) after adjusting for gestational age at birth, maternal BMI and parity. There was a trend towards fresh ET children (especially girls) being heavier and taller with a larger HC at 4 months and taller with a larger HC at 8 months, but after adjusting for age at measurement, maternal BMI and parity, the difference was no longer statistically significant. After adjustments, fresh ET children had a larger HC (p=0.045) at 3 years, but this difference disappeared by 5 years of age. Fresh ET girls were significantly taller than FET girls at 3 years (p=0.034), but no longer at 5 years of age. There were no significant differences in other potential confounding factors such as maternal age, smoking, gestational diabetes, blood pressure disorders

or paternal height between the groups. Accurate pregnancy and growth data, including exact age at measurement, were available, as 99.6% of Finnish children are followed at the municipal child health clinics. Missing measurement values for each growth measurement point varied randomly between 1.4 and 12.4%.

Limitations, reasons for caution: Due to the single-center setting of our study, the study groups are limited in size. This should be taken into consideration when interpreting the results. However, Finland's unique, prospective, high-coverage, free-of-cost child health clinic follow-up made accurate information available for both groups, adding to the reliability of these results.

Wider implications of the findings: Similar childhood growth patterns between FET and fresh ET children give reassurance about the safety and feasibility of embryo freezing practice, thus enabling widespread use of single-embryo transfer policy.

Trial registration number: not applicable

O-037 Risk of higher blood pressure in 3 to 6-year-old singleton born with Ovarian Hyperstimulation Syndrome

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Study question: Does ovarian hyperstimulation syndrome (OHSS) produce an effect on blood pressure (BP) of preschool singleton born following IVF/ICSI?

Summary answer: Preschool singletons born following OHSS in the fresh embryo transplantation (ET) cycle showed higher systolic/diastolic blood compared with spontaneously conceived children and non OHSS children.

What is known already: A large registry-based study found the increasing disorders of cardiovascular and metabolism in IVF children but underlying mechanism is still unknown. OHSS, which shows high estrogen and progesterone during early pregnancy is considered as a good model to study the effects of ovarian stimulation and high sex hormone on the health and development of the offspring. Few studies have investigated any association between OHSS and cardiovascular or metabolic function in subsequent children. Of these studies, some indications of increased cardiovascular and cognitive dysfunction among OHSS-children need further investigation.

Study design, size, duration: The prospective, assessor-blinded study included 1780 women and their corresponding singletons after recruiting women who accepted IVF/ICSI and gave birth to single-births from 2003 to 2014. Follow-up has lasted more than 10 years, and is still ongoing. And we recruited 83 children spontaneously conceived children (SC) as the control group.

Participants/materials, setting, methods: We recruited 83 spontaneously conceived children, 126 children born to OHSS-ET women, 1069 children born to non OHSS-ET women, 98 children conceived by women who developed into moderate or severe OHSS after oocyte retrieval and selected the frozen-thawed embryo transfer (FET), 487 children conceived with non OHSS-FET. BP, heart rate, anthropometrics, and metabolic index of those children were assessed. We applied several multiple regression analyses to investigate the effect of OHSS in the early pregnancy.

Main results and the role of chance: By the single factor analysis, the systemic blood pressure (SBP) and diastolic blood pressure (DBP) in the SC group (SBP: 99.84±8.9; DBP: 55.27±8.8) were significantly lower than the OHSS-ET group (SBP:101.93±8.17; DBP: 58.75±8.48), while the blood pressure was similar between the SC group and other three ART group. Meanwhile the BP were significantly lower in the non OHSS-ET group (SBP: 99.49±8.91; DBP: 56.55±8.02) than in the OHSS-ET group. After using multiple regression analysis to adjust current, early life, parental and ART characteristics, the differences in both SBP and DBP (B (95% confidence interval)) between non OHSS-ET and OHSS-ET remained significant (SBP: -3.257 (-5.231 to -1.284); DBP: -3.084 (-4.920 to -1.248)). And the BP showed no significant difference complementary, when compared non OHSS-FET group with OHSS-FET group or non OHSS-ET group. In addition, the anthropometrics, fast glucose, serum lipid and thyroid index did not differ among the ART groups.

Limitations, reasons for caution: Office BP and heart rate measurements were performed twice on the single day without using gold standard assessment. The study did not perform further mechanism experiments, although OHSS may be an independent risk factor leading to elevated blood pressure in offspring.

Wider implications of the findings: It is the first large sample study to investigate the effect of OHSS on offspring by setting the OHSS-FET and non OHSS-FET control group. Meanwhile, It also provides a clinic evidence of the impact of early environment on the offspring's cardiovascular health.

Trial registration number: not applicable

O-038 Maternal age gradient in children's birth outcomes among mothers conceiving with Medically Assisted Reproduction

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Study question: Are mothers who conceive through Medically Assisted Reproduction (MAR) at higher risk of poorer birth outcomes because they give birth at advanced maternal ages?

Summary answer: Amongst MAR mothers, the risk of poorer birth outcomes does not increase with maternal age at birth until very advanced maternal ages (40+).

What is known already: Existing evidence shows that children conceived through MAR are at higher risk of poorer birth outcomes. As many women undergo MAR treatments at advanced ages, which is itself a well-known risk factor for adverse birth outcomes, it has been suggested that the older age of MAR mothers might play an important role in the observed association between the use of MAR treatments and poorer birth outcomes. However, no existing large-scale study has directly tested this association.

Study design, size, duration: Finnish population register and other administrative registers. The base dataset was a 20% random sample of households with at least one child aged 0–14 at the end of 2000. This study included children who were born in 1995–2000 because the information on whether the child was conceived through MAR or naturally was available from 1995 onward.

Participants/materials, setting, methods: The outcome measures were whether the child had low birth weight (LBW, <2500 g at birth) and whether the child was delivered preterm (<37 weeks of gestation). Conception through MAR was identified from purchases of prescription medication from the National Prescription Register. Linear probability models were used to analyse and compare maternal age gradients in birth outcomes for mothers who conceived through MAR or naturally before and after adjustment for maternal characteristics.

Main results and the role of chance: A total of 56,026 children, of whom 2,676 were conceived through MAR treatments, were included in the study. Amongst mothers who conceived naturally, when compared with maternal ages 30-34 years, maternal ages of 35–39 years and ≥40 years were associated with percentage increases of 1.1 points (95% confidence intervals: 0.6, 1.6) and 1.5 points (95% confidence intervals: 0.5, 2.6), respectively, in the probability of low birth weight. In contrast, amongst mothers who used MAR to conceive, the risk increased by 6 percentage points (95% confidence intervals: 0.2, 12) for maternal ages ≥40 – who represent only 7% of mothers who conceive through MAR. Maternal ages 35-39 were not at increased risk of LBW (-1.3; 95% confidence intervals: -4.5, 1.8). Adjustment for maternal characteristics (i.e. if the mother suffered from acute/chronic conditions before pregnancy, household income and if the mother smoked during pregnancy) only marginally attenuated the associations. The results were similar for preterm delivery.

Limitations, reasons for caution: Limited number of confounders were included in the study because of the administrative nature of the data used in the study.

Wider implications of the findings: This is the first study to analyse and question the role of advanced maternal age in explaining the increased risk of poorer birth outcomes amongst children conceived through MAR. This question is of high importance in light of the widespread and increasing use of MAR treatments, especially among older women.
Trial registration number: Not applicable

INVITED SESSION

SESSION 08: OPTIMIZING ART SUCCESS IN POOR PROGNOSIS PATIENTS

Monday 24 June 2019 Haydn 1 11:45–12:45

O-039 Planning IVF treatment in the context of female ageing

O-040 Poor ovarian reserve: Do adjuvant therapies really work?

F.J. Broekmans[1]

[1]*Broekmans- Frank J., Reproductive Medicine, Utrecht, The Netherlands*

Abstract text
Poor ovarian reserve: Do adjuvant therapies really work?

Frank J Broekmans,
Professor Reproductive Medicine and Surgery

Department for Reproductive Medicine

University Medical Center Utrecht

Assisted reproduction technology is applied as a treatment mode for couples with both explained and unexplained infertility. The first step in this treatment is the creation of multiple follicles with the purpose of obtaining the oocytes held within these follicles, creating embryos in the IVF laboratory and replacing the embryos into the uterine cavity. Ovarian stimulation is mostly applied by using exogenous FSH. The response of the ovaries to this exogenous FSH exposure demonstrates a high degree of variation.

From a clinical significance point of view the low ovarian response defined as the yield of less than 5 oocytes is related to an unfavourable prognosis for live birth. The low responder may either have not more than a few antral follicles available or may suffer from a too low FSH exposure to assure the development of all of a normal number of antral follicles present in the ovaries. This leads to a relevant difference between the 'expected' and 'unexpected' low responder, where the latter often has the better prognosis for live birth, although female age will have a crucial additional role here.

Most clinicians try to foresee the low ovarian response category in order to increase the FSH dosage and bring the ovarian response into the normal range (5-15 oocytes), with the expectation that the prospects of pregnancy for the couple will improve. FSH dosage adjustments will most frequently neither alter oocyte number nor improve live birth prognosis in the low responder, especially when the condition is expected, for instance when the AMH or AFC is very low, or in case of female age above 38 years.

Regarding options to enlarge the number of antral follicles by factors that affect the continuous recruitment of follicles from the primordial follicle pool, research has focussed on the paracrine system that regulates this autonomous process. To subtly interfere herein is not easy, and compounds that could alter paracrine settings have sofar failed to show an obvious benefit. Yet many of adjuvant factors researched in fact lean on indirect changes in FSH exposure, thereby negating the relative limited role for FSH in the continuous recruitment.

For drugs like aromatase-inhibitors, oestrogen receptor modulators, androgens, aspirin, LH or growth hormone, effects on oocyte yield in subsequent or concomitant FSH ovarian stimulation have not been consistent or even clearly absent, as are any benefits for prognosis. This may urge for more in-depth research in the feasibility of outside manipulation of this process. Part of this research focusses on 'rejuvenation' of the ovary from its near-depleted state, simply by mechanical tissue disrupture, or by intra-ovarian instillation of paracrine growth factors. All these options are now awaiting rigorous scientific proof of efficacy.

INVITED SESSION

SESSION 09: ESHRE RECOMMENDATIONS FOR GOOD PRACTICE

Monday 24 June 2019 Haydn 3 11:45–12:45

O-041 Recommendations for good practice in ART: The theory

N. Vermeulen[1], **N. Le Clef**[1], **A. D'Angelo**[2], **Z. Veleva**[3], **K. Tilleman**[4]

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[2]*Cardiff University, College of Biomedical and Life Sciences, Cardiff, United Kingdom*
[3]*Helsinki University and Helsinki University Central Hospital, Department of Obstetrics and Gynecology, Helsinki, Finland*
[4]*Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium*

Abstract text
ESHRE has been developing guidelines since 2010, based on a structured evidence-based approach, which is considered the gold standard of medical guidance. Evidence-based guidelines are primarily based on high quality evidence, and are appropriate for areas where such evidence is available for most of the guideline's key questions. Guideline groups can formulate strong and conditional recommendations, depending on the quality of the supporting evidence and other factors including patient perspective, healthcare context and clinicians' expertise. For some topics in the field of Human Reproduction and Embryology, it became clear that an adapted methodology and nomenclature would be appropriate. Therefore, ESHRE has recently developed a manual for the development of (consensus-based) recommendations for good practice. The methodology described is more applicable in areas where there is an opportunity to reduce uncertainty and improve quality of care, but where evidence for most aspects is absent or limited. Topics for recommendations for good practice are different and often more practically oriented than these for evidence-based guidelines. During the presentation, the ESHRE methodology for development of recommendations for good practice, the rationale, and the differences and similarities with evidence-based guidelines, will be discussed. This presentation will be an introduction to the presentation of 2 papers based on the methodology on transvaginal oocyte pick up and ectopic pregnancy.

O-042 Recommendations for good practice in ultrasound - oocyte pick-up: The practice

A. D'Angelo[1], **C. Panayotidis**[2], **N. Amso**[3], **R. Marci**[4], **R. Matorras**[5], **M. Onofriescu**[6], **A. Turp**[7], **F. Vandekerckhove**[8], **Z. Veleva**[9], **N. Vermeulen**[10], **V. Vlaisavljevic**[11]

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[3]*AMSO, Medi Centre, Cardiff, United Kingdom*
[4]*University of Geneva, Dpt. of Reproductive Health and Research RHR WHO Geneva, Geneva, Switzerland*
[5]*IVIRMA, Ivi, Bilbao, Spain*
[6]*University of Medicine and Pharmacy IASI, Obs & Gynae, Iasi, Romania*
[7]*Harran University School of Medicine, Obs & Gynae, Şanlıurfa, Turkey*
[8]*Universitair Ziekenhuis Gent, Assisted Reproduction, Gent, Belgium*
[9]*Helsinki University, Obs & Gynae, Helsinki, Finland*
[10]*ESHRE, Central office, Brussels, Belgium*
[11]*IVF Adria Consulting, Assisted Reproduction, Maribor, Slovenia*

Abstract text
Study Question: What is good practice in ultrasound, and more specifically during the different stages of transvaginal oocyte retrieval?

Summary Answer: This document provides good practice recommendations covering technical aspects of transvaginal oocyte pick up.
What Is Already Known: Ultrasound guided transvaginal oocyte pick up is a widely performed procedure, but standards for best practice are not available.
Study Design Size, Duration: A working group collaborated on writing recommendations on the practical aspects of transvaginal oocyte pick up.
Participants/Materials, Setting, Methods: This document focused on transvaginal oocyte pick up. Further documents in this series will provide recommendations for other ultrasound procedures in infertility and assisted reproduction.
Main Results And The Role Of Chance: The document presents general recommendations for transvaginal oocyte pick up, and specific recommendations for its different stages, including prior to, during and after the procedure. In addition, information is provided on equipment and materials, possible risks and complications, audit and training.
Limitations Reasons For Caution: The recommendations of this paper were mostly based on clinical expertise as at present only few clinical trials have focused on the oocyte retrieval techniques, and almost all available data are observational. In addition, studies focusing on oocyte pick up were heterogeneous with significant difference in techniques used, which made drafting conclusion and recommendations based on these studies even more challenging.
Wider Implications Of The Findings: These recommendations complement previous guidelines on the management of good laboratory practice in assisted reproduction techniques. Some useful troubleshooting/checklist recommendations were given for easy implementation on clinical practice. These recommendations were aimed to contribute to the standardization of a rather common procedure which is still performed with great heterogeneity.

O-043 Ectopic pregnancy: Classification on imaging

INVITED SESSION

SESSION 10: FERTILITY SOCIETY OF AUSTRALIA EXCHANGE LECTURE

Monday 24 June 2019 Haydn 2 11:45–12:15

O-044 Oocyte-secreted serum biomarkers and reproductive potential in women

A. Riepsamen[1], D.M. Robertson[1,2], R.B. Gilchrist[1], W.L. Ledger[1,3]

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[2]*Hudson Institute of Medical Research, Centre for Endocrinology and Metabolism, Clayton Victoria, Australia*
[3]*IVF Australia, Alexandria, Sydney, Australia*

Abstract text
Current serum biomarkers of reproductive potential, such as follicle-stimulating hormone, oestradiol, and anti-Müllerian hormone (AMH), are used to estimate the number of growing follicles in the ovary and to predict the ovarian response to gonadotropin stimulation during assisted reproduction. However, these biomarkers are not derived from the oocyte, and hence only provide an indirect assessment of oocyte function. They provide no information on oocyte quality, which is the rate-limiting factor in female fertility. Bone morphogenetic protein-15 (BMP15) and growth differentiation factor-9 (GDF9) are essentially secreted solely by the oocyte, and are critical for folliculogenesis, oocyte quality and fertility, making these ideal candidates as biomarkers of oocyte function. However, measurement of BMP15 and GDF9 is difficult as serum concentrations of these proteins are expected to be low/undetectable, as these are locally-acting growth factors, and each adult ovary has only ~300,000 oocytes, with the majority of these in a quiescent state. Furthermore, BMP15 and GDF9 exhibit unusual structural variations, including non-covalent dimersation, and there are few molecular tools available to detect these. Currently, there are no validated means to quantitate their concentrations in serum. Our research program aims to develop and validate assays to measure BMP15 and GDF9 in female serum and to investigate their use as biomarkers of female reproductive function. Enzyme-linked immunosorbent assays (ELISAs) for BMP15 and GDF9 were developed in-house and validated for specificity (<0.01% and <0.03%, respectively), sensitivity (24 and 26 pg/ml, respectively) and reproducibility. Recombinant protein standards diluted in parallel with serum samples in dose-response experiments, and serum BMP15 and GDF9 were stable after 3 repeated freeze thaw cycles (1 and 10% reduction in detection, respectively). Validated ELISAs were applied to serum samples from women undergoing infertility treatments (n=154), and from peri- and post-menopausal women (n=28). BMP15 and GDF9 were determined in women relative to age, AMH and number of oocytes retrieved after superovulation for IVF. Serum BMP15 and GDF9 were detectable in 61% and 29% of women, respectively. BMP15 and GDF9 varied 64- and 15-fold, respectively, between women but did not change within an individual during ovarian stimulation with gonadotropins. Furthermore, there was no difference is serum BMP15 or GDF9 between women relative to ovarian stimulation treatment, or between stimulated and unstimulated women. Serum GDF9, but not BMP15, correlated with the number of oocytes retrieved (p=0.058) and was significantly lower in poor responders (p=0.032). Conversely, and where detectable, serum BMP15, but not GDF9, was significantly lower in women over 55 years, compared with women of reproductive age (p=0.018). There was no association between AMH and either of these growth factors. This is the first study to develop and validate assays to quantitate BMP15 and GDF9 in human serum, and to correlate concentrations with female reproductive potential. As oocyte paracrine factors, predictably, BMP15 and GDF9 concentrations were low in serum, in the pg/ml range, several orders of magnitude lower than AMH, making them undetectable in some women with this first-generation assay. Although assay sensitivities require improvement, this study demonstrates the diagnostic potential of oocyte-secreted BMP15 and GDF9 as serum biomarkers in reproductive medicine.

INVITED SESSION

SESSION 11: FERTILIZATION IN THE RESEARCH AND HUMAN IVF LABORATORIES

Monday 24 June 2019 Haydn 4 11:45–12:45

O-045 Breakthroughs in human fertilization

E. Bianchi[1], G. Wright[1]

[1]*Wellcome Sanger Institute, Cell Surface Signalling Laboratory, Hinxton- Cambridge, United Kingdom*

Abstract text
The Molecular basis of sperm-egg recognition
In sexually reproducing species, fertilization represents the first step toward the generation of a new genetically distinct individual. Male and female gametes, which in mammals possess very distinct features, have to recognise and fuse with one another for fertilization to be successful. Spermatozoa are constantly produced in large numbers: they are small motile cells with a densely packed DNA, very scarce cytoplasm and a tail that propels them along the female reproductive tract. The oocytes are rare, with only a few hundred produced over the entire reproductive life of an individual, have a large cytoplasm to support the early embryonic developmental stages, are immotile, and protected by a mesh made of glycoproteins (the Zona Pellucida, ZP). After migrating along the female reproductive tract, the sperm become capable of penetrating the oocyte, the first gamete interaction is the binding of the sperm to the ZP, followed by the passage through the ZP and into the perivitelline space. The last essential step before fusion is the binding of the cell membranes of sperm and egg. Cell fusion finally begins in the equatorial region of the sperm head and guarantees the delivery of the paternal DNA inside the maternal cytoplasm.

It is remarkable that the accurate descriptive knowledge that we have about fertilization does not correspond to a comparable level of understanding of the molecular events. The interactions established by cell surface proteins are

often weak and transient making them challenging to deal with using standard biochemical methods, in particular biochemical purifications that often employ stringent washing and denaturing conditions. This biochemical feature, together with the paucity of biological material, especially the very limited number of oocytes, have hindered the identification of membrane proteins required for the sperm-egg interaction. Only three cell surface proteins are known to be essential for fertilization: the binding pair JUNO and IZUMO1, and CD9. IZUMO1 is displayed on the head of acrosome-reacted sperm and binds the egg protein JUNO. Soon after fertilization, JUNO is lost from the egg membrane of mouse oocytes suggesting that it might be involved in establishing the membrane block to polyspermy. We have also shown that JUNO and IZUMO are necessary for sperm-egg binding but are not sufficient to induce fusion of the cell membranes, it is therefore likely that other proteins take part in this process. Alongside JUNO and IZUMO, the third protein known to be essential for fertilization is CD9. The similarity with other proteins of the tetraspanin family, its dynamic localization during fertilization, and the aberrant microvilli in mouse eggs lacking CD9, suggest that it plays a structural role in the organization of the egg cell surface.

Recently, a cell surface protein (HAP2) able to induce fusion in lower eukaryotes that is not conserved in mammals has been identified; moreover, a novel molecule named Bouncer required for fertilization in fish has also been discovered. With the interest raised by these discoveries and the development of biochemical methods becoming more and more sensitive, it is likely that the role of novel proteins will be elucidated in the next future. Undoubtedly the biggest unsolved question remains the identity of the molecule(s) inducing fusion of sperm and egg.

O-046 The first 24 hours of human development in vitro

M. Montag[1]

[1] *ilabcomm GmbH, CEO, Sankt Augustin, Germany*

Abstract text

The first 24 hours of the human embryo roughly comprise the journey of the human oocyte and sperm cell, from the time of fertilization until the initiation of the first cleavage generating the 2-cell stage embryo. In preparation for fertilization the oocyte has completed the first meiotic division and extruded the first polar body. The oocyte is arrested at metaphase-II by specific kinases and the paired chromatids are lined up in the metaphase-II spindle. This spindle-chromatid complex is usually located in the ooplasm at a position, which is underneath the first polar body. Fertilization itself is not a singular event – it is a complex course of ordered events that play into each other and are called the fertilization cascade. It is initiated by the presence of the sperm cell and by sperm-specific proteins that activate the oocyte and cause resumption of meiosis. Thus it is the sperm cell that ultimately activates the oocyte and triggers completion of the final maturation step. Oocyte activation causes that the block of the metaphase-II spindle is released enabling the spindle to initiate it´s main function: separation of the aligned chromatids, which gives rise to two sets of chromatids. One set remains inside the oocyte and the other set is extruded into the newly forming second polar body by the microtubules of the spindle. The process of formation of the second polar body usually takes around 1.5 to 3.5 hours the sperm has entered the oocyte. The formation of the female and male pronuclei is initiated at the same time. Both pronuclei can be seen as early as 4 to 5 hours after insemination. The male pronucleus is initially located close to the site where the sperm has entered the ooplasm or had been injected, whereas the female pronucleus is located underneath or close to the position where the second polar body was extruded. Over time, both pronuclei will enlarge and will be positioned by the oocyte´s cytoskeleton in the center of the oocyte. Once the entire DNA of each pronucleus is fully replicated and the pronuclei are positioned next to each other, the cell cycle machinery will get ready for the first cleavage to generate the 2-cell stage embryo. The nuclear membrane of both pronuclei will start to dissolve, the male and the female chromosomal material will be no longer separated, whereby forming the genome of the new embryo. For the process, which has been outlined above, many of the molecular and cellular events have been characterized. Using different imaging technologies we are able to visualize one or the other of these event and to follow the course of the very early development. At the same time we are able to detect deviations from the normal course. Such deviations may be linked to a patient factor, but they can also be an effect of suboptimal conditions in the laboratory. Therefore the normal development of the human embryo in the first 24 hours will be presented in conjunction with some of the key elements that are of prognostic and / or therapeutic importance.

INVITED SESSION

SESSION 12: RCT SESSION - THE LATEST NEWS

Monday 24 June 2019 Strauss 1+2 11:45–12:45

O-047 PRISM trial

O-048 TABLET trial

INVITED SESSION

SESSION 13: UNDERSTANDING MISCARRIAGE AFTER ART

Monday 24 June 2019 Haydn 1 14:00–15:00

O-049 Implications for further ART cycles?

O-050 Is it all in the genes?

INVITED SESSION

SESSION 14: NURSES/MIDWIVES INVITED SESSION: COMMERCIALISATION OF EGG FREEZING: A DEBATE

Monday 24 June 2019 Haydn 3 14:00–15:00

O-051 Pro

Z. Gurtin[1]

[1] *University College London, Institute for Women's Health, London, United Kingdom*

Abstract text

Over the past decade, oocyte cryopreservation for elective reasons, more commonly referred to as 'social egg freezing', has grown exponentially. As we have recently begun to learn from qualitative studies, increasing numbers of women each year - in the US, Britain, Israel, Denmark, and more - are choosing to freeze their eggs, citing not being in a relationship, or "not being in the right relationship to have children", as the main reason for wishing to preserve their fertility for the future. While some women in the US are able to receive company or insurance-funded egg freezing, the great majority of egg freezing cycles around the world are offered in a private market, and paid for out of individuals' own pockets. That egg freezing has become commercialised is an undeniable reality. Indeed, this reproductive technology – appealing and marketed to a much broader population than traditional patients requiring fertility treatments – requires us to think critically about how to respond to such a commercialisation. In this talk, I will outline how a pragmatic and ethical response to social egg freezing must first accept the commercial nature of technology, before suggesting specific guidelines and recommendations for its practice.

O-052 Contra

G. Pennings[1]

[1] *Bioethics Institute Ghent (BIG) Ghent University, Department of Philosophy, Gent, Belgium*

Abstract text

The past decades, egg freezing has evolved from an experimental technique towards a clinically acceptable method to preserve the reproductive potential

of women of reproductive age. Today, it can offer a solution for women who risk to become infertile due to a medical treatment such as chemotherapy. In addition, it can be used by women who want to postpone motherhood for other reasons, for example because they have not found the right partner yet. While there might be several good reasons to freeze eggs to enhance women's reproductive life span, it nevertheless remains a medical procedure that requires proper counseling and the provision of correct, objective information.

Nowadays, there are several worrying tendencies that illustrate that egg freezing is increasingly being commercialized. Aggressive television campaigns and advertisements with flashy slogans are trying to convince women of all ages to freeze their eggs. In some countries, 'egg freezing parties' are being held to promote the technique and some multinationals even publicly announced that they are willing to pay the procedure of egg freezing for their female employees. In Spain, calls have even been made that in the future all women should freeze their eggs. This commercialization is worrying for several reasons.

First of all, the commercialization leads to misinformation and underestimation of the risks of the procedure. It creates the wrong perception as if egg freezing is recommended for women of all ages and situations while it is actually only needed and advisable for a selected group of women. Moreover, egg freezing is often 'sold' as a success formula with an almost 100% success rate, while in reality the chances of success are limited and depend on a number of factors.

Secondly, one can voice serious criticism on the way in which egg freezing is sold as a way to help women to combine their career and child wish. There is indeed a growing tendency for women to postpone having children. This may partly relate to women's individual life course, but it is also linked to social structures that make it difficult for women to have children at a young age. One cannot pretend to solve such a complex social problem with a purely medical solution such as egg freezing. There should be political and societal solutions that enable women and men to combine both a career and a family life.

Thirdly, egg freezing is part of a broader tendency of commercialization in healthcare. However, the decision as to who can use egg freezing should not be made by the free market. Women should have the right to use this technique, but they must be able to trust that it is done in a proper scientific way and within an ethically correct framework. In conclusion, egg freezing is a valuable medical technique that can be used to enhance the reproductive life span of certain women. However, it concerns a delicate and nuanced subject and therefore commercialization should be avoided. Consequently, clear international guidelines are urgently needed.

INVITED SESSION

SESSION 15: OOCYTE AGEING IN VIVO AND IN VITRO

Monday 24 June 2019 Haydn 2 14:00–15:00

O-053 Oocyte quality during reproductive aging: Are the chromosomal abnormalities the only problem

S. Ledda[1], **D. Bebbere**[1]

[1] *University of Sassari, Department of Veterinary Medicine, Sassari, Italy*

Abstract text

The negative correlation between age and fertility is well established in the scientific literature. Reproductive aging involves declines both in oocyte number and developmental capacity. Although part of the declining developmental competence can be ascribed to increased meiotic errors, other factors such as mitochondrial or metabolic dysfunction, abnormal gene expression or epigenetic dysregulation may play important roles. As oocyte maturation, fertilization and the earliest phases of embryo development occur in absence of transcription, they rely exclusively on maternal macromolecules deposited during oocyte growth, and are essentially under "maternal command". In addition, the oocyte is crucial for a proper definition of the embryo epigenome, as it regulates the two major DNA methylation reprogramming events during gametogenesis and early embryogenesis. The crucial remodelling of the oocyte epigenetic baggage during oogenesis often overlaps with potential interfering events, and advanced reproductive age was seen to increase the probability of occurrence. As the underlying molecular mechanisms remain poorly understood, studies in large animal models may deepen current understanding, to be potentially translated to human applications. We focused on the molecular mechanisms that shape oocyte developmental competence and that may be involved in reproductive aging using a sheep model. The expression of Maternal Effect Genes (MEGs), genes involved in epigenetic reprogramming (DNA methylating and demethylating enzymes) and DNA methylation dynamics were evaluated during oogenesis and early embryo development. MEGs code for a special class of maternal transcripts, expressed exclusively in oocytes and early embryos and required for post fertilization early cleavage. The analysed MEG panel comprised the components of the Subcortical Maternal Complex (SCMC), a multiprotein complex uniquely expressed in mammalian oocytes and early embryos, essential for zygote progression beyond the first embryonic cell divisions. The SCMC orchestrates multiple developmental events during oocyte-to-embryo transition, including embryonic genome activation (EGA), F-actin dynamics, genome stability, organelle organization, and DNA methylation. Mounting evidence indicates that these highly conserved maternal genes might contribute to human reproductive disorders and recurrent early embryo loss. We described a fine temporal regulation in MEG storage during folliculogenesis, with gene-specific patterns, and in methylation dynamics in terms of global methylation, hydroxymethylation and expression of the enzymes involved in their remodelling. Analysis over oocyte *in vitro* maturation and in a model of differential quality showed reduced abundance in low competence gametes for transcripts involved in several functions (MEGs, housekeeping genes, genes involved in pluripotency and cell cycle regulation); among them, a SCMC component (*KHDC3*), resembling what observed in MII oocytes of aged women (*NLRP5*). The association between developmental competence and maternal transcript dynamics was most evident in cleavage-stage embryos, as EGA approaches. Early embryos derived from developmentally incompetent oocytes consistently showed delayed transcript degradation, including SCMC components. We propose that the molecular shortage in the low competence oocyte impairs the early pathways of maternal mRNA clearance in the embryo, hindering further transition to EGA. The abnormal transcript persistence interestingly recalls the delayed developmental kinetics often observed in low quality oocytes in vitro. The epigenetic status of these embryos was affected as well, as the expression of genes involved in DNA demethylation (TETs) and relative cofactors (MBD1 and MBD3) was altered. The effect of maternal age on oocyte developmental competence has a high level of complexity and may be influenced by various maternal and environmental factors through interconnected mechanisms. While chromosome abnormalities, mitochondrial and metabolic dysfunction and production of oxygen radicals have already been described as implicated in poor oocyte quality associated with maternal age, less information is available on the molecular picture during oogenesis. Correct molecular storage during folliculogenesis and maternal transcript dynamics during early embryonic development appear to be closely associated with developmental competence.

O-054 The impact of DNA damage on oocytes and eggs

K. Jones[1]

[1] *University of Adelaide, Faculty of Science, Adelaide- SA, Australia*

Abstract text

The impact of DNA damage on oocytes and eggs

Mammalian oocytes are long-lived cells. The time between their creation in the fetal gonad and their eventual fertilization following ovulation can span up to 40-45 years. Here I explore the relationship between DNA damage and aging at distinct points in the life of the oocyte. Programmed DNA breaks occur systematically across the genome early in the life of the oocyte as part the process of meiotic recombination. These breaks, and then their subsequent repair, is an essential feature of mixing chromosomal content and tethering together the two pairs of homologous chromosomes that go on only to separate at the completion of meiosis I. However subsequent to recombination any DNA damage has the potential of being deleterious to the maturing oocyte or the embryo. Here I discuss the impact of DNA damage, principally double strand breaks, at two distinct timepoints in the life of the oocyte. Also, I reflect on the influence of maternal age in these processes. Firstly, in primordial follicles, where acute radio- or chemo-therapy damage can result in loss of oocytes, through activation of pro-apoptotic proteins. Even without exogenous insult primordial follicles appear to experience continual

DNA damage and interestingly the ability of oocytes to repair such damage declines with maternal age. Secondly, in fully grown oocytes at the germinal vesicle stage, where DNA damage will arrest maturing oocytes at metaphase of meiosis I. As such oocytes carrying DNA double strand breaks often fail to fully mature into eggs. Here oocytes appear to have an unusual ability to switch on the Spindle Assembly Checkpoint in response to DNA damage. This cellular checkpoint pathway is present in all cells but is normally activated by incorrect attachment of chromosomes to microtubules on the spindle. We currently do not understand mechanistically how DNA damaged oocytes use this pathway. However interestingly this checkpoint is compromised with maternal aging. As such one can expect an increase in mature eggs with DNA damage from older females. Although many of the above observations have been performed on mice, there are likely important clinically relevant points worthy of considering. These would be with respect to the impact of DNA damage on the ovarian reserve and the quality of oocytes remaining within the ovary at advanced maternal age, as well as the ability to recover more DNA damaged mature eggs from older females.

INVITED SESSION

SESSION 16: ESHRE GUIDELINE ON OVARIAN STIMULATION

Monday 24 June 2019 Haydn 4 14:00–15:00

O-055 Report of the GDG Ovarian stimulation. The LOW responder: Time to stop dreaming!!

F.J. Broekmans[1]
[1]*Broekmans- Frank J., Reproductive Medicine, Utrecht, The Netherlands*

Abstract text
The LOW ovarian responder: time to stop dreaming?

Frank J Broekmans,
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In Assisted Reproduction Technology, the first step is the creation of multiple follicles with the purpose of obtaining the oocytes held within these follicles, creating embryos in the IVF laboratory and replacing the embryos into the uterine cavity. Ovarian stimulation is mostly applied by using exogenous FSH. The response of the ovaries may demonstrate a high degree of variation. The low ovarian response defined as the yield of less than 5 oocytes is generally related to an unfavourable prognosis for live birth. The low responder may either have not more than a few antral follicles available or may suffer from a too low FSH exposure to assure the development of all of a normal number of antral follicles present in the ovaries. This leads to a relevant difference between the 'expected' and 'unexpected' low responder, where the latter often has the better prognosis for live birth, although female age will have a crucial additional role here. Recent studies have revealed that when cumulative live birth rates are studied, the prognosis for the several subgroups of low responders m.ay not be so cumbersome as frequently concluded from single cycle studies. Most clinicians try to foresee the low ovarian response category in order to increase the FSH dosage and bring the ovarian response into the normal range (5-15 oocytes), with the expectation that the prospects of pregnancy for the couple will improve. FSH dosage adjustments will most frequently neither alter oocyte number nor improve live birth prognosis in the low responder, especially when the condition is expected, for instance when the AMH or AFC is very low, or in case of female age above 38 years. Regarding options to enlarge the number of antral follicles by factors that affect the continuous recruitment of follicles from the primordial follicle pool, research has focussed on the paracrine system that regulates this autonomous process. For drugs like aromatase-inhibitors, oestrogen receptor modulators, androgens, aspirin, LH or growth hormone, favourable effects on oocyte yield in subsequent or concomitant FSH ovarian stimulation have not been consistent or even clearly absent, as are any benefits for prognosis for live birth. This may urge for more in-depth research in the feasibility of outside manipulation of this process. Part of this research focusses on 'rejuvenation' of the ovary from its near-depleted state, simply by mechanical tissue disrupture, or by intra-ovarian instillation of paracrine growth factors. All these options are now awaiting rigorous scientific proof of efficacy. Until these new options have become a breakthrough, we may realise that especially the fate of low responder with a low antral follicle reserve cannot be changed.

O-056 Report of the GDG Ovarian Stimulation. The HIGH responder: Start the OHSS free clinic!!

SELECTED ORAL COMMUNICATIONS

SESSION 17: NEW PERSPECTIVES IN DIAGNOSIS AND TREATMENT OF UTERINE PATHOLOGIES

Monday 24 June 2019 Strauss 1+2 14:00–15:00

O-057 The accuracy of three-dimensional ultrasonography and magnetic resonance imaging in the diagnosis of congenital uterine anomalies based on ESHRE/ESGE Classification System

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Study question: What is the accuracy of three dimensional ultrasonography (3D-US) and magnetic resonance imaging (MRI) in diagnosing congenital uterine anomalies (CUA) according to the ESHRE/ESGE classification?
Summary answer: 3D-US is almost as accurate as MRI in diagnosing CUAs with the ESHRE/ESGE classification. However, MRI may have better performance for borderline anomalies.
What is known already: Two-dimensional ultrasound (2D-US) is the primary tool for diagnosing CUA, with a limited accuracy, especially in differentiating anomalies like septate and bicornuate uteri where visualization of the outer uterine contour in the coronal section is necessary. Having the advantage of scanning coronal section, both MRI and 3D-US has increased ability to precisely diagnose CUA. 3D-US has the advantage of being more available and cheaper compared to MRI. Endoscopic evaluation, as either laparoscopy and/or hysteroscopy, which was accepted traditionally as the "gold standard" in diagnosing CUA is being used less after the implementation of these 2 imaging modalities.
Study design, size, duration: Prospectively collected data was retrospectively analyzed derived from 27 patients presumably diagnosed with CUAs for a period of 6 months admitting to the gynecology department. The presence and type of uterine malformation was made in accordance with the ESHRE/ESGE classification system. American Society of Reproductive Medicine (ASRM) classification was also used for comparison.
Participants/materials, setting, methods: Patients with history of infertility, recurrent abortion or having suspicious findings for presence of CUA on examination or hysterosalpingography were selected. After performing 2D-US, patients were referred to the radiology department for the evaluation by 3D-US and MRI. Final diagnosis was provided by hysteroscopy and/or laparoscopy in indicated patients. Patients with uterine septum (U2) hysteroscopic septum resection was done. Cervical septum was preserved in patients with C1 anomaly, however longitudinal vaginal septa (V1) were excised.
Main results and the role of chance: The most common CUA was septate uterus (U2A-B; 66.67%, n=18). The incidences of other anomalies were: normal uterus (U0; 7.41%, n=2), dysmorphic uterus (U1A; 3.70%, n=1), bicorporeal uterus (U3a-b-c; 14.81%, n=4), unicorniate uterus (U4; 3.70%, n=1) and aplastic uterus (U5; 3.70%, n=1). Compared with MRI, 3D-US had

a sensitivity of 96.3%, and a false positivity of 3.7% in diagnosing uterine anomaly. One patient misdiagnosed as partial uterine septum (U2aC0V0) by 3D-US turned out to be normal (U0C0V0) under MRI. Cervical anomalies were: 3 (11.11%) septate (C1), 1 (3.70%) double cervix (C2), 2 (7.41%) unilateral dysplasia (C3) and 1 (3.70%) aplasia (C4). Three patients (11.11%) had longitudinal non-obstructing vaginal septum (V1), one (3.70%) had transverse vaginal septum (V3) and another (3.70%) had vaginal aplasia (V4). 3D-US and MRI both detected these anomalies excellently giving 100% accuracy. Nine cases (33.3%) of arcuate uterus (Class VI) were diagnosed according to ASRM classification, which were all diagnosed as partial uterine septum by ESHRE/ESGE classification. Four patients (14.8%) had partial bicornuate (Class IVb), which were diagnosed as complete septate, partial bicorporeal (n=2), and bicorporeal septate with the ESHRE/ESGE system. Diagnostic accuracy of 3D-US in detecting CUAs was excellent with a Kappa index of 0.93 ($p<0.001$).

Limitations, reasons for caution: Present study implies that 3D-US is as accurate as MRI, but latter may have better diagnostic performance in borderline cases or in rare type of anomalies. MRI has the advantage of diagnosing associated renal anomalies. Small number of anomalies other than septate and bicorporeal was the limitation of the study.

Wider implications of the findings: 3D-US displays a satisfactory level of agreement with MRI in the diagnosis of CUAs based on ESHRE–ESGE system. Our results are in accordance with the existing literature showing the non-inferiority of 3D-US. Therefore, as being cheaper and more available than MRI, 3D-US may be proposed as the non-invasive "gold-standard".

Trial registration number: 2019/01-50 is the trial registration number obtained from the clinical ethical committee of Dokuz Eylul University

O-058 Septum resection in women with a septate uterus: a cohort study

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Study question: Does hysteroscopic septum resection improve reproductive outcome in women with a septate uterus?

Summary answer: In women with a septate uterus, septum resection does not increase live birth, nor does it decrease miscarriage and preterm birth compared with expectant management.

What is known already: A septate uterus is the most common uterine anomaly with an estimated prevalence of 2.5% in women of reproductive age. Women with a septate uterus are at increased risk for miscarriage, infertility and preterm birth. Currently guidelines recommend to surgically remove the intrauterine septum, but the underlying evidence is missing.

Study design, size, duration: We performed an international multicentre cohort study for which we collected data of 245 women with a septate uterus in 21 centres in the Netherlands, USA and UK. We identified women both prospectively (from September 2015 to July 2018) and retrospectively (from 2000 to August 2018) by searching electronic patient files.

Participants/materials, setting, methods: We included women with recurrent miscarriages, subfertility, previous preterm birth or without reproductive problems, who were diagnosed with a septate uterus. Women without an active wish to conceive or with a contra-indication for surgery were not eligible. Primary outcome was live birth. Secondary outcomes were miscarriage and preterm birth. To account for differences in patient population and follow up time we used multivariable Cox regression analysis and expressed differences as hazard rate ratios (HRR) .

Main results and the role of chance: In total, 260 women were included in the cohort. At the time of writing the abstract we missed complete case report forms of 12 women, such that 248 women were eligible for analysis.

Among the 148 women with a septum resection mean age was 32.2 years (SD 4.4), mean BMI was 25.5 (SD 5.1), 11 smoked (7.5%), 90 had at least one previous miscarriage (60%) and 36 (24%) had a previous live birth. Among the 100 women without a septum resection, mean age was 31.1 years (SD 4.7), mean BMI was 24.8 (SD 5.2), 5 smoked (5.1%), 50 had at least one previous miscarriage (50%) and 37 (37%) had a previous live birth. The live birth rates were 51% (76/148) following resection versus 71% (71/100) following expectant management (HRR 0.71, 95% CI 0.49 to 1.04). The number of women with at least one miscarriage was 49/148 (33%) versus 28/100 (28%) (HRR 0.98, 95% CI 0.59 to 1.64). Pre-term birth occurred in 25/76 (33%) versus 28/71 (39%) of the live births (RR 1.18, 95% CI 0.52 to 2.67). Hazard rates were adjusted for female age, BMI, smoking, previous miscarriages and previous live birth.

Limitations, reasons for caution: This was a non-randomised study. Despite correcting for the most important patient characteristics, our estimates might be confounded.

Wider implications of the findings: Our results suggest that in women with a septate uterus septum resection does not lead to improved fertility and pregnancy outcomes compared to expectant management.

Trial registration number: not applicable

O-059 A systematic review on reproductive outcomes after surgical treatment of asherman syndrome (AS)

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Study question: To assess the pregnancy outcomes in women who conceived following hysteroscopic surgical for AS

Summary answer: Pregnancy following surgical treatment of AS was associated with a number of adverse obstetric complications, including but not limited to placenta accrete syndrome.

What is known already: The reproductive outcome of women who underwent hysteroscopic adhesiolysis has been reported in a number of studies. However, the results are rather variable for a number of reasons. Firstly, there are a number of confounding variables including the severity of AS, recurrence of the adhesions after surgery, duration of follow up and co-existence of other infertility factors. Secondly, many of the reported series consisted of small numbers with a relatively wide confidence interval.

Study design, size, duration: This is a systematic review of 54 papers of variable quality searched from 3 databases including PubMed, Web of Science and Cochrane Library from the inception to April 2018 without restriction of regions, publication types or languages. Studies of pregnancy after AS, which included complications in the antenatal, intrapartum and postnatal period as well as neonatal complications were included in this review.

Participants/materials, setting, methods: Fifty-four studies including 4953 (4640 follow up) cases fulfilled the inclusion criteria and were included in the final analysis. All of the publications were full-text studies.

Main results and the role of chance: The pooled rate of pregnancy was 50.7% (95% CI [confidence interval]: 49.1 to 52.3) in 53 studies, early pregnancy loss was 17.7% (95% CI : 15.9 to 19.6) in 31 studies, ectopic pregnancy was 4.2% (95% CI: 2.8 to 6.3) in 9 studies, mid-trimester loss was 11.5% (95% CI: 7.6 to 17.8) in 7 studies, cervical incompetence was 12.5% (95% CI: 3.3 to 33.5) in 2 studies, placenta accrete syndrome was 10.1% (95% CI: 8.6 to 11.8) in 23 studies. The pregnancy rate in women with severe adhesion was significantly (P= 0.021) lower than that of women with mild adhesion.
Limitations, reasons for caution: The lack of consensus with regard to the use of post-operative adjuvant treatment to prevent adhesion reformation preclude a meta-analysis at the present time.
Wider implications of the findings: The findings may be used to counsel women with AS before surgical treatment and for planning antenatal care after conception.
Trial registration number: No.

O-060 Ultrasound guidance of hysteroscopic metroplasty, resection of intrauterine adhesions and hysteroscopic myomectomy: analysis of 1104 cases of intraoperative three-dimensional sonohysterography

J. Guerrero[1], J.P. Rouleau[1], D. Vasquez[1], R. Vicario[2], M. Costa[2], L.M.A. Pirollo[2], A. Rodriguez-Fuentes[1], A. Palumbo[1,2]

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Study question: What is the benefit of intraoperative three-dimensional (3D) sonohysterography performed during hysteroscopy?
Summary answer: Intraoperative 3D sonohisterography is an excellent method to monitor surgical correction of uterine anomalies, resection of intrauterine adhesions and submucous fibroids.
What is known already: Normal uterine anatomy and endometrium are prerequisites for normal implantation. Evaluation of the uterine cavity is therefore a crucial step for the diagnosis of infertility patients. 3D sonohysterography has been shown to be an excellent non-invasive diagnostic procedure. Hysteroscopic correction of intrauterine pathology is frequently performed prior to fertility treatment, but the role of ultrasound guidance to monitor these procedures has not been adequately evaluated. There is debate in the literature regarding the possible role of minor uterine anomalies such as arcuate uterus, but there is some evidence that an indentation >5.8 mm is associated with reduced implantation.
Study design, size, duration: Retrospective analysis of 1104 operative hysteroscopies performed in patients with uterine maltormations, intrauterine adhesions or submucous fibroids in a single private infertility clinic and a satellite office between September 2004 and January 2019. Surgeries were performed under ultrasound guidance using a Volusoni, VolusonE8 or Voluson730 ultrasound device with a 3D-RAB 4-8 transabdominal probe and a 3D-RIC 5-9 transvaginal probe (GE Healthcare, Austria). Surgeries were videotaped and ultrasound pictures/videos were recorded and subsequently reviewed.
Participants/materials, setting, methods: Surgeries were performed with either a compact hysteroscope (3,8 or 5 mm) with an operative channel using cold scissors, forceps or a bipolar electrode or with a resectoscope (7 or 9 mm) (Wolf, Germany). Intraoperatively a sonohisterography was performed either transabdominally or transvaginally with the hysteroscope in place in order to monitor the progress of the operation. Surgeries were performed with a full bladder. A foley catherer was left in place for 7 days postoperatively.
Main results and the role of chance: A total of 2151 hysteroscopies were performed during the study period. In 1047 cases the indication for surgery was polyp and ultrasound guidance was not required. The remaining 1104 were performed with 3D ultrasound guidance. Of these, 608 cases where uterine malformations including 95 complete septa, 168 incomplete septa, 269 arcuate uteri with an indentation >5.8mm, and 76 "T- shaped uteri"; 363 cases were severe intrauterine adhesions; 133 were myomectomies. Of the total, 147 patients had more than one pathology. In all cases the volumes acquired intraoperatively, which are identical to those obtained when performing office 3D sonohisterography, perfectly illustrate the progress of the operation, showing the limits of the uterus and avoiding the serosa . There were no cases of uterine perforation.
Limitations, reasons for caution: This technique requires expert ultrasonographers and high resolution 3D ultrasound devices. A potential limitation is the poor quality of image observed in obese and overweight patients.
Wider implications of the findings: Hysteroscopic correction of uterine malformations and severe intrauterine adhesions and submucous fibroid resection can be performed safely and effectively under 3D ultrasound guidance. Intraoperative 3D sonohysterography allows monitoring the progress of hysteroscopic procedures minimizing complications and indicating when the surgical procedure is complete.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 18: GENETIC AND CELLULAR DETERMINANTS OF EMBRYONIC FUNCTION

Monday 24 June 2019 Mozart 15:15–16:30

O-061 An integrated chromatin accessibility and transcriptome landscape of human pre- and post-implantation embryos

Z. Shang[1], L. Liu[1], L. Leng[2], C. Liu[1], X. Dai[1], Q. Wang[1], S. Wang[1], T. Li[3], F. Chen[1]

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Study question: Molecular regulation and cell fate decision during human pre- and post-implantation development
Summary answer: We mapped the chromatin accessibility and transcriptome profiles for human pre- and post-implantation embryos.
What is known already: The human pre- and post-implantation development is a delicately balanced and orchestrated process that involves extensive changes in chromatin structure and transcriptional activity. However, a genome-wide survey of chromatin structure and its association with molecular regulation in this process have been impeded by the scarcity of the required materials.
Study design, size, duration: For pre-implantation development study, we adopted LiCAT-seq, a technique that allows simultaneous profiling of chromatin accessibility and gene expression with ultra-low input of cells, cell stages including oocytes, 1-cell, 2-cell, 4-cell, 8-cell, morula and blastocyst. For post-implantation development study, we generated single-cell RNA and single-cell ATAC-seq data of E6, E7, E8, E9, E10, E12, E13.5 cells isolated from an *in vitro* culture system.
Participants/materials, setting, methods: Here, by developing LiCAT-seq, we mapped the chromatin accessibility and transcriptome profiles for human pre-implantation embryos. In addition, combined with single-cell transcriptome and epigenome maps of human post-implantation *in vitro* culture system (before E14) allowed us to reveal the underlying regulatory mechanism of cell fate decision during this complex developmental time.
Main results and the role of chance: We mapped the chromatin accessibility and transcriptome profiles for human pre- and post-implantation embryos. Integrative analysis between the two omics layers revealed a strong association between the establishment of accessible chromatin and the related genes up-regulated during embryonic genome activation (EGA) and epiblast development. Furthermore, combined analysis of transcription factor accessibility identified putative novel transcription factors in regulating EGA and cell fate decision. In addition, we identified massively expressed endogenous retrovirus (ERVs) during EGA and epiblast disc.
Limitations, reasons for caution: Relatively small dataset.
Wider implications of the findings: Our results thus offer new mechanistic insights into the molecular events inherent to human pre- and post-implantation embryo development.
Trial registration number: none.

O-062 Heterozygous loci used for evaluation of sgRNA off-targeting

A. Koski[1], D. Liang[1], N. Marti Gutierrez[1], S.W. Park[2], H. Ma[1], H. Darby[1], Y. Lee[1], E. Kang[1], R. Ahmed[1], T. Hayama[1], C. Van Dyken[1], Y. Li[1], P. Amato[3], J.S. Kim[2], S. Mitalipov[1]

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Study question: Can heterozygous loci be used for easy assessment of sgRNA off-targeting effects?
Summary answer: Targeting of heterozygous loci can be used to evaluate single guide RNA (sgRNA) specificity and provide simple informative data on off-targeting of opposite alleles.
What is known already: CRISPR/Cas9 is a powerful gene-editing tool; however, sgRNA mismatch tolerance and off-target mutations on homologous regions are serious safety concerns. Existing off-target screening approaches are based on extensive sequencing of most potential loci but can be expensive and time consuming. The results are cumbersome to analyze, mostly predictive and can contain false positives. Here we provide a simple and reliable method to assess the specificity of genome editing tools by targeting heterozygous loci.
Study design, size, duration: Human embryos carrying three different heterozygous loci were generated containing mutant (MT) and wildtype (WT) loci: *MYBPC3* 4-bp GAGT deletion (g.9836_9839 del., NC_000011.10), *MYH7* 1bp substitution (g.15819 C>T, NG_007884.1), and *LDLRAP1* 1bp substitution (g.24059 G>A, NG_008932.1). Multiple sgRNAs were designed and pre-tested in heterozygous iPSCs, one sgRNA for each locus with the highest indel rate on MT allele and lowest on WT allele were selected for embryo experiments. Indel mutations on alleles were compared.
Participants/materials, setting, methods: Three sperm and 12 oocyte donors' carriers of the *MYBPC3*, *MYH7* and *LDLRAP1* mutations were recruited and consented to provide gametes, skin and blood. Each blastomeres from injected embryos were isolated, whole genome amplified and evaluated by sequencing. The sgRNA and Cas9 protein were injected into heterozygous zygotes and on (MT) and off-target (WT) specificity were evaluated in cleaving day 3 embryos. Indel mutations were compared to assess targeting specificity of sgRNA.
Main results and the role of chance: The sgRNAs selected for *MYBPC3* and *MYH7* mutations exhibited exceptional fidelity as 55/55 and 91/91of analyzed blastomeres, for the each locus respectively, carried indels on MT allele but all WT alleles were intact. Targeting the *LDLRAP1* locus with the first selected sgRNA introduced indels on 56/74 (76%) of MT alleles. However, indels were also found in 18/74 (24%) of WT alleles; We designed, and selected another sgRNA that demonstrated a 43% (3/7) on-target and 57% (4/7) off-target indels. These results demonstrate that targeting heterozygous loci allows simple and reliable evaluation of sgRNA specificity.
Limitations, reasons for caution: Data presented is limited to the three loci and mutation types tested. Results suggest the nitrogenous base positions and nucleotide chain interactions may affect sgRNA specificity. Research into how nucleotide bases influence sgRNA specificity is needed.
Wider implications of the findings: Off-targeting effects of genome editing pose a concern for therapeutic applications. Heterozygous loci provide rapid and precise confirmation of specificity prior to conducting expensive next generation sequencing to search for genome wide off-targeting.
Trial registration number: not applicable

O-063 Treatment Factors and the Risk of Specific Congenital Heart Defects

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Study question: Are there specific congenital heart (CH) defects associated with ART treatments?
Summary answer: Patent Ductus Arteriosus was associated with Fresh IVF cycles aOR=2.69, and frozen ICSI cycles aOR=4.59. Clomiphene citrate was associated with coarcation of the aorta aOR=5.90.
What is known already: CH defects are a leading cause of death in the first year of life and occur in around 8 per 1,000 births natural conceptions, but in 16 per 1,000 after ART. Approximately half of children diagnosed will require surgery, with more complex conditions often requiring lifelong medical care. As we and others have reported previously that certain ART is associated with an increased risk of cardiac defect, further reporting of specific defect types is an important step in identifying aetiologic pathways.
Study design, size, duration: Cohort study of all deliveries (n=302,811) and terminations of pregnancy in South Australia for the period Jan 1986-Dec 2002 that were linked to all cycles of ART (6,163 births) for the same time period. These data were linked to a State-wide birth defect registry.
Participants/materials, setting, methods: The South Australian Birth Cohort is based on all registrations of birth and terminations of pregnancy, linked to all cycles of ART, and to all congenital anomalies notified to the 5th birthday (ICD-9 British Paediatric Association codes). Logistic regression was used to investigate associations between parental factors, treatment modality and the presence of CH defects. Adjustment for multiple testing was not performed at this investigatory stage.
Main results and the role of chance: Compared to the fertile population, and after adjustment:

a) For IVF and ICSI combined, risk for Any cardiac defect (BPA 74500-74799) was increased aOR=1.42, 1.13-1.80; as was Congenital Aorta Valve Stenosis aOR=3.20;, 1.26-8.11; Patent Ductus Arteriosus aOR=1.88, 1.18-3.00.

b) Increased risk of Patent Ductus Arteriosus was associated with Fresh IVF cycles aOR=2.69, 1.32-5.48 and frozen ICSI cycles aOR=4.59, 1.84-11.44.

c) Ovulation induction with clomiphene citrate was associated with Coarctation Of Aorta aOR=5.90, 1.05-33.11.

d) Comparison of 4 culture medias revealed one with an increased risk of Patent Ductus Arteriosus for both fresh IVF aOR=5.56, 1.31-23.53, and for fresh ICSI aOR=5.57, 1.08-28.71

e) 'Spontaneous' conceptions to ART patients post-treatment were associated with Endocardial Cushion Defects aOR=4.06, 1.43-11.50.

f) A medical history of "infertility" with no IVF clinic contact was associated with Cardiac Septal Closure Anomalies aOR=1.90, 1.12-3.23; Atrial Septal Defect, 3.65, 1.88-7.12; Patent Ductus Arteriosus aOR=3.09, 1.26-7.56.

g) As there were no protective associations for any ART procedure for any cardiac defect, the distribution of multiple increased risks with ART is unlikely to be random.

Limitations, reasons for caution: Analyses were limited in statistical power for infrequent events. Increased power may reveal further associations that are boarder line in the present analysis. Replication of these findings elsewhere is important as some individual significant associations may not be robust.
Wider implications of the findings: Serious cardiac defects are associated with ovarian stimulation with clomiphene citrate, mode of fertilisation, cryopreservation with ICSI, and culture media. These observations can inform both mechanistic studies on cardiovascular development, and further targeted epidemiological research on specific clinical and pharmacological exposures and cardiac outcomes.
Trial registration number: N/A

O-064 Lipids are modulators of embryonic metabolism and stress response: insights from the diabetic rabbit model

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Study question: Our aim was to analyse embryonic lipid metabolism in embryoblast and trophoblast, and to determine potential consequences for cellular stress adaptation in rabbit blastocysts.

Summary answer: Rabbit embryoblast and trophoblast cells handle hyperlipidaemic conditions in lineage-specific ways, leading in consequences to a different endoplasmatic and oxidative stress response.

What is known already: During preimplantation development embryos take up nutrients from the surrounding milieu. The uterine concentrations depend on maternal metabolism and can be affected by maternal diseases, such as diabetes mellitus. We have shown that an insulin-dependent diabetes mellitus leads to maternal hyperlipidaemia and to higher amounts of intracellular lipid vesicles in rabbit blastocysts, especially in embryoblast cells (doi:10.1210/en.2013-1760). Lipids and fatty acids are able to regulate central transcription factors, like PPARα and PPARγ. Alterations in PPARs signalling lead to changes in antioxidant defence and redox status.

Study design, size, duration: To study effects of a maternal diabetes mellitus on early embryo metabolism, we induced an insulin-dependent diabetes experimentally by alloxan in female rabbits. Blastocysts were collected on day 6 post coitum and separated into embryoblast and trophoblast for the metabolic profiling in vivo. To evaluate the effect of an increased lipid level more in detail, blastocysts from healthy rabbits were cultured for 6h in vitro with a specified lipid mixture (Gibco, *Chemically Defined Lipid Concentrate*).

Participants/materials, setting, methods: Expression of relevant marker molecules involved in lipid metabolism (PPARα, PPARγ, PGC1α, SREBP1c, FASN, CPT1, FATP4 and FABP4) and endoplasmatic and oxidative stress response (ATF4, NRF2 and SOD2) were analysed by qRT-PCR and Western Blot, separately in embryoblast and trophoblast. Intracellular lipid accumulation was visualised by Oil Red-O staining. Amount of the ketone body 3-hydroxybutyrylcarntine (BHBA) which is synthesised via the metabolism of fatty acids was analysed by Metabolon® analysis in the blastocysts' cavity fluid.

Main results and the role of chance: Maternal diabetes increased BHBA levels in the embryo almost 3-fold. Expression of genes encoding for fatty acid uptake (FATP4) and binding (FABP4) as well as for fatty acid metabolism (PGC1α, PPARα and PPARγ) and β-oxidation (CPT1) was increased in embryoblasts from diabetic rabbits. Trophoblastic genes encoding for fatty acid and lipid biosynthesis (SREBP1c) and PGC1a were elevated. CPT1 and fatty acid synthesis (FASN) were down-regulated in trophoblast cells from diabetic rabbits. Furthermore, only in the embryoblast from diabetic rabbits two markers for endoplasmatic (ATF4) and oxidative stress (NRF2) were increased. SOD2, a marker for cellular redox status, was again differentially regulated with a reduction in embryoblast and increase in trophoblast cells from diabetic rabbits. These results show that metabolic changes in a diabetic pregnancy are differently processed in embryoblast and trophoblast gastrulation. Consistently with our observation in the diabetic model, rabbit embryos cultured with a lipid mixture showed a higher amount of intracellular lipid droplets. The expression of PPARs and fatty acid transporters was increased in embryoblast and decreased for CPT1 and FASN in trophoblast cells. Lipid exposure induced the expression of NRF2, ATF4 and SOD2 in embryoblast cells, indicating that the embryoblast is more sensitive towards metabolic stress.

Limitations, reasons for caution: The embryogenesis of the rabbit reflects human development only during blastocyst formation. Therefore, statements are limited to the embryonic stages investigated.

Wider implications of the findings: The results of the current study provide further experimental evidence that in a diabetic pregnancy maternal lipids have an impact on metabolism of the preimplantation embryo. In addition, our study point out that especially the embryoblast is vulnerable to metabolic stress by dysregulation of endoplasmatic and oxidative stress response.

Trial registration number: not applicable

This work was supported by the German Research Council (DFG ProMoAge, GRK 2155), Deutsche Diabetes Stiftung (DDS) 371/05/15 and the EU (Epihealth, EpihealthNET).

O-065 L-carnitine restores mitochondrial function of human embryos decreased with female donor age

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Study question: Does L-carnitine restore an adverse effect of female donor age on the mitochondrial function of their embryos?

Summary answer: Mitochondrial function at morula stage of human embryos decreased with their female-donor age and L-carnitine restored the mitochondrial function and the development of human embryos.

What is known already: Although the rates of oxygen consumption (OCRs) of human embryos increases with their development, the relationship between female donor age and mitochondrial function of their embryos remains obscure. L-carnitine plays important roles in reducing the membranous toxicity of free fatty acids by forming acyl-carnitine and promoting beta-oxidation, leading to alleviation of cell damage. Recent studies have shown that L-carnitine also plays important roles in in vitro oocyte growth, oocyte maturation, and embryonic development. However, whether such beneficial effects of L-carnitine lead to an improvement of mitochondrial function remains unknown.

Study design, size, duration: Fourteen oocytes and 106 embryos were used to assess mitochondrial DNA (mtDNA) copy numbers and OCRs and to examine the effect of L-carnitine. All specimens were obtained between July 2004 and June 2016, and donated from couples after they had given informed consent. The development of 374 embryos was retrospectively analyzed to assess the effects of female donor age. The development of 1308 zygotes cultured in medium with or without L-carnitine was prospectively analyzed.

Participants/materials, setting, methods: Mature oocytes and developed embryos to 6-8 cell, morula and blastocyst stages were used to assess their OCRs and mtDNA copy number. The relationships between female donor age and OCRs, and female donor age and mtDNA copy number were analyzed. Effect of L-carnitine was also assessed similarly. Using clinical data, the developmental rate from morula to blastocyst was compared among 3 different age groups and effect of L-carnitine on the blastocyst development was examined.

Main results and the role of chance: Although there were no relationships between female donor age and mtDNA copy number in any stages, the OCRs of morulae decreased with female donor age ($r^2 = 0.45$, $P < 0.01$). An addition of l-carnitine increased the OCRs of morulae (1.18 fmol/sec) compared with their sibling embryos (1.08 fmol/sec, $P < 0.05$). In clinical data analysis, the developmental rate from morula to blastocyst decreased with female donor age ($P < 0.05$, < 35 yo: 75.6% vs. 35-40 yo: 70.4% vs. > 40 yo: 46.8%). An addition of L-carnitine into a culture medium significantly improved the morphologically-good blastocyst rate per fertilized ova ($P < 0.01$, 15.1%) compared with their sibling embryos (8.8%). Twenty healthy babies were born from blastocysts cultured in L-carnitine-supplemented medium after single blastocyst transfer.

Limitations, reasons for caution: Large-scale studies should be required to assess whether an addition of L-carnitine improves the development of embryos obtained from older women. Aneuploidy has not been assessed in accordance with clinical guideline of the Japan Society of Obstetrics and Gynecology.

Wider implications of the findings: The data of the present study revealed that mitochondrial function (OCRs) at morula stage of human embryos decreased with their female donor age. L-carnitine restored the mitochondrial function and the development of human embryos.

Trial registration number: This study was approved by the IRB of IVF Namba Clinic and registered by Japan Society of Obstetrics and Gynecology (Registry numbers 135 and 138).

SELECTED ORAL COMMUNICATIONS
SESSION 19: FREEZE ALL FOR ALL?
Monday 24 June 2019 Haydn 1 15:15–16:30

O-066 Pregnancy and perinatal outcomes of 521 fresh and frozen cycles resulting in pregnancy: a secondary outcome of a RCT comparing GnRH antagonist and agonist protocols

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Study question: Are pregnancy and perinatal outcomes different after treatment with the GnRH antagonist versus the long GnRH-agonist protocol for IVF?
Summary answer: Pregnancy and perinatal outcomes are similar after GnRH-antagonist or long GnRH-agonist protocols in IVF treatments.
What is known already: The goal of assisted reproductive technology (ART) is to achieve a healthy live born child with the least complications during pregnancy. However, several studies have shown that ART pregnancies are associated with higher risks of obstetric and perinatal complications compared to spontaneously conceived pregnancies.
Study design, size, duration: Pregnancy and perinatal outcome were secondary outcomes in a large phase IV, dual-centre, open-label, randomized controlled study, with the objective to compare the GnRH-antagonist and long GnRH-agonist protocols in an unselected population of women <40 years of age referred for their first ART treatment. Women (n=1050) were randomized in a ratio 1:1 from January 2009 to December 2013 and were followed until December 2016.
Participants/materials, setting, methods: All fresh and frozen embryo transfer (FET) cycles from one single oocyte aspiration, leading to pregnancy (hCG > 10 IU/L), were included (n=521). Data were analyzed to compare miscarriage, ongoing pregnancy, stillbirth and live birth rates. Preterm birth (<37 weeks), very preterm birth (VPTB) (<32 weeks), low birth weight (LBW) (<2500 grams) and very LBW (<1500 grams) rates were compared among singleton live births.
Main results and the role of chance: Similar pregnancy outcomes were found in the GnRH-antagonist and GnRH-agonist protocols in cycles with fresh embryo transfer: miscarriage (32.0% vs 34.3%; p=0.73), ongoing pregnancy (65.2% vs 64.5%; p=0.89), live birth (64.1% vs 63.9%; p=0.96) and stillbirth (1.1% vs 0.6%; p=0.61) rates. After fresh ET, the singleton mean gestational age at delivery was similar in antagonist and agonist protocol (39.1±2.49 vs 39.3±1.90; p=0.67). Very early PTB rates (0.95% vs 0 %; 95% CI (-0.086; 0.286); p=0.31) were also similar. Mean birth weight was 3264 ± 662g in the antagonist and 3341 ± 562g in the agonist group (p=0.37). LBW was found in 9.5% vs 6% (95%CI (-0.038; 0.108); p=0.35) and very LBW in 2.9% vs 1% (95% CI (-0.019; 0.056); p=0.34). For FET cycles, all pregnancy and perinatal outcomes were similar for antagonist vs. agonist protocol except that mean birth weight was significantly lower in the GnRH-antagonist protocol (3404g ± 642 vs 3641g ± 485; p=0.05).
Limitations, reasons for caution: Twins account for poor perinatal outcomes and were excluded from the analyses to avoid bias. Perinatal outcomes were secondary outcomes of an RCT and the study was not powered for these outcomes.
Wider implications of the findings: This is the first RCT comparing GnRH antagonist and agonist protocols reporting perinatal outcomes. It strengthens the importance of large follow-up studies on children after ART. In addition, the choice of GnRH-analogue for ART treatments should be based only on optimizing clinical aspects.
Trial registration number: EudraCT #: 2008-005452-24, ClinicalTrial.gov: NCT00756028.

O-067 Relationship between low ovarian reserve and p53 family members polymorphisms

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Study question: Is low ovarian reserve (LOR) associated with polymorphisms in the p53 family members?
Summary answer: TP53C>T(rs1625895), TP63A>G(rs7619549) and TP73G>A(rs3765730) polymorphisms are associated with ovarian reserve. TP53-TT and TP73-GG genotypes increase, but TP63-AA decreases the likelihood of a woman presenting LOR.
What is known already: Knowledge regarding a patient's potential ovarian reserve may assist clinicians in adjusting medication dosages to reduce adverse effects. However, the ovarian reserve is a complex clinical phenomenon, influenced not only by age but also by environmental and genetic variables. The p53 family members are powerful transcription factors and small variations in their DNA structure can modify gene expression patterns. We know that the p53 family is involved in blastocyst implantation and female germ cell integrity, but we still do not understand its influence on ovarian function.
Study design, size, duration: All of the recruited women (n: 145) met the following inclusion criteria: age ≤37years, normal karyotype, ultrasound evidence of the two ovaries and no ovarian surgery, endometriosis, hydrosalpinx, infections or endocrine problems. The women were divided into two groups according to their Anti-Müllerian hormone/AMH (ng/ml) levels and antral follicle count/AFC (2-9mm), evaluated during the early follicular phase:
-Low ovarian reserve (LOR/n=86): AMH<1+AFC≤9
-Normal ovarian reserve (NOR/=59): AMH≥2+AFC≥15
Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from LOR and NOR groups. DNA was sequenced on MiSeq(Illumina) to search for single nucleotide polymorphisms(SNPs) in the p53 family members (TP53/TP63/TP73). SNPs were identified using TruSeq Custom Amplicon (TSCA) Panel (DesignStudio Illumina). This design was performed in order to sequencing the exons, 3' and 5' *untranslated regions* (UTRs) and some intronic regions SNPs found by Next-Generation Sequencing were analysed to find a possible association with LOR
Main results and the role of chance: The TP53 C>T (rs1625895), TP73 G>A (rs3765730) and TP63 A>G (rs7619549) SNPs were identified. The results observed an association between TP53-TT genotype and TP73-GG genotypes with women presenting LOR and TP63-AA genotype with women presenting NOR(Table1). Logistic regression (Table 2) showed that patients with TP53-TT and TP73-GG genotypes had 4.0-fold and 2.5-fold (respectively) increase in the chance of being included in the LOR group. On the other hand, the presence of TP63-AA genotype was associated with an 81% decrease in the chance of being included in the LOR group.
Limitations, reasons for caution: Additional validation of the SNPs analysed would be important to provide more information about the relationship of theses polymorphisms and ovarian reserve, once sample size was limited despite recruiting all eligible participants during the study period. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: The SNPs identified might provide an additional tool to test ovarian reserve and help in the individualization of ovarian stimulation protocols. To the best of our knowledge, this is the first study associating these SNPs and ovarian reserve.
Trial registration number: Not applicable. The local ethics committee authorised the study. Merck Grant for Fertility Innovation (GFI-2014-16) supported this study.

O-068 Comparison of the ongoing pregnancy rate of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle: a prospective randomized controlled trial

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Table 1 Distribution of genotype/allele frequency in normal and low ovarian reserve groups.

Genes	Low ovarian reserve	Normal ovarian reserve	*P*
TP53			
Genotypes			
TT	**45.0%**	17.0%	0.001
TC	21.2%	25.4%	
CC	33.8%	57.6%	
Alleles			
T	55.6%	29.7%	<0.0001
C	44.4%	70.3%	
TP73			
Genotypes			
GG	**62.8%**	40.7%	0.03
GA	27.9%	44.1%	
AA	9.3%	15.3%	
Alleles			
G	76.7	62.7	0.01
A	23.3	37.3	
TP63			
Genotypes			
AA	73.8%	**93.6%**	0.04
AG	14.3%	2.1%	
GG	11.9%	4.3%	
Alleles			
A	81.0%	94.7%	0.005
G	19.0%	5.3%	

Table 2 Logistic regression. Genotypes X Chance of low ovarian reserve.

Genotypes	Odds Ratio	95% Confidence Interval	*P*
TP53:TT	4.0	1.78-9.01	0.0008
TP73:GG	2.5	1.25-4.85	0.009
TP63:AA	0.19	0.05-0.75	0.01

Study question: To compare the ongoing pregnancy rate of immediate versus delayed frozen-thawed embryo transfer (FET) following a stimulated (in-vitro fertilization) IVF cycle.

Summary answer: Immediate FET following a stimulated IVF cycle had a significantly higher ongoing pregnancy rate than delayed FET.

What is known already: FET has become an increasingly important part of IVF but there is still no good evidence to support when to perform FET following a stimulated IVF cycle. Since all published studies are retrospective and the findings are contradictory, a randomized study is needed to provide level 1 evidence to guide the clinical practice.

Study design, size, duration: This is a prospective randomized controlled trial of 724 women recruited between August 2017 and December 2018. The primary outcome is the ongoing pregnancy defined as a viable pregnancy beyond 12 weeks' gestation.

Participants/materials, setting, methods: Women aged ≤43 years having the first FET cycle after a failed stimulated IVF cycle or undertaking the freeze-all strategy were randomized to either: (1) the immediate FET group in which FET was performed in the first cycle following the stimulated IVF cycle, or 2) delayed FET group in which FET will be performed at least in the second cycle following the stimulated IVF cycle.

Main results and the role of chance: At the time of abstract submission, 687 women completed the study, 1 patient waiting pregnancy result and 36 women dropped out. We expect to have completed results by the time of the conference. The age of women was significantly younger in the immediate group than the delayed group (30.5±4.0 years vs 31.4±4.1 years respectively, p=0.004). No significant differences in other baseline characteristics were observed between the two groups. Intention to treat analysis showed the ongoing pregnancy rate was significantly higher in the immediate group [170/362, 47.0%] vs in the delayed FET group [142/362, 39.2%], odds ratio 0.729 (95% CI 0.543–0.979)]. Per protocol analysis showed similar results. The ongoing pregnancy rate was significantly higher in the immediate group [170/345, 49.3%] vs in the delayed FET group [142/342, 41.5%], odds ratio 0.731 (95% CI 0.541–

0.988)]. After a multivariable logistic regression, the ongoing pregnancy rate is still higher in the immediate group [adjusted odds ratio 0.721 (95% CI 0.529–0.983)]. The positive HCG and miscarriage rate are comparable between the two groups.

Limitations, reasons for caution: The researchers, doctors and the participants cannot be blinded to treatment allocation.

Wider implications of the findings: Immediate FET following a stimulated IVF cycle had a significantly higher ongoing pregnancy rate than delayed FET. The findings of the study support immediate FET after a failed fresh ET cycle or a freeze all cycle.

Trial registration number: NCT03201783

O-069 Freeze-all versus fresh embryo transfer in ART: A multicentre randomised controlled trial in normo-ovulatory women

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Study question: Is the ongoing pregnancy rate in a freeze-all strategy involving GnRH agonist trigger superior to the ongoing pregnancy rate in conventional fresh embryo transfer strategy?

Summary answer: The ongoing pregnancy rate per randomised patient after the first single blastocyst transfer is comparable between freeze-all and fresh embryo transfer.

What is known already: In recent years, growing clinical evidence has emerged suggesting similar or even better pregnancy rates in elective frozen embryo transfer cycles compared to conventional fresh transfer, thus encouraging a further implementation of a freeze-all strategy as part of standard ART in patients at risk of ovarian hyperstimulation syndrome (OHSS) or where impaired endometrial receptivity is suspected. Importantly freeze-all including the use of GnRH agonist trigger has the potential to virtually eliminate the risk of OHSS alongside encouraging an eSET policy diminishing multiple birth rates overall improving safety aspects of ART care.

Study design, size, duration: Multicenter, randomised, double-blind trial including 460 women allocated 1:1 to either (1) GnRH agonist trigger and single vitrified-warmed blastocyst transfer in a subsequent natural cycle or (2) hCG trigger and single blastocyst transfer in the fresh cycle. Patients at risk of OHSS(>18 follicles >11mm)on trigger day had their embryo transfer postponed to a subsequent natural cycle. A computerized randomization program was used. Participants were enrolled over a two-year period from May 2016 to September 2018.

Participants/materials, setting, methods: A total of 460 normo-ovulatory women aged 18 to 39 referred to their first, second or third ART treatment in 8 clinics in Denmark, Sweden and Spain were included. Participants were randomised on the day of inclusion and blinded of the randomisation result until the day of ovulation trigger. After randomisation 23 patients were excluded from analyses on criteria of AMH and TSH. Ongoing pregnancy was determined by transvaginal ultrasonography at gestational weeks 8 –10.

Main results and the role of chance: All analyses were based on the ITT principle. In the primary endpoint, the ongoing pregnancy (OPR) per randomised patient after the first potential blastocyst transfer was 26.1 % (57/218) in the freeze-all group compared with 28.8 % (63/219) in the fresh transfer group (OR 0.88, 95% CI 0.58-1.34; p =0.54). OPR per transfer (only including patients who had a transfer) was 36.1% (57/158) in the freeze-all group compared with 36.8 % (63/171) in the fresh embryo transfer group (OR 0.97, 95% CI 0.62-1.52; p = 0.89). Positive hCG rate per randomised was 32.1% (70/128) vs. 38.4% (84/219) in freeze-all and fresh group, respectively (NS) and positive hCG per transfer was 44.3% (70/158) vs. 49.1% (84/171) in freeze-all vs. the fresh group (NS). In the fresh embryo transfer group, 25 participants were converted to freeze-all due to risk of OHSS. These patients were all included in the ITT analysis as part of the fresh embryo transfer group. One patient in the fresh embryo transfer group had an OHSS related hospital admission. Cancellation of transfer due to lack of blastocyst development occurred in 18.8% (n=41) and 15.1% (n=33) of patients per randomised in the freeze-all and fresh embryo transfer group, respectively.

Limitations, reasons for caution: In this design the effect of GnRH-agonist trigger and freeze-all cannot be separated, however aimed to compare an OHSS-free strategy with conventional fresh embryo transfer. The study is powered to detect a 12 % difference in OPR between the two groups, thus smaller differences may be overlooked.

Wider implications of the findings: Our findings give no support for a general freeze-all strategy in normo-ovulatory women but encourage further adaptation of a freeze-all strategy including GnRH agonist trigger in patients where this may be beneficial as part of individualised patient care. The results are in agreement with the latest meta-analysis on the area.

Trial registration number: Clinicaltrials.gov identifier: NCT02746562

O-070 Fresh versus elective frozen embryo transfer: The cumulative live birth rates for 7132 in vitro fertilization cycles

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Study question: Is elective frozen embryo transfer, or the 'freeze-all' strategy, associated with better clinical outcomes compared to fresh embryo transfer in *in vitro* fertilization (IVF) cycles?

Summary answer: The freeze-all strategy is associated with improved cumulative live birth rates in normal and hyper-responders, but not in suboptimal and poor responders.

What is known already: Many questions have arisen regarding the adverse effects of controlled ovarian stimulation (COS) on the endometrium and its consequences on endometrial receptivity. Thus, many practitioners have advocated for the use of elective frozen embryo transfer (eFET) to overcome the potentially deleterious effects of COS while improving clinical outcomes during an IVF cycle. However, evidence in support of this approach is not unequivocal. Further, less is known about which groups of patients would benefit most from the freeze-all strategy, as most studies have not evaluated this approach in different subgroups of patients based on their ovarian response to COS.

Study design, size, duration: This is a retrospective analysis of 7132 IVF cycles that were followed by a fresh or elective frozen embryo transfer between 2012 and 2017. The patients were submitted to COS with a gonadotropin-releasing hormone (GnRH) antagonist protocol and cleavage-stage embryo transfer. Embryo cryopreservation was performed on day 3 by vitrification using an open system.

Participants/materials, setting, methods: We performed a comparison of cumulative outcomes between the eFET (n=3920 cycles) and fresh embryo transfer groups (n=3212 cycles). The analysis was performed in four groups of patients based on the number of retrieved oocytes: **Group 1**– poor responders (1–3 oocytes); **Group 2**– suboptimal responders (4–9 oocytes); **Group 3**– normal responders (10–15 oocytes); and **Group 4**– hyper-responders (>15 oocytes). The primary outcome was the cumulative live birth rate (CLBR) per stimulated cycle.

Main results and the role of chance: There was a total of 7132 IVF/intracytoplasmic sperm injection (ICSI) cycles and 10314 embryo transfers (n=5621 in the eFET group; n=4693 in the fresh group). In **Group 1**, there were 343

IVF cycles and 373 embryo transfers (ET) in total, and the CLBR was 16.5% and 17.9% (P=0.75) for the eFET and fresh group, respectively (relative risk [RR]=0.92; 95% confidence interval [CI]: 0.56–1.52). In **Group 2**, there were 1990 IVF cycles and 2398 ET in total, and the CLBR was 27.7% and 24.9% (P=0.16) in the eFET and fresh group, respectively (RR=1.11; 95% CI: 0.96–1.29). There was a significant difference in the CLBR in Groups 3 and 4, favoring the eFET strategy. In **Group 3**, 2211 IVF cycles and 3225 ET were performed. The CLBR was 43.4% in the eFET and 38.9% in the fresh group (P=0.03; RR=1.11; 95% CI: 1.01–1.23). In **Group 4**, there were 2588 IVF cycles and 4318 ET in total, and the CLBR was 54.6% and 49.8% (P=0.03) in the eFET and fresh group, respectively (RR=1.10; 95% CI: 1.01–1.19). The number needed to treat to achieve one additional live birth was 22.5 in Group 3 and 21.0 in Group 4.

Limitations, reasons for caution: The main limitation of this study was its retrospective design, which may be subject to bias.

Wider implications of the findings: This is the most extensive study comparing the CLBR between eFET and fresh cycles. Moreover, comparisons among different groups of patients based on ovarian response were conducted for the first time. The findings suggest that the freeze-all strategy should not be implemented for all patient groups given the clinical outcomes.

Trial registration number:

SELECTED ORAL COMMUNICATIONS
SESSION 20: SPERMATOGENESIS

Monday 24 June 2019 Haydn 3 15:15–16:30

O-071 Germ cells synthesis of all-trans retinoic acid (ATRA) is dispensable for spermatogenesis but cooperates with Sertolian synthesis to initiate and propagate spermatogenic waves during puberty

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Study question: What is the role of germ cells or Sertoli cells-derived ATRA in spermatogenesis?

Summary answer: Sertoli cell (SC)-derived source of ATRA is sufficient to ensure a normal adult spermatogenesis while germ cells (GC)-derived ATRA is dispensable in steady-state conditions. Both sources are involved during puberty.

What is known already: In mammals, ATRA is instrumental to spermatogenesis. ATRA is synthesized by dedicated enzymes, RALDHs and binds to and activates nuclear ATRA receptors (RARA, RARB, and RARG) either within the ATRA-synthesizing cells or in the neighboring cells. We established previously that during the first, prepubertal, spermatogenic cycle RALDH-dependent synthesis of ATRA by Sertoli cells, the supporting cells of GC lineage, is indispensable to initiate differentiation of A aligned into A1 spermatogonia. Moreover, ATRA of SC origin is no longer necessary for the subsequent spermatogenic cycles but essential to spermiation.

Study design, size, duration: In order to determine the relative contributions the somatic, Sertoli, and germ cell sources of ATRA to spermatogenesis and spermiation, we have generated mutant mice lacking all RALDH activities in germ cells and compared their phenotype with that of mice lacking RALDH in Sertoli cells.

Participants/materials, setting, methods: To study the role of RALDH1 and RALDH1 in GC, mice expressing the Cre recombinase under the control of *Stra8* promoter were crossed with mice bearing loxP-flanked alleles for *Raldh1*, *Raldh1* and *Raldh3*. The Stra8-Cre transgene yields an efficient excision of loxP-flanked genes in GC.

Main results and the role of chance: We show that ATRA both sources cooperate to initiate and propagate spermatogenic waves at puberty. In homeostatic adult spermatogenesis, the GC and SC sources exert redundant functions and, against all expectations, the former does not perform any specific role. The production from SC, although representing the minor fraction of ATRA in the seminiferous epithelium, is sufficient to maintain, throughout life, the normal periodic expression of genes in these cells as well and the cycle and wave of the seminiferous epithelium which account for the constant and steady production of sperm. ATRA source of SC is also specifically required for spermiation. Altogether, these results suggest that during spermatogenesis key transitions in germ cells differentiation are regulated by distinct threshold values of ATRA concentrations.

Limitations, reasons for caution: These results were obtained in mice and therefore the relevance for human spermatogenesis remains to be demonstrated.

Wider implications of the findings: Pharmacological studies strongly support a role of the ATRA signaling pathway in human spermatogenesis. However, vitamin A deficiency, although common in developing countries, does not include male infertility among its clinical manifestations. Whether intratesticular impairment of ATRA (the active metabolite of vitamin A) synthesis might be a cause of azoospermia or oligozoospermia remains to be explored in clinical research.

Trial registration number: N.A.

O-072 Molecular profiling different stages of human sperm maturity based on transcriptome analysis and in situ hybridizations of testicular spermatozoa in comparison to mature ejaculated spermatozoa

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Study question: The major objective of this study was to generate a transcriptomic profile of human sperm maturity by microarray analysis and in situ hybridization.

Summary answer: Gene expression profiles between testicular and ejaculated spermatozoa are provided as RNA-based marker panels for the distinct stages of sperm maturity in humans.

What is known already: Although the testicular spermatozoa of azoospermic men are successfully used for in vitro fertilization and newborns were produced by microinjection of testicular spermatozoa into oocytes, there is not much data available on human testicular spermatozoa, including their potentially different gene expression profile in comparison to mature, ejaculated spermatozoa. The fact that in all previous studies only transcriptomics of the whole human testicular tissue or ejaculate, consisting of different cell types, have been dissected, may represent a bias.

Study design, size, duration: In this study, we selected 100 single human testicular or ejaculated spermatozoa per sample using the micromanipulation system and analyzed them on gene expression profiles by microarrays. At least samples from 3 different patients per group were used for the in situ hybridizations.

Participants/materials, setting, methods: For the microarray samples from 3 different patients per group were provided and for the in situ hybridizations at least samples from three different patients per group were used.

Main results and the role of chance: The microarrays revealed different expression profiles related to different cellular components with various molecular and biological ontological functions. While molecular functions of testicular spermatozoa are locally restricted to cellular components of the nucleus and intracellular membrane-bound organelles, the molecular features of ejaculated spermatozoa are more limited to cytosolic large ribosomal subunits related to the synthesis of proteins. Some differentially expressed transcripts in testicular spermatozoa were closely connected to spermatogenesis, germinal lineage, transcription regulation, epigenetics, DNA repair and chromatin, development and differentiation. We further performed the comparison of testicular spermatozoa in situ (in testicular tubules) and ejaculated spermatozoa in humans by in situ hybridization (ISH) for the genes CRISP2, RCOR3, DDX57, and RAD23B.

Furthermore, we compared by ISH the expression of these proteins/genes in non-malignant ("healthy") tissue vs. malignant tissue (testicular cancer), and comparison of different cancerous tissues (testicular cancer): seminoma, non-seminoma, teratoma.

Limitations, reasons for caution: Technical limitations of microarray and in situ hybridization.

Wider implications of the findings: The results of this study might provide exciting varieties in gene expression profiles between testicular and ejaculated spermatozoa, which might serve as RNA-based marker panels for the distinct stages of sperm maturity in humans.

Trial registration number: none

O-073 Microarray analysis in human testis reveal the critical roles of long non-coding RNAs in non-obstructive azoospermia via immune signaling pathway.

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Study question: The function of long non-coding RNAs (lncRNA) in the underlying etiology of non-obstructive azoospermia (NOA) which are not systematically understood.

Summary answer: The significanty altered lncRNAs may lead to impaired spermatogenesis, paving the way for deciphering the pathogenesis of NOA.

What is known already: Spermatogenesis is an intricate yet highly sequential process, which involves three distinct phases: mitotic proliferation of spermatogonia, meiotic divisions of spermatocyte, and spermiogenesis. Damage to any link in the process may cause spermatogenic failure. NOA is one of the most severe male-related reproductive disorders, which results from spermatogenic failure, affecting approximately 10% of infertile men in the world. LncRNAs engaged in numerous important biological process. There is abundant evidence that lncRNAs were found to present highly specific expression in the testis by genome-wide transcriptome analyses. Furthermore, increasing evidence indicates that lncRNAs play pivotal roles in spermatogenesis.

Study design, size, duration: The study analyzed the expression profile of lncRNAs and mRNAs in NOA. Four pairs of NOA patients and obstructive azoospermia (OA) with normal spermatogenesis (controls) were screened by microarray. In details, adult human testis specimens were obtained from NOA and OA patients, voluntarily recruited from the Reproductive Medicine Center of Nanfang Hospital, Southern Medical University, from 2017 to 2018. Furthermore, all subjects were selected on the basis of comprehensive andrological testing.

Participants/materials, setting, methods:

1 Hematoxylin and Eosin Staining(H&E)

2 RNA extraction and qualification3 Microarray analysis

4 Quantitative real-time PCR (qPCR) validation

5 Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

6 LncRNA and mRNA co-expression network

7 Statistical analysis

Main results and the role of chance: Our data showed that 2108 lncRNAs and 2021 mRNAs that were differentially expressed (fold change ≥2.0-, p value < 0.01) among NOA compared with controls. Several GO terms including spermatogenesis, male gamete generation, cell cycle, were enriched in gene lists, suggesting a potential correlation with NOA. Pathway analysis demonstrated that apoptosis pathway, lysosome and phagosome pathway play important roles in spermatogenesis. Through co-expression analysis, differently expressed transcripts were divided into unique modules, and lncRNA were functionally annotated. Furthermore, We analyzed all the differentially expressed lncRNAs and constructed three separate networks to show the connections of lncRNAs with GO annotations. The core biological process GO terms of each separate system were:GO:0000003, reproduction;GO:0007049, cell cycle; GO:0002376, immune system process. Referring to the results of GO annotations for differentially expressed mRNAs, the core GO terms for lncRNAs correctly reflect the biological process of NOA. Finally, three candidate lncRNAs were verified by qPCR and indentified for further study.

Limitations, reasons for caution:

1 The biological functions of the candidate lncRNAs need to be further validated by additional studies.

2 For the limit of ethics, testis samples from OA patients with normal spermatogenesis were used as controls, which could lead to minor variations as expected.

Wider implications of the findings: Our findings paved the way for elucidating the pathogenesis of NOA with unknown etiologies. Furthermore,the study provided clues for further diagnosis and investigation of male-related reproductive disorders.

Trial registration number: not applicable

O-074 In vitro Induction of different stages of spermatogenesis from immature mice by colony stimulating factor-1 using methylcellulose as a 3-dimension culture system

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Study question: What is the effect of colony stimulating factor-1 (CSF-1) on the development of mouse spermatogonial cells in 3-dimension (3D) in vitro culture system?

Summary answer: CSF-1 induced the proliferation of spermatogonial cells (VASA positive stained cells) and their differentiation to BOULE and ACROSIN positive stained cells.

What is known already: CSF-1 is a hematopoietic growth factor that induce the development of macrophages from hematopoietic stem cells. It is produces by fibroblasts and by endothelial cells. In the testis it is mainly produced by Leydig cells. The receptor for CSF-1 (CSF-1R) is present on spermatogonial cells. It was shown that CSF-1 induced the proliferation of spermatogonial cells.

Study design, size, duration: Cells were enzymatically isolated from the seminiferous tubules of 7-day-old immature mice. Cells were cultured in methylcellulose (as a 3D in vitro culture system) that contained 10% KSR and GDNF, LIF and EGF with/without CSF-1 (0.1-100 ng/ml). After 4-6 weeks of culture, the developed cells were collected.

Participants/materials, setting, methods: The developed cells were evaluated by immunofluorescence staining using specific antibodies for VASA (a marker for premeiotic cells), BOULE (a marker for meiotic cells) and ACROSIN (a marker for meiotic/postmeiotic cells).

Main results and the role of chance: Isolated cells from seminiferous tubules that grow in methylcellulose that contained KSR and GDNF, LIF, EGF lead to development of colonies after 4-6 weeks (control). These cultures contained cells that expressed VASA, BOULE and ACROSIN positive cells. Addition of CSF-1 (0.1-100 ng/ml) to those cultures significantly increased the percentage of VASA positive cells (from 15% in the control to 25%) ($p<0.001$), in a dose-dependent manner compared to control. Furthermore, addition of CSF-1 to the cultures significantly increased the percentages of BOULE positive cells (from 17% in the control to 28%) ($p<0.001$) and ACROSINE positive cells (from 15% in the control to 27%) ($p<0.001$) compared to control.

Limitations, reasons for caution: The experiments were performed in vitro and need to be confirmed in vivo.

Wider implications of the findings: This is the first study to show a direct effect of CSF-1 on the differentiation of spermatogonial cells in vitro, in addition to its effect on their proliferation. These results may suggest the possible involvement of CSF-1 that is mainly produced by Leydig cells, in the microenvironment of spermatogonial cells.

Trial registration number: NA

O-075 The influence of AZF deletions on sperm retrieval rate (SRR) by TESE and ART results

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Study question: For men with AZFc deletion, sperm can be collected by TESE. Does AZFc deletion affect SRR and clinical outcome of ART?
Summary answer: SRR is significantly higher in the gr/gr and b2/b4 deletion groups. However, in b2/b4, clinical outcome of ART was extremely poor.
What is known already: AZF is used as a predictor of the possibility of sperm retrieval by TESE, and it is said that sperm can be collected for patients with AZFb partial deletion, AZFc deletion, and microdeletion. Since gr/gr deletion is a common deletion for Asians especially Japanese, it does not affect the sperm retrieval rate by TESE. Although there are many papers showing SRR according to the results of AZF deletion, there are few references to the clinical outcome of ART.
Study design, size, duration: From April 2014 to December 2017, a screening test for detecting AZF deletion was performed on 294 patients with azoospermia (AZO) or cryptozoospermia (CS). Among them, we surveyed 191 cases who had TESE. Patients were divided into obstructive azoospermia (OA) and non-occlusive azoospermia (NOA) by testicular size, medical history, serum hormone level, chromosome examination, and AZF test result before TESE.
Participants/materials, setting, methods: The relationships among AZF deletion type, sperm retrieval rate, and ART results-fertilization rate (FR), blastocyst rate (BR), good blastocyst rate (GBR), and pregnancy rate (PR)-were retrospectively studied in OA, NOA and CS.
Main results and the role of chance: Out of 294 cases, 249 with AZO and 45 with CS appeared. 118 cases had AZF deletion (40.1%): 89 gr/gr (30.3%), 12 b2/b4 (4.1%), 4 AZFb+c, 3 AZFa, 3 Ym-3, and 3 Y-chromosome long arm deletion, 2 indeterminate finding, 1 AZFb and 1 Y-chromosome complete deletion. 37 OA (No deletion (ND): 25, gr/gr: 12), 145 NOA (ND: 88, gr/gr: 51, b2/b4: 3, others: 3), and 10 CS (ND: 6, gr/gr:1, b2/b4:3) received TESE. In OA, sperm was retrieved in all cases. FR (ND vs gr/gr: 62.5% vs 62.2%), BR (49.6% vs 39.5%), GBR (26.1% vs 21.9%), and PR (49.0% vs 25.9%) showed no significant difference. In NOA, sperm was retrieved in ND, gr/gr, and b2/b4 (25.0% vs 39.2% vs 66.6%), and b2/b4 tended to be higher than others. No sperm was retrieved in the Ym-3 and indeterminate finding groups. FR (61.0% vs 58.5% vs 23.9%), BR (43.24% vs 44.17% vs 0.0%) and GBR (20.5% vs 20.8% vs 0.0%) were significantly lower in b2/b4. PR (46.2% vs 50.0% vs 0%) showed no significant deference in ND and gr/gr, but no pregnancy was achieved in b2/b4. In CS, FR of gr/gr was comparatively high at 53.1%, but GBR (11.8%) and FR (22.2%) tended to be lower.
Limitations, reasons for caution: In the CS group, the AZF test was performed because it was diagnosed as AZO in the first semen examination. In b2/b4, the number of cases is small, and statistical significance cannot be obtained in this study.
Wider implications of the findings: In b2/b4 of CS, FR was lower than ND but other factors saw no difference. In b2/b4 of NOA, although SRR was high, FR and embryogenesis rate were extremely low. It is suggested that the AZF test can predict not only SSR but also clinical outcome of ART.
Trial registration number: none

SELECTED ORAL COMMUNICATIONS
SESSION 21: INSIGHTS ON EMBRYO SELECTION AND PGT/IVF OUTCOMES
Monday 24 June 2019 Haydn 2 15:15–16:30

O-076 Selection of single euploid blastocysts for transfer with high resolution next-generation sequencing significantly improves live birth outcome for IVF patients: a prospective randomized study

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Study question: Is selection of single euploid blastocysts for transfer with high resolution NGS (hrNGS) an efficient mean to improve live birth outcome for young IVF patients with good prognosis?
Summary answer: NGS has demonstrated an efficient and robust high-throughput technology for selecting single euploid blastocysts for transfer in patients with good prognosis to reduce multiple pregnancies.
What is known already: Early randomized clinical trials of preimplantation genetic testing for aneuploidy (PGT-A) with FISH detected a limited numbers of chromosomes and produced disappointing pregnancy and live birth outcomes. Recent studies on PGT-A with aCGH, SNP array and qPCR testing of 24 chromosomes have resulted in a significant increase in ongoing pregnancy and implantation rates for IVF patients. More recent advancement in high resolution next-generation sequencing has provided new method for PGT-A in IVF treatment. However, there is still limited information about the efficiency of hrNGS in selecting single blastocysts for transfer for IVF patients with good prognosis in a randomized study.
Study design, size, duration: A total of 267 IVF patients at mean age 32.4 ± 3.5 years who met the inclusion criteria (≤37 years old, presence of both ovaries and with normal uterine lining) were enrolled in this prospective, single-blind interventional study in our multiple IVF clinics from June 2015 to June 2017. The enrolled patients with good prognosis requested single embryo transfer (SET) and singed consents for selecting single blastocysts for transfer with hrNGS to reduce multiple pregnancies.
Participants/materials, setting, methods: The enrolled patients were randomized into either hrNGS plus morphology assessment (Group A, n=134) or morphological assessment alone (Group B, n=133). For both groups, all embryos were cultured to blastocyst stage and were vitrified after biopsy on day 5 (up to 10pm). Single blastocysts were selected for transfer to individual patients based on NGS results primarily in Group A or morphological assessment only in Group B. Live birth rates were compared between the two groups.
Main results and the role of chance: Data analysis revealed that there were no significant differences in female patient's mean age, day 3 FSH, AMH, E_2, antral follicle number between the two groups ($p > 0.05$). The fertilization (2PN) and blastocyst rates were comparable between the two groups ($p > 0.05$). Clinical pregnancy rate was significantly higher in Group A compared to Group B (72.1% vs. 51.9%, respectively, $p < 0.05$). The live birth rate in Group A was also higher than that of Group B (69.8% vs. 46.6%, respectively, $p < 0.05$). There was no significant difference in miscarriage rate between Group A and Group B (2.3% vs. 5.3%, respectively, $p > 0.05$) probably due to the limited numbers in this category. There were no twin or higher-order multiple pregnancies in Group A and Group B. While hrNGS has been recently introduced to IVF treatment, this is the first randomized study showing clinical benefits of hrNGS on selection of single euploid blastocysts for transfer in good prognosis patients in comparison to morphology assessment alone. With the resulting high clinical pregnancy and live birth rates, NGS has demonstrated an efficient and robust high-throughput technology for selection of single euploid blastocysts for transfer in IVF patients with good prognosis to reduce multiple pregnancies.
Limitations, reasons for caution: Although PGT-A with hrNGS brings distinct benefits for many IVF patients seeking single embryo transfer, the approach is not for all patients, especially for those with diminished ovarian reserve and/or poor responders. Further randomized clinical trials with a larger sample are required to confirm its clinical benefits for these patients.
Wider implications of the findings: With the observed high accuracy of 24-chromosome testing and the resulting high live birth rate, hrNGS has demonstrated an efficient and reliable technology for selecting single blastocysts for transfer. With high sensitivity and specificity, hrNGS may be used to detect embryonic aneuploidies, imbalance translocations and mosaicism in routine clinical practice.
Trial registration number: No applicable.

O-077 New evidence on mosaic developmental potential: two hundred mosaic embryos transferred prospectively in a single clinic

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Study question: Can chromosomal constitution of mosaic embryos influence the clinical outcome of in vitro fertilization treatments?
Summary answer: Mosaic monosomy embryos have significant higher chances to result in healthy babies born compared to mosaic trisomy, mosaic segmental and mosaic complex aneuploidy embryos.
What is known already: Chromosomal mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. In a recent published study, we have demonstrated that mosaic embryos hold the potential to implant and result in the birth of healthy babies. Therefore, the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF) resulting in only mosaic embryos but no euploid embryos for transfer. However, there is still few data concerning the impact of mosaicism on viability, and the classification of such embryos in relation with their reproductive potential is unclear.
Study design, size, duration: The transfer of mosaic embryos was offered to 200 women for whom IVF had resulted in no euploid embryos between May 2016-May 2018. All embryos were cultured to blastocyst stage; trophectoderm (TE) biopsy was performed on Day-5 of development or Day6/7 for slow growing embryos. The clinical outcome obtained after transfer of mosaic embryos with different chromosomal constitution was compared with each other and with that obtained from a control group of 500 euploid blastocysts.
Participants/materials, setting, methods: Comprehensive chromosome screening PGT-A was performed using high resolution next generation sequencing (NGS) methodology. TE biopsies were classified as mosaic if they had 20%-80% abnormal cells. For statistical analysis mosaic embryos were divided in four groups based on chromosomal constitution: mosaic monosomy (single and double monosomies; MM), mosaic trisomy (single and double trisomies, MT), mosaic complex aneuploidy (more than two different aneuploidies; MC) and mosaic segmental aneuploidy (single and double deletion or insertion >5Mb, MS).
Main results and the role of chance: MM showed significant higher birth rate compared to MT (46% vs 24% p=0.02), MC (46% vs 23% p=0.03) and MS (46% vs 22% p=0.02). No significant difference was observed in clinical outcome between the groups MT, MC and MS. A comparison of the clinical outcomes with control euploid group showed no significant difference between euploid control and MM, while a significant low implantation rate (55.4% vs 37% MT, vs 31% MC, vs 23% MS, $p<0.05$) and live birth rate (48.4% vs 24% MT, 23% MC, vs 22% MS, $p<0.05$) between the euploid control and rest of mosaic groups was observed (Table). The highest biochemical pregnancy rate (31%) and early abortion rate (13%) was observed in MS, and MT, respectively.
Limitations, reasons for caution: Additional clinical data must be obtained to evaluate the contribution of different chromosomal constitution before this approach can be evaluated as an additional tool to choose mosaic embryos for transfer.
Wider implications of the findings: The study demonstrated that embryos with MT, MS and MC aneuploidies have lower chances of resulting with birth of healthy babies compared to embryos with MM. More interestingly, no difference has been find in clinical results between MM and euploid control group. These findings should be considered for genetic counseling.
Trial registration number: not applicable.

O-078 Preimplantation genetic testing is not associated with adverse perinatal and obstetric outcomes in singletons

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Study question: Does the embryo biopsy for preimplantation genetic testing (PGT) is associated with adverse obstetric and perinatal outcomes IVF/ICSI cycles singletons?
Summary answer: The PGT in blastocyst stage in GnRH antagonist protocol ICSI cycles is not associated with increased risk of adverse obstetric and perinatal outcomes.
What is known already: Pregnancies arisen from IVF/ICSI treatments are associated with a higher risk of adverse perinatal and obstetric outcomes when compared to naturally conceived. It has been suggested that the manipulation and culture of gametes and embryos would be one of the factors leading to increase risks of adverse outcomes. PGT during IVF/ICSI treatments is increasing worldwide due to advanced maternal age and for embryo selection that may improve live birth per embryo transfer. However, there are few data available evaluating if embryo manipulation associated with PGT cycles may add risk to obstetric and perinatal complications when compared to cycles without PGT.
Study design, size, duration: This is a cohort study evaluating singletons born following ICSI cycles with and without PGT in a private center between 2011 and 2015. Data were collected from 996 newborns following fresh (n= 490 singletons) and frozen-thawed (FET) (n=506). All the embryo transfers and biopsies were performed in the blastocyst stage. The cycles were performed in GnRH antagonist protocols and the FET following a hormonal replacement endometrium priming.
Participants/materials, setting, methods: Outcomes measure were: low birthweight (LBW), small-for-gestational-age (SGA), large-for-gestational-age (LGA), preterm birth, congenital anomalies, preeclampsia, gestational diabetes, and perinatal mortality. Multiple logistic regression analysis was performed to evaluate the association of the number of the oocytes retrieved with the outcomes mentioned above. Adjusted odds ratio (aOR) was obtained for each outcome after adjusting for confounding factors such as female age and BMI, infertility cause, number of oocytes retrieved.
Main results and the role of chance: The singletons from a fresh embryo transfer with PGT (n=113) vs. no-PGT (n=377) presented no differences in the obstetric and perinatal outcomes. After fresh embryo transfer, the singletons mean birthweight was 2971.97±501.16 and 3016.41±546.69 grams (P=0.4382), in PGT and no-PGT cycles, respectively. The mean gestational age was 37.55±1.95 and 37.79±2.15 weeks (P=0.2848) following PGT and no-PGT cycles, respectively. There were no statistical differences in the aOR of LBW, SGA, LGA, preterm birth, congenital anomalies, preeclampsia, gestational diabetes, and perinatal mortality when comparing PGT to no-PGT cycles with fresh embryo transfers. In singletons following FET with (n=127) and without (n=379) PGT, no differences were found. The singletons mean birthweight was 3109.26±528.44 and 3080.23±542.67 grams (P=0.4801) in PGT and no-PGT cycles, respectively. The mean gestational age was 38.37±2.13 and 37.98±2.27 weeks (P=0.0807) following PGT and no-PGT cycles, respectively. The aOR of all the other outcomes were not statistical different in FET cycles with and without PGT.
Limitations, reasons for caution: As this is a cohort study, although the outcomes were adjusted for confounding factors, some unknown confounders may affect the outcomes.
Wider implications of the findings: For the first time, this study compared the obstetric and perinatal outcomes among singletons with and without PGT after blastocyst embryo transfer during a GnRH antagonist protocol in fresh and FET cycle with hormonal replacement therapy for endometrial priming.
Trial registration number: Not applicable.

		Mosaic			
	Euploid	MM	MT	MC	MS
No. Of embryos	500	72	38	62	32
No. of transfer	500	71	38	62	32
No. of positive bhCG	325 (65%)§	41 (58%)	18 (47%)	26 (11%)	7 (54%)
No. of Biochemical pregnancies	48 (9.6%)	6 (8%)	4 (11%)	7 (11%)	4 (31%)
No. of embryos implanted	277 (55.4%)§	35 (49%)	14 (37%)	19 (31%)	3 (23%)
No. of Early abortion	35 (7%)	3 (4%)	5 (13%)	4 (6%)	1 (3%)
No. of Babies Born	242 (48.4%)§	33 (46%)*	9 (24%)	14 (23%)	7 (22%)

MM=single/double monosomies;MT=single/double trisomies;MC=more than two different aneuploidies;MS=single/double deletion or insertion.

*p<0.05MM vs MT,MC,MS
p<0.05Euploid vs MT,MC,MS

O-079 Predictors of poor prognosis in preimplantation genetic testing for monogenic disorders (PGT-M)

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Study question: How many oocytes are needed for a single PGT-M treatment cycle to achieve a sufficient cumulative live birth rate (CLBR).
Summary answer: When assessing both age and number of oocytes together the predicted CLBR remained >10% whenever female age was ≤40 and the number of oocytes ≥7.
What is known already: The number of oocytes is an important contributory factor in the prognosis of PGT in order to obtain a sufficient number of embryos. PGT treatment is more likely to bear the negative effects of suboptimal ovarian stimulation, fertilisation and embryo culture than conventional IVF. A part of the obtained embryos will be affected by the disease tested, a part of the embryos will fail to have a genetic diagnosis; these embryos may not be used for embryo transfer. The loss of embryos available for embryo transfer as a result of PGT varies between 25-81% according to the indication for PGT.
Study design, size, duration: This was a single centre retrospective comparative cohort study. The principal outcome measure were predictors of poor CLBR (predicted CLBR<10%) in patients undergoing PGT-M. The secondary outcome measure was to compare these predictors of poor prognosis to non-PGT women undergoing ART. 2265 PGT-M treatment cycles were compared to 2833 conventional ICSI cycles from January 2011 to December 2015. Treatment without known live birth outcome were considered negative. IVM and donor treatment cycles were excluded.
Participants/materials, setting, methods: Multivariable regression analysis was applied to account for the following potential confounding factors: female age, rank, oocytes, embryos analysed and dose of ovarian stimulation. Post-hoc power calculations (alpha of 0.05, power of 0.80) indicated that our study was powered adequately to demonstrate significant difference of 3.8% in CLBR per retrieval.
Main results and the role of chance: Main demographic characteristics (female age, cycle rank) and ICSI treatment characteristics (total gonadotrophins, oocytes retrieved, embryos available for PGT-M, blastocyst development) were not significantly different between both groups. In the PGT group the CLBR of 10% was surpassed from 2 oocytes retrieved or 1 embryo available per single treatment cycle. Both the crude and predicted CLBR reached a maximum at the age of 27 and decreased thereafter with increasing age. Relative to the number of oocytes in the PGT group the CLBR reached 30% at 11 oocytes and increased to a maximum of 47%. When assessing both age and number of oocytes retrieved together, the predicted CLBR remained >10% whenever female age was ≤40 and the number of oocytes ≥7. Neither the crude nor calculated cumulative live birth delivery rate changed substantially with increasing rank of treatment. CLBRs following PGT were significantly lower than without PGT (29.4% vs 35.0%, respectively; p<0.001) even following confounder adjustment. Both in the PGT and the non PGT group the CLBR increased with increasing numbers of oocytes retrieved, leveling off in both groups from 14 oocytes. The CLBR leveled off beyond 4 PGT useable embryos and beyond 6 useable embryos in the non PGT group.
Limitations, reasons for caution: Despite the large sample size, the findings are confined by limited confounder adjustment and lack of specific PGT comparators.
Wider implications of the findings: In a PGT-M programme patients need to be informed on the limited prognosis if ovarian response is low. Nevertheless, two or more oocytes retrieved per single treatment cycle offer a mean CLBR of over 10%. The results diagram produced from this study is a useful tool in counseling PGT patients.
Trial registration number: not applicable

O-080 miRNAs as novel biomarkers of mucous membrane permeability associated to infertility

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Study question: Is there any biochemical parameter that allows us to identify microbiota dysbiosis related to immunological disbalance in women with unexplained infertility and implantation failure?
Summary answer: Aberrant expression of miRNAs regulating macrophages and mucosal tight junctions could be associated to microbiota disbiosis, increased mucous permeability, recurrent candidiasis and autoimmunity.
What is known already: Autoimmunity is associated to an increase in intestinal permeability as well as reproductive failure. Mucous membranes (gut, endometrium, reproductive tract) contain commensal bacteria that modulate physiological host functions. Dysbiosis of commensal bacteria triggers dysfunction of the intestinal epithelial barrier, leading to the induction or aggravation of intestinal inflammation. In addition increased tight junction (TJ) barrier permeability may lead to the defects in TJ barrier and subsequent development of inflammation. Recent evidence suggests that different miRNAs are implicated in inflammatory diseases. However, the physiological role of miRNAs in infertility remains elusive.
Study design, size, duration: Diagnostic test study. Analytical, prospective, experimental. microARN transcriptional vaginal test were performed in 17 fertile women and 21 unexplained primary infertility (UI) patients between March and December 2018. We investigated whether the single or combined expression of miR21, miR155, miR193b, and miR141 was able to identify infertile patients with increased risk of mucosal immune system deficiency, increased intestinal permeability, recurrent candidiasis and autoimmunity.
Participants/materials, setting, methods: Vaginal swab and peripheral blood samples were collected from 38 women between the ages of 25 and 39. MiRNAs (miR21, miR155, miR193b, miR141) were determined with specific TaqMan probes by real time PCR. Quantification of thyroid antibodies, gastrointestinal autoantibodies, antiphospholipid syndrome and anti-nuclear antibodies where determined together with insulin and glucose blood levels by standard protocols.
Main results and the role of chance: MiR-21 which is associated to tight junctions disruption, was up regulated (*p=0.0030) in infertile patients showing altered integrity and functionality of mucous membranes. Moreover, miR-155, which is strongly associated with inflammation, positively correlate with miR21 level in those patients (*p<0,0001). Receiver-operator characteristic (ROC) curve analyses suggest that these serum miRNAs may be useful markers for discriminating patients with gastrointestinal autoimmunity and infertility. ROC curve areas for miR-21, and miR-155 were 0.844 (95% CI: 0.773–0.947; *p<0.001), and 0.838 (95% CI: 0.765–0.944, *p<0.001), respectively.

According to the selected cutoff values , sensitivity and specificity were 81% and 92%, and 81% and 83%, respectively.
Limitations, reasons for caution: Sample size should be increased and the analysis of local microbiota by NGS which is ongoing at this moment should be include in order to correlate with miRNAs biomarkers.
Wider implications of the findings: miRNAs should be considered as new innovative not invasive diagnostic test in UI. Improving knowledge in maternal microbiome could inform future research about modifications to the individual's microbiome as a potential strategy to prevent adverse outcomes and to develop a healthy microbiome in the mother and their offspring.
Trial registration number: Not apply.

SELECTED ORAL COMMUNICATIONS
SESSION 22: PREVENTING INFERTILITY: WHAT WORKS?
Monday 24 June 2019 Haydn 4 15:15–16:30

O-081 Too little, too late: A Systematic Review of Clinical Practice Guidelines Informing Counselling on Female Age-Related Fertility Decline

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Study question: What are the high-quality clinical practice guidelines (CPGs) informing patient counselling on female age-related fertility decline, and what is their content?
Summary answer: Few high-quality guidelines address counselling on female age-related fertility decline, and existing guidance is both inconsistent and incomplete.
What is known already: Natural fertility declines with increasing female age, with fecundity significantly diminished by age 40; however, women often underestimate the effects of age-related fertility decline. As women delay childbearing, this misunderstanding may lead to unintended childlessness. Providers do not routinely counsel patients on the effects of reproductive aging and may lack accurate knowledge of female age-related fertility decline and how to counsel. Healthcare providers rely on guidelines to inform care, and poor quality or insufficient recommendations may contribute to inconsistent counselling and a knowledge gap among healthcare professionals.
Study design, size, duration: A systematic review was performed of professional organization guidelines published in English between 2006 and 2018 addressing patient counselling on age-specific content relating to preconception, fertility decline, or reproductive life planning. Guidelines were assessed for quality and bias using the AGREE II guideline appraisal instrument and were considered high-quality if $\geq$3 domains had a composite score $\geq$60%.
Participants/materials, setting, methods: We identified 2036 records through a systematic database search and 872 records through a hand-search of professional organizations. We identified 18 of those records as guidelines addressing age-related fertility decline counselling. The content of each guideline was evaluated and compared.
Main results and the role of chance: Of the 18 guidelines identified, 6 focused on reproductive aging, and 12 covered related topics of contraception, preconception, infertility, and general women's healthcare. Only 11 (61%) met our criteria for high-quality, and all of these high-quality CPGs scored >60% in the AGREE II domain assessing rigor of development. Among the 11 high-quality guidelines, 9/11 targeted primary providers. Though 10/11 explicitly stated an age when female fertility declines, that age varied across guidelines from 30-"late 30s." Only one guideline recommended an age at which patients should be counselled, and 4/11 loosely advised counselling prior to onset of fertility decline. Nearly half (5/11) of the high-quality guidelines did not address the risks of advanced maternal age.
Limitations, reasons for caution: AGREE II does not endorse a specific threshold for considering a guideline high-quality, thus our criteria for high-quality guidelines is not validated. Inconsistent and limited guidance could be related to a lack of clear evidence regarding the benefits of counselling on patient knowledge and reproductive decision-making.
Wider implications of the findings: Recommendations vary regarding who and when to counsel about age-related fertility decline, and guidelines present conflicting ages at which fertility is compromised. Weak and inconsistent guidelines may contribute to insufficient counselling, leading to inconsistent care. Improvement in CPGs, including incorporation of age-specific recommendations, may lead to improved patient anticipatory guidance.
Trial registration number: N/A

O-082 Concerns on future fertility among users and non-users of combined oral contraceptives

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Study question: To what extent are current users and non-users of combined oral contraceptives (COC) concerned about effects of COC usage on future fertility?
Summary answer: Overall, 28% of COC-users vs. 19% among non-users believed COC could impair ovarian reserve, and COC-users expressed considerable concerns about impact on future fertility.
What is known already: The COC-pill is one of the most commonly used contraceptive methods in the Western world; approx. 45% of Danish women use COC during their fertile life. To our knowledge no previous studies have explored the thoughts and concerns on future fertility among COC-users. Due to the frequent use of COC and increasing use of fertility assessment and counselling it is important to uncover concerns, well-founded or not, to optimize fertility counselling. Therefore, we conducted this large-scale study to investigate the extent of thoughts and myths about fertility among women of reproductive age, including 1332 current COC users.
Study design, size, duration: The study was performed as a population-based on-line questionnaire during the period from 2016 to 2018. Eligible women were between 18 to 41 years either with or without a current use of COC. Non-users (prior users and never users) constituted a comparison group. In total, 2534 women completed the electronic questionnaire. The analyses comprised 1332 current users of COC and 1202 non-users.
Participants/materials, setting, methods: Respondents (users and non-users) were recruited either from the Fertility Assessment and Counselling (FAC) Clinics in Copenhagen, Denmark (Hvidman et al. 2015), (n=1,075) or via an internet-based survey addressing women with interest in the FAC-Clinics webpages (n=1,410). The respondents were asked about contraceptive history and thoughts/concerns on various aspects on future fertility. Data were analyzed using age-adjusted logistic regression analyses to identify potential differences between current COC-users and non-users (non-users being the reference group).
Main results and the role of chance: Mean age of COC-users were 24.8 years vs. 30.8 among non-users ($p<0.0001$). Due to the age-difference all analyses were age-adjusted. The duration of COC-use spanned from 1-20 years among COC-users; 52% had used COC between 6-10 years. A total of 65.6% of COC-users reported having thoughts about whether COC-use could affect future fertility versus 52.1 among non-users (OR=1.6, 95%-CI 1.3-1.9). Overall, 27.9% of COC-users vs. 19% among non-users believed COC could impair the ovarian reserve (OR=1.2, 95%-CI 0.96-1.52). Oppositely, 14.1 % of COC-users and 10.5% in the non-COC group thought that lack of ovulation could "save" the eggs (OR=0.9, 95% CI 0.7-1.3). Nearly half of both groups thought that COC-use could make it more difficult to become pregnant after discontinuation (45.5% among COC-users, 53.3% among non-users, OR=0.7 (0.6-0.8). Two thirds of both groups were concerned it could be harmful to disturb the natural hormonal balance. Primary reasons for non-use were concerns on disturbance of the natural hormone-system (36.2%), and concerns on future fertility (26.4%). In conclusion, the results clearly show a

need for information on risks as well as "non-risks" when prescribing COC pills. The large sample size means that it is unlikely that the results is caused by chance.
Limitations, reasons for caution: There is a risk for recall bias regarding contraceptive and reproductive history. It cannot be excluded that questions on potential harmful effects of COC use could induce these concerns. However, comments from respondents suggest that many had pre-existing concerns.
Wider implications of the findings: Health care professionals prescribing contraception must be aware of concerns and myths on future fertility from COC usage to perform better counselling of these women, especially since this is one of the most widely used contraceptive methods in the Western world.
Trial registration number: Not applicable.

O-083 Does watching an educational video increase Fertility Awareness (FA)? Results from a randomised controlled trial with partnered people desiring to become parents

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[5]University of Porto, São João Hospital, Porto, Portugal
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Study question: What are the short and long-term effects of a video aiming to increase fertility awareness (FA) among partnered people desiring to become parents?
Summary answer: The video intervention increased FA levels 1-month later and the effects were maintained 1-year later for almost all variables.
What is known already: FA interventions have been shown to be effective on increasing FA in short-term. However, these studies had been using heterogeneous samples regardless of their age, marital status, parity, and desire to have children. Less is known about the long-term effectiveness of these educational interventions on childless and presumably fertile people who are already committed in a heterosexual relationship and who express the desire to have children in a near future.
Study design, size, duration: A double-blind randomised controlled trial was initiated in October of 2016. Participants were randomly allocated into the intervention group (IG) and control group (CG) and answered to an online questionnaire before(T0), 1-month(T1), 6-months(T2) and 1-year(T3) after the intervention. Seven questions assessed FA about infertility definition, risk factors, chance of pregnancy if the woman is 25-30, 35-40 years old and the chance of pregnancy with fertility treatment if the woman is 35, 40, 45 years old.
Participants/materials, setting, methods: 369 participants (243 women) were invited in gynaecology/fertility clinics, religious pre-marital courses and through social media. IG participants (n=189) were exposed to a 5-min video delivering information on age-related fertility decline, infertility risk factors and pregnancy chances according to women's age and conception mode after answered the T0 questionnaire; participants in CG (n=180) received no stimulus. Mixed Anovas using conservative F-tests were performed to test the interaction between time and group on FA variables.
Main results and the role of chance: Participants were aged 29 years old and were partnered for 6 years, on average. Attrition analyses didn't reveal significant differences between those who had answered and those lost to follow-up in T1 (n=217) regarding age, gender, relationship length, education, reproductive status (being trying to conceive or not) and belonging to IG or CG. Participants at T2 (n=111) and T3 (n=102) were older and T3 participants desired to have children in a shorter time. Significant interaction between group and time were found for infertility definition, lifestyle risk factors, chance of spontaneous pregnancy (if the woman is 25-30 and 35-40 years old) and treatment pregnancy if the woman is 35 and 40 years old ($p<.005$), demonstrating an increase in six of the seven FA variables in IG participants from T0 to T1. When looking at follow-ups times (T2,T3), significant interaction effects between group and time were found for the chance of spontaneous pregnancy if the woman is 25-30 and 35-40 years old and treatment pregnancy if the woman is 35 and 40 years old ($p<.005$). No significant interaction effects were found for intentions to adopt fertility-optimizing behaviours, intended time to start trying to conceive and desired age of first and last child.
Limitations, reasons for caution: Although we had randomly allocated people to CG and IG, the high attrition rate may limit the generalization of our results. Future studies should include larger samples to account for this. Some bias might have occurred due to high education level and volunteer participation since younger participants withdrawer more often.
Wider implications of the findings: This video might be a cost-effective and easy to access tool to raise FA among reproductive-age people in short and long-term. Information regarding fertility should be more easily spread (using videos, text messages, online tools) and presented in different contexts (health services, personalized appointments) in order to improve FA levels.
Trial registration number: NCT02813993

O-084 Women's perceptions of fertility assessment and counselling six years after attending the Fertility Assessment and Counselling clinic

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Study question: What are women's perceptions of fertility assessment and counselling six years after attending the Fertility Assessment and Counselling (FAC) clinic?
Summary answer: Personalized fertility knowledge and advice were viewed as important aids to decision-making but the findings emphasize the need for guidance related to desired family size.
What is known already: Many young people wish to become parents in the future and desire an average of two children. There is a need for fertility awareness in women and men to increase fertility knowledge and support informed fertility decision-making to achieve parenthood and family size goals. The FAC clinic in Copenhagen, Denmark is a personalized fertility awareness intervention based on clinical examination and evaluation of individual risk factors (Hvidman et al., 2015). It opened at the end of 2011. Available qualitative research showed that attending the FAC clinic increased fertility awareness and served as a catalyst for change in women one-year post-consultation.
Study design, size, duration: A six-year follow-up qualitative study of 24 women who attended the FAC clinic between January and June 2012. All women were interviewed during a two-month period from February to March 2018 at Rigshospitalet, Copenhagen, Denmark. Interviews were held in English and ranged between 60 and 94 minutes (average 73 minutes).
Participants/materials, setting, methods: Invitations to participate in an interview-based follow-up study were sent to 120 women who attended the FAC clinic in 2012. In total 95 women read the invitation, 35 confirmed interest in participating and 16 declined to participate. Twenty-five interviews were booked and 24 interviews held. Interviews followed a semi-structured format regarding reasons for attending the FAC clinic, if/how their needs were met, and perceptions of fertility assessment and counselling. Data was analyzed using thematic analysis.
Main results and the role of chance: At the present interview, women were on average 38 years old. In total, 10 were currently single or dating and 14 were married/common-law. All were childless when they attended the FAC clinic. At the interview, 21 women were parents (14 women with 1 child; 6 with 2; 1 with 3) and 3 women intended to have children in the future. The most common reason for originally attending the FAC clinic was to determine how long they could delay childbearing. The majority of women now believed their needs had been met by attending the FAC clinic. Those who were

dissatisfied cited a desire for concrete information as to their remaining years of fertility, although acknowledged that this was likely not realistic. Women stated that they had felt reassured as to their fertility status after attending the FAC clinic whilst receiving the message that they could not delay childbearing "too long". Women viewed personalized fertility knowledge as an important aid to decision-making but cautioned about developing a false sense of security based on the results. Although many had achieved their goal of becoming a parent, several had not achieved their family size goal due to starting their family at an advanced age.

Limitations, reasons for caution: Participants were self-selected and interviews were conducted in English, which could have introduced a selection bias. Importantly, the study group included a broad spectrum of women who achieved parenthood through different means (heterosexual/lesbian relationship, single parent with donor, co-parent) with various family sizes, and women who were currently childless.

Wider implications of the findings: The findings suggest that women who attended the FAC clinic are generally satisfied with their experience and achieved their goal of becoming a parent. Information regarding timing of parenthood in terms of desired number of children should be emphasized in future consultations to support achievement of family-size goals.

Trial registration number: N/A.

O-085 Women's decision-making process about the use of fertility preservation (FP) to prevent age-related fertility decline: a two-year prospective study

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[3]Cardiff University, Department of Psychology, Cardiff, United Kingdom

Study question: How does decision-making about the use of FP in childless women aged between 28 and 35 years unfold over a two-year period?

Summary answer: Few women show evidence of progressing in their FP decision-making process with only 14% reaching a decision; all of which deciding not to use FP.

What is known already: During the last decade (2010-16), women's use of egg freezing increased by 460%. The optimal age-range to freeze oocytes is between 28 and 35 years, but around 30% of the women do it at 38 years or later, therefore compromising their reproductive goals. Additionally, although a considerable number of women consider its use (31.5-34.5%) only a minority (4%) actually use it, and evidence shows that this decision-making process takes on average two years. Thereby, it is critical to understand how this FP decision-making process changes over time, in order to better advise women about if and when to use FP.

Study design, size, duration: A theoretically informed online survey was conducted in 2014, to investigate the factors associated with women's intentions to use FP (Health Belief Model, HBM) and their stage of decision-making (Transtheoretical Model, TTM). The present study is the two-year follow-up to investigate how women's intentions and stage of decision-making changed over time. 257 women participated in the initial survey, among which 214 consented to be contacted for future research.

Participants/materials, setting, methods: The original inclusion criteria were being childless, aged 28-35 years, having a child-wish and not being/trying to get pregnant. The questionnaire used in the follow-up study assessed sociodemographic factors, parenthood goals (intention and desire to have a child, number of children wanted), fertility knowledge; HBM variables (perceived susceptibility, severity, barriers and benefits); FP intentions (Likert-scale from 1=*strongly disagree* to 7=*strongly agree*); FP decisional stage and steps regarding behavioural engagement (according to the TTM); and decision.

Main results and the role of chance: 115 women completed the questionnaire (53.74% response rate). Women were on average 32 years, the majority were living with their partner, were heterosexual, employed, resident in UK and had university education. Since the baseline assessment, 16% (n=17) of women delivered a baby or were pregnant and 7% (n=7) were trying to achieve pregnancy, without FP. Inferential statistical ($p<.05$) showed that women's intention and desire to have a child and number of desired children significantly decreased over time. Compared to baseline, fertility knowledge and perceived susceptibility to infertility significantly increased, perceived severity of infertility significantly decreased and perceived barriers/benefits toward FP did not change. Women's intentions to use FP remained low (around three). Most women (n=81, 77.14%) stayed in the first decisional stage of the TTM (pre-contemplation of whether or not to use FP), 10% (n=10) progressed through the stages, with 14% (n=15) reaching a decision, all of which deciding not to use FP. Around 40% (n=44) of women discussed FP, mostly with friends/partner, 30% (n=32) actively sought information about FP, with google being the most used source, and 3% (n=3) sought specialised advice. Women's baseline intentions to use FP did not significantly predict their decision at follow-up (β=-.12, p=.26).

Limitations, reasons for caution: This was a self-selected sample of childless women with parenting desire who may have been prompted to engage in FP decision-making by being invited to participate. Therefore, FP intentions and progression through FP stages may be overestimated. The sample size might have constrained the power to detect small effect sizes.

Wider implications of the findings: Women at the optimal age-range to freeze oocytes do not actively engage in decision-making about it. This lack of engagement could be related with achieving parenthood goals; downwards revision of desired number of children that could reduce required period of childbearing; and/or less appraisal that infertility is a threat.

Trial registration number: not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 23: ENDOMETRIOSIS AND ENDOMETRIUM: NEW INSIGHTS IN DISEASE MECHANISMS

Monday 24 June 2019 Strauss 1+2 15:15–16:30

O-086 A Genome-wide Association Study of Endometriosis: Data from the UK Biobank

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Study question: *Study Aim* To perform a genome-wide association study in a population of European ancestry to investigate genetic factors contributing to endometriosis and to explore existing endometriosis-associated variants in the literature.

Summary answer: Identified variants implicate genes involving steroidogenesis, ovarian follicular development, cell signalling, maintenance of cell morphology, angiogenesis, regulation of sex hormone levels in development of endometriosis.

What is known already: The risk loci that have been identified from published GWAS so far implicate certain WNT protein signalling, cell adhesion, cell migration, angiogenesis and inflammatory and hormone-metabolism pathways as significant in the pathogenesis of endometriosis.

Study design, size, duration: A genome-wide association study using data from the UK Biobank including 3820 endometriosis cases and 240731 controls. Genotyping was performed using the Applied Biosystems™ UK BiLEVE Axiom™ Array by Affymetrix and the Applied Biosystems™ UK Biobank Axiom™ Array, covering 807,411 and 825,927 markers respectively, in GRCh37 coordinates.

Participants/materials, setting, methods: Data from the UK Biobank including 3820 endometriosis cases and 240731 controls were analysed in the GWAS using genotypes imputed from the 1000 Genomes reference panel and BOLT-LMM v2.3 software. Multivariate logistic regression analysis was performed in 12 top signals identified.

Main results and the role of chance: One novel variant and two previously published endometriosis signals reached genome-wide significance: Chr4:55998379_TAA_T deletion (p=2.90x10-8), rs11031005 (p= 1.7x10-9) and rs61768001(p= 8.1x10-10). A variant rs58415480 was also moderately associated with endometriosis (p= 2.9x10-6). Genes related to these loci include KDR, WNT4, SYNE1, ESR1, and FSHB. Association with previously

published endometriosis SNPs (rs1250241, rs1537377, rs10167914, rs6546324 and rs11674184) was replicated.
Limitations, reasons for caution: Further research to assess the functional impact of these variants is required. The process by which phenotype data was collected in the UK Biobank study for this condition risks reporting bias. Conditional analysis assessing rs1903068 and chr4:55998379 is required to ascertain if this is a truly novel variant.
Wider implications of the findings: Insight into the genomic pathways leading to endometriosis will potentially advance the development of treatments better targeted to processes underlying the condition, and the identification of markers that can be used to facilitate clinical diagnosis of the condition - possibly avoiding resort to surgery and the risks associated with this.
Trial registration number: not applicable.

O-087 Excess germline mutations in four genes in unrelated women with surgical endometriosis

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Study question: To identify any genes with a large burden of mutations in subjects with endometriosis compared to normal population controls.
Summary answer: Four genes (ZNF586, LUZP4, POP4 and UNC5CL) show significant mutational burden associated with endometriosis.
What is known already: Genome-wide association studies in endometriosis have implicated several genomic regions, and whole exome sequencing studies have identified coding variants associated with endometriosis. Although some mutations have segregated with the disease in familial endometriosis, studies to date have not identified genes with significant mutational burden in unrelated patients with endometriosis.
Study design, size, duration: DNA was extracted from blood or saliva collected from 2,596 unrelated women with surgically-confirmed endometriosis using standard methods. Whole Exome Sequencing was performed using Ion Proton platform.
Participants/materials, setting, methods: Variants were called using Torrent Variant Caller (ThermoFisher). We calculated the cumulative genetic burden for each gene by adding the minor allele frequencies for all the rare coding variants identified in cases (frequency <0.01 in the gnomAD database). We used Fisher's Exact Test to determine excess burden in endometriosis subjects compared to the Non-Finnish European cohort (n=64,000) in gnomAD database.
Main results and the role of chance: We identified 4 genes (ZNF586, LUZP4, POP4 and UNC5CL) with significant gene burden in subjects with endometriosis ($p<5 \times 10^{-8}$). One in four women with endometriosis carry a mutation while the population prevalence is only 8%. 141 different coding mutations were observed in these 4 genes; the G518T mutation in the POP4 gene was seen in 2.9% of the endometriosis subjects. None of the protein encoded by these genes have a known role in endometriosis.
Limitations, reasons for caution: Replication of these results in additional cohorts is warranted.
Wider implications of the findings: Discovery of gene mutations underlying endometriosis may lead to new pathophysiology insights, improved diagnostics, and novel treatment approaches.
Trial registration number: N/A

O-088 Do women with endometriosis have an increased risk for immunological diseases? A systematic review of the literature and study of 273,404 women from UK Biobank

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Study question: Are women with endometriosis more susceptible to have immunological diseases, and how robust is the evidence with regards to temporality, confounders, effect mediators and disease ascertainment?
Summary answer: Preliminary analysis results among 273,404 women from UK Biobank suggest that women with endometriosis are more likely to develop certain immunological diseases than those without.
What is known already: Aberrations in the immune system of endometriosis patients have been proposed, preventing clearance of ectopic endometrial cells and allowing for their implantation, survival and maintenance outside the uterus. Also, an association between endometriosis and immunological diseases at population level has been observed by several epidemiological studies, though the robustness of the evidence is uncertain. We investigate the association between endometriosis and 29 immunological diseases identified from UK Biobank including 9 autoinflammatory diseases, 16 classic autoimmune diseases and 4 mixed-pattern immunological diseases.
Study design, size, duration: The UK Biobank includes 273,404 women aged ≥40 years old at recruitment, linked to hospital records, of whom 8,223 are diagnosed with endometriosis and 64,620 with immunological diseases (52,027 autoinflammatory; 14,764 classic autoimmune; 4,379 mixed-pattern diseases).We investigate the association between endometriosis and immunological diseases through: 1) A systematic review of published population-based studies; 2) epidemiological analysis of UK Biobank data using both a nested case-control and a retrospective cohort study designs.
Participants/materials, setting, methods: The systematic review included 26 population-based studies (6 cross-sectional, 14 case-control, 6 cohort); fixed-/random-effect meta-analysis was employed for 5 endometriosis-immunological disease associations. The nested case-control study of UK Biobank data will be stratified by disease ascertainment and ethnicity, and accounting for confounders such as age, lifestyle or hormonal factors using logistic regression modelling. The retrospective cohort analysis will be conducted using Cox proportional hazards regression models of incident immunological diseases in association with endometriosis history.
Main results and the role of chance: The systematic review and meta-analysis suggested endometriosis to be significantly associated with systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and coeliac disease. However, quality of the evidence was generally poor due to study design issues such as self-reported outcomes, small sample size, unrepresentativeness of the control group or whole study population, limited or no adjustment of confounding factors, and reporting no confidence intervals for risk estimates. Preliminary analysis results of the UK Biobank data suggested a significantly increased risk among women with endometriosis of: systemic lupus erythematosus (odds ratio (OR):1.56,95% confidence interval (CI):1.10-2.15;P=0.009), multiple sclerosis (OR:1.31,95%CI:1.00-1.67;P=0.042), inflammatory bowel disease (OR:1.23,95%CI:1.02-1.46;P=0.023), coeliac disease (OR:1.26,95%CI:0.99-1.58;P=0.048), myasthenia gravis (OR:2.19,95%CI:1.12-3.85;P=0.012) and Behcet's syndrome (OR:9.22,95%CI:3.38-21.51;P<0.001). A 9% (OR:1.09, 95%CI:1.03-1.14;P=0.002) significantly increased risk is found for all immunological diseases combined; 6% (OR:1.06,95%CI:1.00-1.12;P=0.037) for autoinflammatory diseases; 15% (OR:1.15,95%CI:1.05-1.26;P=0.003) for classic autoimmune diseases; and 19% (OR:1.19,95%CI:1.01-1.40;P=0.031) for mixed-pattern diseases in association with endometriosis. Further analyses of UK Biobank data to test for the robustness of these associations with adjustment for confounders, stratification on effect modifiers and consideration of temporality are underway and will be presented.

Limitations, reasons for caution: While endometriosis ascertainment is most reliable by hospital diagnosis/surgeries, 3,197(38.88%) out of the 8,223 UK Biobank endometriosis cases were identified by self-reports; 3,982(48.43%) by hospital diagnosis; 1,044(12.70%) by both self-reports and hospital diagnosis. Although sensitivity analysis by diagnostic ascertainment, separate analyses highlight the validity of self-reported diagnosis will be presented.
Wider implications of the findings: The potential association between endometriosis and immunological disease will help to understand the causes and consequences of both disorders to provide reference for clinical and research practice, and opens up the opportunity for novel diagnosis or treatment methods for endometriosis.
Trial registration number: not applicable.

O-089 The Hippo pathway transcription coactivator Yes-associated protein 1 inhibits endometriosis stromal cell invasion through repression of estrogen receptor β

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Study question: The aim of this study was to investigate the regulatory mechanism of YAP1 in terms of ERβ and YAP1 regulation of ESC invasion in endometriosis.
Summary answer: Decreased levels of YAP1 in endometriotic tissues enhance ERβ expression via a YAP1-NuRD complex, which further binds to ESR2 promoters. Furthermore, YAP1 inhibits ESC invasion.
What is known already: Endometriosis is an estrogen-dependent disease, and estrogen receptor β (ERβ) plays a critical role in the pathogenesis of endometriosis by promoting cell invasion. Yes-associated protein 1 (YAP1) plays suppressive roles in several types of tumors. The relationship between YAP1 and ERβ is not fully understood.
Study design, size, duration: In vitro experiment.
Participants/materials, setting, methods: Paired ectopic endometrium (EC) and eutopic endometrium (EU) from patients with endometriosis and primary cultured paired ESCs were used. RT-PCR and western blotting were used to quantify gene expression. SiRNA-targeted knockdown of gene expression and YAP1 overexpression by plasmid transfection were performed. Chromatin immunoprecipitation was used to determine whether YAP1 binds to the ERβ promoter. The association between proteins was assessed by coimmunoprecipitation (Co-IP). Transwell analysis was carried out to observe cell invasion.
Main results and the role of chance: YAP1 mRNA and protein levels in EU tissues were higher than that in paired EC tissues. ESCs transfected with siYAP1 exhibited a significant increase in both ESR2 mRNA levels and ERβ protein expression. Simultaneously, YAP1 overexpression in ESCs exhibited the opposite results. Co-IP assays demonstrated YAP1-NuRD complex formation by YAP1, CHD4 and MTA1 in ESCs. YAP1 bound to two sites (-539, -533 and -158, -152) upstream of the ERβ transcription initiation site. YAP1 binding to the two sites of the ESR2 promoter in ESCs was significantly lower than that in EMs. ESCs transfected with siYAP1 exhibited profoundly increased invasion activity, while ESCs transfected with siERβ showed marked inhibition of invasion. However, transfection with siYAP1 and siERβ together decreased the number of invading cells compared with transfection with siYAP1 alone.
Limitations, reasons for caution: only in vitro.
Wider implications of the findings: new mechanisms in endometriosis
Trial registration number: not applicable.

O-090 A multi-methodological approach defines sub-populations in human endometrial epithelium

A. Maclean[1], J. Bhullar[1], O. Vaseiva[2], A. Valentijn[3], J. Drury[1], N. Tempest[1], C. De Lelis Medeiros-de-morais[4], F. Martin[4], S. Miwa[5], T. Von Zglinicki[5], D. Hapangama[1]

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[5]*Institute for Cell and Molecular Biosciences- Newcastle University, Ageing Research Laboratories- Campus for Ageing and Vitality, Newcastle, United Kingdom*

Study question: Can endometrial epithelial cell sub-populations be characterised using a multi-methodological approach?
Summary answer: Key differences in expression of hormone metabolizing enzymes provides evidence for the existence of endometrial epithelial sub-populations, and novel techniques offer opportunities for further characterisation.
What is known already: The human endometrium has the remarkable capacity to regenerate after menstruation. It is composed of epithelial and stromal cells, which are organised into functionally distinct layers; the luminal epithelium, the superficial functionalis, and deeper basalis. Endometrial epithelial cells (EEC's) are the defining cell type of the endometrium, and EEC's from the three compartments are known to respond differently to the same ovarian hormones, suggesting the existence of sub-types. We have previously shown that SSEA1 isolates basalis EEC's that have regenerative ability. This study aimed to optimise novel methods to contribute to the characterisation of EEC's.
Study design, size, duration: A prospective observational study, analysing full-thickness human endometrial biopsies from 30 healthy women undergoing hysterectomy for benign conditions, including 20 samples from premenopausal women, and 10 from postmenopausal women. Full thickness samples were obtained to enable investigation of the three endometrial compartments: the luminal epithelium, the functionalis, and the basalis. *In silico* study analysing all published whole endometrial microarray datasets (n=16) identified a list of hormone metabolising enzymes (HME's) likely to reflect cyclical hormone responsiveness.
Participants/materials, setting, methods: Transcriptional profiling of purified freshly sorted SSEA1-enriched and depleted EECs from full-thickness endometrium (n=8) identified differentially regulated HMEs. The expression of AKR1C3 and 17βHDS2 was investigated with immunohistochemistry (n=30), and corroborated by RT-qPCR in region specific EEC's isolated by laser capture microdissection (LCM) (n=3). Raman microspectroscopy investigated structural differences between EEC's (n=1). Hyperion^{TM} Imaging was used for the first time in endometrium (n=6) to identify and compare a panel of markers of cellular differentiation.
Main results and the role of chance: Analysis of published microarray data identified 1539 differentially expressed genes in SSEA-1 enriched basalis EEC's, including the HME's AKR1C3, which was upregulated, and 17βHSD2 which was down-regulated. This expression pattern was confirmed both at transcript and protein level in the endometrium. We observed significantly stronger AKR1C3 immunostaining in the basalis than the functionalis ($P<0.05$) and increased mRNA expression in the secretory phase basalis EEC's isolated by LCM. 17βHSD2 immunostaining was stronger in the functionalis than the basalis ($P<0.05$), and mRNA expression was absent in basalis, but present in functionalis. Computational analysis of the obtained Raman spectra identified key structural differences between functionalis and basalis endometrium. MatLab analytical models applied accurately distinguished between the basalis and the functionalis (AUC 98%, sensitivity 0.97, specificity 0.95 and AUC 94%, sensitivity 0.95, specificity 0.97, respectively). Key biomarkers of differentiation were identified by Raman spectra. Mass cytometry using CyTOF technology and Hyperion imaging was optimised for the first time in the human endometrium, providing comprehensive analysis of the expression profile of cell differentiation markers (SSEA1, N-cadherin). This identified significant differences between region specific EEC's.
Limitations, reasons for caution: This is a descriptive study focusing on the optimisation of novel methodology.
Wider implications of the findings: This study has confirmed that the above techniques are feasible adjuncts to improve our understanding of endometrial epithelial cell biology, and identified epithelial cell specific markers at protein level that discern the diversity of epithelial cell sub-populations, which contributes to identification of EEC sub-population specific abnormalities in endometrial disease.
Trial registration number: This work was funded by the Wellbeing of Women project grant RG1073, and Entry-Level Scholarship FTR706.

INVITED SESSION

SESSION 24: ARE IVF CHILDREN DIFFERENT?

Monday 24 June 2019 Haydn 1 17:00–18:00

O-091 Alarming findings when using administrative databases and national registries

O-092 ART children-long term reassurance?

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[1] *Sahlgrenska University Hospital, Department of Obstetrics and Gynaecology, Göteborg, Sweden*

Abstract text

ART children-long term reassurance? IVF is an ever increasing strategy to help childless people to get an own child with now more than 8 million children born worldwide. New and more advanced techniques are steadily developed and brought into clinical practice. Children follow-up studies have so far showed that a majority of children born after ART are healthy even though some adverse outcomes are found in comparison to children born after spontaneous conception. While international registries, such as EIM and ICMART on ART only monitor pregnancy and delivery rates, child morbidity is best caught by large national/international registry studies where possibilities exist to crosslink registries on ART children with other health registries and perform comparison with children born after spontaneous conception. The main risk for adverse perinatal outcomes in ART, which includes ICSI and conventional IVF techniques, are associated with ART's higher rates of multiple pregnancies. In ART singletons, the rate of very preterm birth and very low birth weight is about two to three times higher than in the general population. These increased risks exist for conventional IVF as well as for ICSI, even though it seems some lower for the ICSI techniques. For children born after cryo preservation, a technique which is increasing worldwide, due to better freezing methods, more recent studies have found a lower risk for low birth weight and preterm deliveries compared to fresh IVF while higher rates of large for gestational age and macrosomia have been detected, both in comparison to fresh IVF and compared to singletons born after spontaneous conception. Another important neonatal outcome that has been in focus for studies are birth defects where a higher overall risk has been detected for ART, being between 30-70%. Turning to long-term effects of ART on children outcomes, much fewer studies of high quality exist. Studies of growth and physical health are few and limited to childhood where general physical health including hospitalizations, childhood illnesses, surgical interventions and medical therapies, are similar for age and gender-matched controls in the general population. For childhood cancer, the two largest studies, one from UK and one the Nordic countries, both including around 100 000 ART children do not show any increase in childhood cancer in ART children compared to children born after spontaneous conception and after adjustment for relevant confounders. Most studies on neurocognitive development, autism and autistic disorders show no increased risks if adjusted for multiple birth. In addition, school performances of 15-16 year old adolescents have been investigated in two large registry studies from Denmark and Sweden. Both studies showed better school performance for ART children in crude analyses but after adjustment for relevant confounders, particularly parental education, no differences of clinical importance were observed. There are some recent concern regarding cardiovascular parameters. Case-control studies have detected altered blood vessel structure and increased blood pressure, both systolic and diastolic in ART singletons compared to matched controls and further that these differences remain in adolescence. For diabetes type 1, very limited data exist but large studies are ongoing. A few longitudinal reports on reproductive health in adolescents and young adults have been published. While onset of puberty and pubertal development were similar for ICSI and spontaneously conceived boys and girls, ICSI-conceived men seemed to have lower sperm concentrations and total sperm counts than age-matched, spontaneously conceived controls. Further studies on long-term health of ART children are needed, both in view of the widespread use of ART and that new and advanced technique are rapidly implemented in this arena.

INVITED SESSION

SESSION 25: NAVIGATING BETWEEN HOPE AND HYPE IN SCIENCE COMMUNICATION: ETHICAL ISSUES IN PUBLICISING RESEARCH

Monday 24 June 2019 Haydn 3 17:00–18:00

O-093 The problem of science hype and what to do about it

T. Caulfield[1]

[1] *University of Alberta, Health Law Institute, Edmonton, Canada*

Abstract text

This presentation will explore the evidence that suggests both the existence and source of exaggerated claims about scientific advances and benefits. I will map the sources of hype to explore how they influence the representation of science in laboratory, the research institutions and, finally, in the popular press. While science hype is not a new phenomenon, it is arguably becoming both more common and more problematic. At a minimum, it adds an element of inefficiency to the corrective tendencies of the scientific process. Worse, it may facilitate the premature implementation of technology, have an adverse impact on the distribution of research resources and erode public trust.

O-094 Research finding, hype and public policy: Presenting evidence to policy makers

S. Norcross[1]

[1] *Progress Educational Trust, London, United Kingdom*

Abstract text

Title: Hype, Hope and Headlines: The Impact Factor Subject: The influence of 'impact' on patients, the public and policy in relation to ART Making an impact is important to everyone involved in research, whether in academia or in the clinical setting. But the significance of impact is wider than this – it involves clinics, patient support groups, charities (including the Progress Educational Trust - PET) and both the lay and specialist media (including BioNews). Institutions, companies, publications and broadcasters compete for profile, to attract funding and to increase their market share. The impact factor (IF) is making an impact, and like it or not it is here to stay. In a research context, IF is a measure of the frequency with which the average article in a journal has been cited in a particular year. It is used to measure the importance or rank of a journal, by calculating the number of times its articles are cited. IF is frequently used to rank the relative importance of a journal within its field. Journals with higher impact factors are often deemed to be more important than those with lower ones. Journals, keen to have a better IF than their rivals, use print and digital media – including social media – to promote papers. This promotion maybe coordinated with the authors' academic institution, but tensions can arise. In the UK, the Research Excellence Framework (REF) is the system for assessing the excellence of research in higher education institutions. Two of the elements assessed are quality of outputs (including publications and IF in its formal sense) and the impact of these outputs beyond academia (including IF in a more general and informal sense). Outside academia, news websites gather real-time data on their traffic, writing copy and using images to give readers what they want (or think they want) and encouraging them to click through and be exposed to advertising. The longer people spend on websites, the more those websites can charge for their advertising space. Attention-grabbing, sensational headlines can entice people to buy a print publication or click on a link. Headlines are often necessarily brief and lacking in nuance, and may promise more than the article below will deliver. Clinics and charities take to social media, issue statements and write blogs to attract attention. Of course, this is done to provide information to patients. But it is also often done with one eye on attracting paying clients and/or donors, and this means that hype can creep in. Over the years, many so-called IVF 'add-on' treatments have been reported uncritically in the media, giving patients what some have criticised as false hope. PET has been critical of this trend, and held two key events highlighting the issue in 2014, 2015 and 2017. Several media outlets have conducted undercover investigations into 'add-ons',

until in 2019 the UK's fertility regulator – together with leading professional bodies, including ESHRE – published a consensus statement advising that IVF clinics should not charge patients for add-on treatments that are not proven effective by clinical trials.Sometimes however, academics, clinicians, the media and charities work together to make a positive impact. A good example of this is the long-running and ultimately successful campaign to change UK law to permit the clinical use of mitochondrial donation. Concerted efforts were made throughout the campaign not to overpromise, and not to push for use of the relevant techniques outside a clearly and narrowly defined purpose.

What responsibilities fall upon all the actors who seek to make in impact, on professional audiences and on the wider public, in relation to ART?

INVITED SESSION

SESSION 26: DONOR IDENTITY - WHO IS TELLING WHO?

Monday 24 June 2019 Haydn 2 17:00–18:00

O-095 Lessons from three decades of non-anonymity

C. Lampic[1]

[1]*Reproductive Health, Women's and Children's Health, Stockholm, Sweden*

Abstract text

Lessons from three decades of non-anonymity

More than three decades ago, in 1985, Sweden was the first country in the world to abolish anonymous gamete donation. This initiative was met with some resistance by clinicians who feared a drop of sperm donors, but these concerns have been largely refuted. Initially, sperm donation was available only for heterosexual couples but in the past 15 years also oocyte donation, as well as sperm donation for lesbian couples and single women, has been permitted. In general, the donor is totally anonymous to the recipient couple or woman, and the adult offspring is the sole person who has the right to access the donor's identity. Donation treatment is only provided to recipients who plan to share information about the donor conception with the child, and they are encouraged to start the disclosure process from an early age. The child's conception with donor gametes is not visible in any official records and the parents have the full responsibility to share this information with the child. The psychosocial aspects of gamete donation are investigated in the multicenter longitudinal 'Swedish Study on Gamete Donation' that recruited large groups of recipient couples as well as oocyte and sperm donors in 2005-2008. Overall, the results indicate that couples want to be honest with their children and intend to share information about the donor conception with their offspring. In compliance with professional advice provided at the clinics, a majority of couples had already started the disclosure process when their children had reached school age. Similarly, oocyte and sperm donors were found to have neutral or positive attitudes towards potential contact by offspring from their donation. Half of the donors would prefer to receive notification when an offspring from their donation requests information about them, which is a service not routinely provided by the clinics. Since 1985 approximately 5000 children have been born following oocyte or sperm donation in Sweden. Among these, more than 700 offspring following sperm donation to heterosexual couples have now reached adult age, and thus have the legal right to obtain identifying information about their donor. However, the number of donor conceived persons who have come forward to request this information is surprisingly low. Preliminary results from an ongoing interview study with these adult offspring and their parents indicate a wide variety of experiences regarding both the disclosure process and the situation when the donor conceived person requested donor information. In some families, information about the donor conception was shared with the child from an early age, while the disclosure in other families was prompted by biology lessons at school or by adult offspring who used DNA-tests to find out about their genealogy. A majority of the offspring had contacted the donor and several were in regular contact with him and/or genetic half-siblings. While parents who received donation treatment in recent years appear to be open with their child , there is reason to believe that many of the persons conceived in the late 1980s and 1990s are unaware of their conception with donor sperm. It is also likely that parents are concerned about the risk of accidental revelation, e.g. through available resources to search for genetic linkage. In conclusion, introducing legislation that abolishes donor anonymity and informing recipients about the importance of early disclosure does not seem to be sufficient to ensure that donor conceived persons have access to information about their genetic origin. Sweden, which was a pioneering country in terms of legislation, could today learn from international initiatives to provide targeted resources and support to families following donor conception, as well as to donors.

O-096 Donor detectives

A. Indekeu[1]

[1]*Fiom- Expert Center for Ancestry and University of Leuven, Department of Sociology, Hasselt, Belgium*

Abstract text

"Donor detectives": The exchange of information/ contact between donor-offspring and their donor Already a longer time donor-conceived individuals have shown an interest in information about their donor and/or started searching for them. Two social developments have facilitated this request: the increasing removal of donor anonymity and the rise of online genetic testing sites. The first development concerns a social-legal movement in which more and more countries in the last two decades have abolished donor anonymity and have given donor-conceived people the child access to identifying information about their donor at a certain age or at maturity. This change demonstrates a growing recognition of donor-conceived peoples' interest in learning more about their donor(s). A second development relates to the technological advancement and establishment of online commercial 'direct-to-consumer' (DTC) genetic testing, (such as 23andMe DNA and FamilyTReeDNA), which enables to find genetic relatives. Increasing numbers of donor-conceived individuals (and/or parents) are seeking individuals genetically related through donor conception and one route they use is DTC DNA testing. The availability of DTC and the opportunities created by the internet are reshaping the field of assisted reproduction. Interest in the exchange of information/contact between donor-offspring and their donor have raised many concerns: What kind of information is searched for? What kind of expectations do people have from these searches and possible contact and what are the experiences? Do donor-offspring, donors and parents want exchange of information? What are the consequences of these searches and exchanges for donors, donor-offspring and parents themselves and their (wider) relationships? How should this exchange of information and possible contact be organised? This presentation reviews the existing empirical data, addressing differences and similarities between exchange for information and possible contact within the context of anonymous and identifiable, sperm, egg, or embryo donation and hetero-sexual, lesbian, or single mother families. It will also address how exchange of information is organised in practice and the legal, ethical, social challenges that arise from this new development.

INVITED SESSION

SESSION 27: PGT DATA REPORTING

Monday 24 June 2019 Haydn 4 17:00–18:00

O-097 PGT Data reporting
O-098 Recommendations for good practice in PGT : What's new

M. De Rycke[1]

[1]*UZ Brussel, Centre for Medical Genetics, Jette- Brussels, Belgium*

Abstract text

The field of PGT is evolving fast. PGT began as an experimental procedure in 1990 with PCR-based methods used for the detection of monogenic diseases. Interphase fluorescence in situ hybridization (FISH) was introduced a few years later and became the standard method for sexing embryos and for detecting chromosomal numerical and structural aberrations. Genome-wide technologies began to replace the gold standard methods of FISH and PCR over the last decade and this trend was most apparent for PGT-A. Based on the pace of development, up-to-date best practice advise is essential for

regulation and standardisation of diagnostic testing. The previous guidelines on best practice for PGT, published in 2005 and 2011, are considered outdated and the development of new series of papers outlining recommendations for good practice in PGT was necessary. Under supervision of the PGT consortium steering committee, four recommendations papers were prepared. Working groups were composed of people with relevant and hands-on experience. Based on the previous guideline papers and after several online discussions, recommendation papers were prepared by the working groups, consistency of the papers was checked, and stakeholder review organised. The final series of 4 papers will be published shortly. The series includes a paper on the organisation of PGT covering aspects of patient selection and counselling, basic requirements for PGT, quality management, pregnancy and children follow-up and transport PGT. Complementary to this paper on organisation of PGT, 3 technical papers provide recommendations on technical aspects of (1) polar body and embryo biopsy for PGT, (2) detection of structural and numerical chromosomal aberrations, and (3) detection of monogenic disorders. These technical papers provide general recommendations on training of staff, laboratory infrastructure, equipment and materials, and preclinical work-up, validation and (post) examination process, before providing specific recommendations for the different techniques or testing strategies. Together, the 4 recommendations papers provide best practice recommendations for organisational and technical aspects of a PGT service, based on experience and available evidence. The overall aim of the series is to take PGT to the same high-quality level as routine genetic testing.

SELECTED ORAL COMMUNICATIONS
SESSION 28: NURSING AND MIDWIFERY

Monday 24 June 2019 Strauss 1+2 17:00–18:00

O-099 Through women's eyes: The female experience of infertility following a recent diagnosis of severe male factor infertility

E. Stevenson[1], K. McEleny[2], E. Moody[2], D. Bailey[1]

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[2]*Centre for Life, Newcastle Fertility Centre, Newcastle-upon-Tyne, United Kingdom*

Study question: How do the partners of men who have recently been diagnosed with severe male factor infertility (MFI) manage during diagnosis and treatment?
Summary answer: Partners perceive men with MFI experience psychological distress about the diagnosis. Partners use intentional strategies to help partners cope. The relationship is affected during treatment.
What is known already: Men with MFI experience psychosocial and informational challenges during diagnosis and treatment. They also experience higher incidences of problems that can impact upon their well-being, such as depression and sexual dysfunction. While the female experience of female factor infertility is well documented and understood, little is known about women's experiences of infertility following a partner's diagnosis of severe MFI. There is very limited understanding of how they perceive their partner's experience, how they support their partner during fertility evaluation/treatment, and the impact of the process upon the functioning of their relationship.
Study design, size, duration: This was a prospective qualitative study among female partners of American and British men with severe MFI (azoospermia, or sperm concentration < 5Million/ml) seeking care from a U.S. or U.K. Urologist for family building, between December 2015 and April 2017. Individual in-depth qualitative interviews were conducted 3 and 6 months after a MFI urology consultation. Each interview lasted between 35 and 60 minutes and was digitally recorded and transcribed verbatim.
Participants/materials, setting, methods: Inclusion criteria were the female partners (married or co-habitating) of men recently diagnosed with severe MFI and 40 years or younger. Exclusion criteria included (living) biological or adopted children, or a partner with a history of vasectomy. Eleven (7 U.S., 4 U.K.) participants were recruited immediately after their urology consultation for MFI. Interviews were conducted via phone. Data were analyzed using content analysis.
Main results and the role of chance: The analysis resulted in three major themes: 1) Women reported that the men's emotional response to receiving the diagnosis included a variety of negative responses (shock, disappointment, internalization/non- disclosure to social network); 2) Women employed intentional strategies to help their partner cope with the diagnosis and treatment, such as the use of prayer/humor, by offering reassurance, support, love, and by active management to reduce negative male feelings; and 3) Women reported that fertility assessment/treatment had an impact on their relationship quality: For those who offered reassurance to partners of their love/support, women perceived their relationship as stronger. For those who worked to reduce men's negative impact on their feelings/ego, women perceived their relationship as strained. Women who perceived infertility as a shared experience reported improved relationship quality. The results demonstrate that while men faced negative emotional challenges throughout the process, their partners worked to manage these emotional experiences, and the strategy employed, may have impacted upon their perception of the quality of their relationship.
Limitations, reasons for caution: The majority of the women lived in the same two areas (a small metropolitan area of the U.S. South and a city in Northern U.K.). As the results are based on qualitative data from 11 partners of men with MFI, the results cannot readily be generalized to larger populations.
Wider implications of the findings: This is the first study seeking to understand the psychosocial impact of MFI from the partner's perspective. The findings highlight a connection between partner's perception and active management to help men cope. More research is needed to understand the dyadic impact on psychological functioning of men during diagnosis and treatment.
Trial registration number: not applicable.

O-100 Cross-border reproductive care: A quantitative study of patients' experiences of care in the Netherlands and the motivation for treatment at a Belgian University Hospital

A. Jongbloet[1], S. Somers[1], M. Schoonenberg[2], A. Geril[1], D. Braat[3], P. De Sutter[1]

[1]*Ghent University Hospital, Department of Reproductive Medicine, Gent, Belgium*
[2]*Nij Geertgen Fertility Center, Fertility Center, Nijmegen, The Netherlands*
[3]*Radboud University Medical Center, Department of Obstetrics and gynaecology, Nijmegen, The Netherlands*

Study question: How do Dutch patients experience the quality of reproductive care in their own country? What is their motivation for treatment at a Belgian University Hospital?
Summary answer: The reason why Dutch patients opted for fertility treatment at a Belgian University Hospital was a combination of emotional factors and real differences in care.
What is known already: Over the years, we saw an increase in the number of Dutch patients going to Belgium for fertility care. Previous research showed that Dutch patients think treatment in the Netherlands is highly protocol-oriented (Van Hoof et al., 2014). An experienced lack of patient orientation would be the main reason for patients to change hospitals (van Empel et al., 2011). Also, patient-oriented care is associated with better clinical outcomes and increased patient satisfaction (van Empel et al., 2010). There is a need for a strategy to effectively increase the quality of care, particularly the patient-centeredness of care (Dancet et al., 2011).
Study design, size, duration: From August 2016 until mid-February 2017 a cross-sectional study was conducted. A consecutive sample was taken of Dutch adult women planning to visit the same Belgian University fertility center (an appointment was scheduled, but the department was not yet visited). This method guaranteed good representativeness of the entire population. Around 508 women received an email with a link to an online questionnaire. In the end 192 women filled in the questionnaire (response rate of 37.8%).
Participants/materials, setting, methods: A four-part questionnaire was constructed. It focused on: 1) the motivation to go abroad for fertility care, 2) the experiences with Dutch fertility care (based on the Patient-Centredness Questionnaire-Infertility [PCQ-I]), 3) Quality of life [QoL] during treatment (based on the FertiQol-questionnaire) and 4) background of the participants. SPSS 23 was used for statistical analysis (descriptive analysis and Pearson

correlations). The Netherlands was divided into 3 regions for a more profound analysis of the results.

Main results and the role of chance: The PCQ-I was filled in by those who had already been treated in the Netherlands (n = 159). The average total score was 1.90 (range 0-3) which means that patient-centeredness was experienced better than mediocre. The results showed that Dutch fertility care can be improved at the level of the organization (continuity and transition (1.59)) and at the individual level (patient involvement (1.71), respect for patient's values (1.59)). Because the PCQ-I does not focus on the individual expectations of the patient, the questionnaire was expanded. The FertiQol was only completed by non-pregnant women (n = 162). The final score was 63.80 (range 0-100). This means that the QoL in individuals with fertility problems was better than mediocre. The possibility to undergo new and/or experimental investigations (86.0%), the higher level of knowledge and expertise (83.8%) and the higher success rates (82.1%) were the main reasons for choosing Belgian fertility care (n = 179). A significant link was found between the reasons for visiting a Belgian fertility clinic and three Dutch regions. 59.3% of the participants from the second border region (having the widest choice of IVF centers in the neighborhood) indicated that they were not satisfied with Dutch fertility care.

Limitations, reasons for caution: Women were asked to complete the questionnaire alone. Future studies should focus on both partners. In this study, 39.4% of women had already wanted children for more than four years. This duration could have evoked negative feelings which could have negatively influenced how experienced care was scored.

Wider implications of the findings: The significant relationship between the PCQ-I and the FertiQol ($p<0.01$) shows the importance of coping with negative emotions caused by subfertility. Dutch fertility care should focus on the emotional reasons of why patients visit a Belgian hospital. This can lead to a higher quality of care in the Netherlands.

Trial registration number: NA

O-101 A systematic review and meta-synthesis of mobile device fertility applications: More than a glitch in the matrix

S. Costa[1], A. Yazdani[1]

[1]Eve Health, Fertility, Spring Hill, Australia

Study question: The aim of this meta-synthesis was to determine the utility and validity of current mobile device fertility applications and the medical advice given in these applications.

Summary answer: Despite a significant increase in the number of fertility applications, apps have limited validity, make unrealistic claims of efficacy, and have no established review cycle

What is known already: Fertility and cycle tracking applications are consistently among the most popular applications on both Android and iOS platforms. Up to 70% of women initiating fertility treatment track their cycles. There is limited published information on how applications are featured or promoted in either store. Furthermore, little systematic assessment has been performed on the validity of the advice offered despite frequent claims of efficacy. Despite data supporting the belief of health professionals that patient apps promote autonomy and and understanding of health, primary healthcare providers have limited familiarity with these applications, and the features frequently used by patients.

Study design, size, duration: The research protocol followed the published methodology for Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P). The research protocol was developed through a staged, iterative process prospectively registered through (PROSPERO ID: 124109). The literature search was conducted of online databases, with a predefined search strategy. All applications up to and including January 8th 2019 were included. Additional applications were identified through snowballing and manual searches of published reviews.

Participants/materials, setting, methods: iTunes and Google Play were systematically searched for iOs and Android applications, respectively. The analysis included paid and free applications, unrestricted by age but limited to English. Each application was reviewed and scored independently by each researcher based on a predetermined matrix. Descriptors were sorted and categorized in EndnoteX7 (Thomson Reuters). Data analysis and synthesis was performed by computer-assisted qualitative data analysis (NVIVO12, QSR International) and analytic induction in a staged independent framework approach.

Main results and the role of chance: 399 applications were identified in the initial search strategy. 381 applications were included after review of the descriptors. 9 non-English applications and 41 duplicates were removed from the final analysis. 332 applications were included in the systematic review. Between January 2015 and January 2019 there has been a 47% increase in the number of mobile fertility applications (from 225-332). Commonly included features are: conception and contraception tracking, health education, flow and ovulation symptom tracking, cycle length information and prediction. Less then 10% of applications cited published literature or professional guidelines. A significant number of applications made unrealistic and unsubstantiated claims with respect to efficacy.

Limitations, reasons for caution: Application sources are unreliable, incomplete and subject to commercial imperatives. There is limited transparency in app development, functionality, ownership, and store ranking. This study did not assess user experience and reproductive outcomes.

Wider implications of the findings: End users, patients and healthcare professionals, are at risk of accepting the advice and information provided by these applications unchallenged..

Trial registration number: Not required however PROSPERO ID: 124109

O-102 Does information changes patient's preference for double embryo transfer between year 2015 and 2018?

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Study question: Does information on multiple pregnancy risks changes patient's decision on the number of embryos to transfer in year 2015 and 2018?

Summary answer: Information seems to influence patient's preference for number of embryos to transfer in in-vitro fertilisation (IVF) in 2018 compared to 2015

What is known already: Increased multiple birth following an IVF has been a rising concern. When transferring more than one embryo in IVF patients are at risk for maternal complications, fetal mortality and morbidity. These complications leads to higher costs due to perinatal and neonatal expenditures, not including lifelong disabilities and increased needs for social and medical support. However, in Asia, where patients pay out of pocket to do IVF, they are more likely to ask for multiple embryos to be implanted to save their cost and getting pregnant at their first attempt.

Study design, size, duration: A cross sectional study, where 151 patients seeking fertility treatment in KL Fertility Centre were randomly chosen to answer a self-administered questionnaire during a 3-month survey in 2015 and 168 patients in 2018.

Participants/materials, setting, methods: Patients were recruited for a self-administered questionnaire which consists of the same 15 questions. They were asked to fill out the questionnaire before counselling and again after the counselling on multiple pregnancy risks. They were also asked about personal preference in number of embryos to be transferred. Other questions included were their sociodemographic data, pregnancy history, awareness on the risk of multiple pregnancy and knowledge on the risk of more than single embryo transfer (SET).

Main results and the role of chance: This cross-sectional study has gathered information that there was no significant difference in patient's awareness on the risk of multiple pregnancy in year 2015 and 2018. In both years, patients were aware that multiple pregnancy carries a high risk to both mothers and their babies. There was also no significant difference in patient's knowledge on multiple pregnancy risks and the ideal outcome of IVF in both years. Patients agree that single baby is the best outcome. However, patient's personal preference to have SET after counselling being given was significantly higher in 2018 compared to 2015 ($p<0.05$). In 2015, after being counselled, 85.7% still opted for double embryo transfer (DET) but in 2018, only 59.2% wished for DET.

Limitations, reasons for caution: Patients with various backgrounds, language barrier and culture may affect results. However, all patients are able to give full cooperation on completing the survey.
Wider implications of the findings: This study suggests that continuous education and counselling would be able to encourage more patients to choose SET. Clinicians, nurse counsellors and embryologists will advocate patient but until legislation implemented, patients will still decide what they believe is the best for them based on their culture, family background and finance.
Trial registration number: not applicable

INVITED SESSION
SESSION 29: THE PRESENCE OF MOSAICISM. WHAT DO WE DO?
Tuesday 25 June 2019 Mozar 08:30 - 09:30

O-103 Mosaicism in the embryo

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Abstract text
Mitotic errors during embryo development can result in chromosomally distinct cell populations. Mosaicism can occur as early as the 2-cell stage, although detection at the blastocyst stage is more common because more trophectoderm (TE) cells can be simultaneously analysed. The introduction of next generation sequencing (NGS) allows the identification of different levels of mosaicism in TE biopsies, but proper validation is required to define detection levels. The concordance between the TE biopsy the inner cell mass (ICM) and the whole embryo can be related to factors such as mosaicism type and degree, biopsy location, the number of cells biopsied, and cell loading. Also, different levels of mosaicism have been described according to the infertility aetiology, the insemination technique, stimulation, culture conditions and embryo quality. In summary from different studies published so far, blastocysts diagnosed as mosaics in TE biopsies were confirmed in 50%–90% of the secondary analysis performed in ICM biopsy, additional TE biopsies, or whole-blastocyst analysis.

Different levels of mosaicism have been reported in preimplantation human embryos, but the incidence in early miscarriages can help to understand the real incidence at blastocyst stage and their capability to implant. Classic cytogenetic studies of miscarriage POCs have reported only a 5%–6% incidence of mosaicism. Most of the mosaic aneuploidies found in established pregnancies are confined to placenta. In the same trend, using both hysteroembryoscopy and molecular analysis using aCGH or NGS, our group has described an incidence of 2% fetoplacental discrepancy in 46 early miscarriages. In ongoing pregnancies, most mosaic aneuploidies are also confined to the placenta and may be associated with a poor perinatal outcome. In rare cases, the placental karyotype is normal and foetal cells show an abnormal karyotype. The frequency of mosaicism in live births is undetermined, because chromosomal analysis in live births, children, and adults are mostly performed only when there is a clinical indication or a strong suspicion for a chromosomal disorder. In fact, the clinical manifestations are represented by a spectrum of phenotypes, and their relationship with different syndromes has been widely described.

Therefore, mosaic embryos can be considered to represent a distinct category in terms of viability, lying in between euploid and fully abnormal embryos. Several groups have described the possibility of healthy livebirths with the transfer of mosaic embryos, mostly when no euploid embryo after the TE biopsy. Mosaic embryos are characterized by decreased implantation and pregnancy potential, as well as increased risk of miscarriage rate and adverse perinatal outcomes. Whether to or not to transfer a mosaic embryo depends on key factors such as the methodology used, the degree of mosaicism, the chromosomes affected, the cohort of sibling embryos, and finally, the medical history and expectation of the patient. If a patient proceeds with transfer of an embryo diagnosed as mosaic, counselling about the benefits, risks, and limitations should be properly explained. In case of an ongoing pregnancy after the transfer of a mosaic blastocyst, amniocentesis should be highly recommended due to the a priori increased risk of foetal aneuploidy.

O-104 Mosaicism in the baby

INVITED SESSION
SESSION 30: NEW FRONTIERS
Tuesday 25 June 2019 Haydn 1 08:30 - 09:30

O-105 Piwi/piRNA dysregulation and human male infertility

L.T. Gou[1], **J.Y. Kang**[1], **P. Dai**[1], **X. Wang**[2], **M.F. Liu**[1]
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Abstract text
PIWI proteins, as well as their interacting piRNAs (PIWI-interacting RNAs), are specifically expressed in animal germlines and play essential roles in germ cell development and gametogenesis in animals. Human PIWI proteins are specifically present in testes; however, the function of PIWI or/and piRNAs in human spermatogenesis and male infertility has remained largely unexplored. Our recent studies identified the ubiquitination-deficient mutations, i.e. D-box mutation, in human *Piwi* (*Hiwi*) from azoospermia patients and further revealed that such mutations cause male infertility by impairing Histone-to-Protamine exchange during spermiogenesis. This, for the first time, demonstrate that *Piwi* mutations play a causative role in human male infertility. Of note, we revealed that *Piwi* D-box mutations act to impair sperm formation in a piRNA-independent manner. To pursue whether the piRNA-dependent role of PIWI is also of functional importance in human male fertility, we further screened for potential mutations in the PAZ and MID domains of *Hiwi* in human male infertility. PAZ and MID domains are responsible for piRNA loading in PIWI proteins. Interestingly, from a cohort of patients with idiopathic oligo/astheno/azoospermia and fertile controls, we identified a specific mutation in the PAZ domain of HIWI protein in 5 patients, but none in controls. Importantly, by modeling the mutation in mouse *Piwi* (*Miwi*) in mice, we demonstrate that this genetic defect is directly responsible for male infertility. This supports a functional importance of piRNA-dependent role of PIWI in human male fertility. Together, our findings identify *Piwi* as a new factor in human male infertility and indicate that it functions in multiple ways in regulating male germ cell development.

O-106 Understanding the immune cells of the endometrium and decidua using single cell methods

A. Sharkey[1], **A. Moffett**[2]
[1] *University of Cambridge, pathlogy, Cambridge, United Kingdom*
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Abstract text
Understanding the immune cells of the endometrium and decidua using single cell methods
During early pregnancy the endometrium transforms into the decidua. Extravillous trophoblast cells (EVT) from the fetus invade into this tissue and remodel maternal spiral arteries to increase blood flow to the placenta and support fetal growth. The correct degree of invasion is required; excessive invasion must be avoided, while reduced invasion is associated with major disorders of pregnancy including pre-eclampsia, and fetal growth restriction (FGR).

This process brings together cells from two genetically different individuals and recent work strongly suggests that recognition of trophoblast by maternal immune cells plays a role in determining the depth of placentation. Because

Table 1 FSHR Asn680Ser (rs6166) genotypes X population characteristics, semen parameters, DNA damage parameters

Parameters	FSHR Asn680Ser genotypes			
	Asn/Asn	Asn/Ser	Ser/Ser	*P*
n	126	164	57	
Age (years)	38.3±5.7	37.9±5.4	38.6±5.6	0.63
BMI (Kg/m^2)	27.9±4.0	28.4±4.3	28.6±4.8	0.57
Smoking (%)	10.3%	9.1%	12.3%	0.78
Regular alcohol drinking (%)	62.7%	62.8%	61.4%	0.97
Vitamin supplementation (%)	14.3%	18.3%	15.8%	0.98
Abstinence (days)	3.2±2.2	3.8±3.8	3.7±2.0	0.20
pH	8.1±0.3	8.0±0.2	8.0±0.2	0.62
Volume (ml)	2.9±1.6	2.7±1.3	2.8±1.5	0.54
Concentration (mlx10^6)	80.8±55.1	78.2±59.0	83.6±59.1	0.75
Progressive motility (%)	54.8±15.4	55.7±13.8	56.1±12.4	0.90
Total motility (%)	61.7±15.1	62.8±13.9	62.5±12.0	0.91
Leukocytes (x10^6/ml)	0.3±0.6	0.5±1.2	0.3±0.3	0.62
Vitality (%)	64.6±12.9	64.8±12.3	64.2±11.5	0.93
Normal sperm structure (%)	0.7±0.8	0.7±0.8	0.5±0.6	0.56
DNA fragmentation (%)	14.5±8.7	13.7±7.7	15.2±7.1	0.29
Apoptosis (%)	18.7±7.0	20.1±8.6	20.1±6.7	0.44
CMA3 positivity (%)	56.3±16.6	54.4±16.4	56.4±15.7	0.56
Abnormal MMP (%)	25.9±19.3	24.9±16.1	25.9±14.6	0.84

mother and father generally differ in their HLA class I and class II types, there is the potential for allo recognition of EVT. Immune cells in the decidua that can mediate allo-recognition include T cells and Natural Killer (NK) cells, but they do so by quite different mechanisms. EVT has a unique HLA profile: they do not express HLA-A or HLA-B, the dominant T cell molecules, but do express HLA-E, HLA-G (which are essentially monomorphic) and the polymorphic HLA-C molecules. T-cells that are able to recognise EVT directly are thus likely to do so using HLA-C restricted T cell receptors (TCRs).

In contrast, uterine NK cells (uNK) which comprise ~70% of decidual leucocytes, recognise HLA-C via activating and inhibitory receptors of the KIR gene family. Of all human gene families, HLA and KIR exhibit extreme polymorphism, so each pregnancy is characterized by different combinations of maternal KIR and fetal HLA-C genes, resulting in variable uNK inhibition or activation. Our genetic studies have established that that certain maternal KIR/fetal HLA-C combinations increase the risk of disorders of placentation such as pre-eclampsia and FGR. KIR/HLA-C combinations that result in excessive uNK inhibition are associated with low birth weight and increased risk of pre-eclampsia, while activating KIR/HLA-C combinations may result in large babies. However the biological mechanisms underlying these genetic results are unclear.

To better understand how interactions between maternal immune cells and trophoblast in the decidua might regulate EVT invasion, we have collaborated in the development of a single cell atlas of the maternal–fetal interface. Single-cell transcriptome profiling of decidual and trophoblast cells has revealed new types of decidual stromal and perivascular cells and allowed the identification of novel subsets of maternal immune cells. This has also permitted the creation of a database of potential ligand-receptor interactions between decidual cells and EVT (www.CellPhoneDB.org/). This analysis shows multiple mechanisms exist within the decidua to prevent harmful innate or adaptive immune responses. The RNA studies have been combined with high resolution phenotypic and functional analysis of uNK cells using a 43 marker mass cytometry (CyTOF) panel which has revealed in unprecedented detail the heterogeneity of the uNK and decidual T cell compartments. We have also developed an organoid culture system for human trophoblast cells, and these cells can be stimulated to differentiate into invasive EVT in vitro.

Together we hope that these tools will permit investigation of the cellular and molecular interactions between the cells of the decidua and EVT that are important for successful placentation. In particular, the trophoblast organoid model will allow us to determine how uNK responses triggered by different KIR/HLA-C combinations regulate trophoblast differentiation and function, to understand how interactions between immune cells and EVT result in different pregnancy outcomes.

INVITED SESSION

SESSION 31: 3D REPRODUCTIVE ORGANS

Tuesday 25 June 2019 Haydn 3 08:30 - 09:30

O-107 Advanced tissue engineering to promote female fertility

O-108 3D testicular cultures: Novel tools for the study of human spermatogenesis

J.B. Stukenborg[1]

[1]*Karolinska Institutet, Dept. of Women's and Children's Health- NORDFERTIL Research Lab Stockholm, Stockholm, Sweden*

Abstract text

Title: 3D testicular cultures: Novel tools for the study of human spermatogenesis

Fertility in males relies on the spermatogenic process leading to the production of fertile sperm. It comprises the proliferation of

spermatogonial stem cells (SSCs), meiotic division, and the differentiation and finally the maturation of haploid spermatids. The fact that spermatogenesis is a continuous process which relies on stem cells offers men the opportunity to produce sperm until late in life. However, the production of sperm highly depends on the presence and functionality of SSCs.

Attempts to translate the complex process of spermatogenesis including all maturation steps from SSCs to functional sperm *in vitro* resulted in different strategies, including numerous culture conditions, which have been employed to explore the spermatogenic progress in detail (Alves-Lopes and Stukenborg, 2017). Despite this, understanding of this highly complex process, which is mainly influenced by endocrine and paracrine actions of somatic cells (e.g. Sertoli and Leydig cells), is still inadequate (Stukenborg, et al., 2018). The lack of detailed understanding of mammalian and especially human gametogenesis also results in a paucity of treatment options for patients, who cannot produce sperm and are at the risk of becoming infertile, due to their medical treatment or disease itself. This highlights the need for novel strategies to both understand the process of human spermatogenesis and develop *ex vivo* approaches to differentiate mature gametes from immature germ cells or even pluripotent stem cells.

In recent decades, a broad range of *in vitro* strategies have been applied to study the testicular microenvironment. However, a robust culture condition, suitable to investigate the formation of the human gonad and specifically the formation of the SSC niche has not been reported so far. In addition to first reports on successful differentiation of immature germ cells into functional sperm using explant tissue cultures, the generation of tissue-specific organ-like structures called organoids has created a new possibility, allowing researchers to study paracrine effect in more controlled manner (Alves-Lopes and Stukenborg, 2017). Organoids are small cell aggregates similar to organs found *in vivo*, but generated from single cell suspension containing stem cells. These organ-like structures are also defined by their functionality, which makes them a valuable research model and most likely a useful clinical tool in future. Today only few scientific publications are available focusing on the generation of testicular organoids (Alves-Lopes, et al., 2017, Baert, et al., 2017, Pendergraft, et al., 2017). However, together with other three-dimensional approaches, these novel cell-culture methodologies might provide new tools for more defined research approaches regarding gametogenesis and its failures.

References:

Alves-Lopes JP, Soder O and Stukenborg JB. Testicular organoid generation by a novel in vitro three-layer gradient system. *Biomaterials* 2017; **130**: 76-89.

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Baert Y, De Kock J, Alves-Lopes JP, Soder O, Stukenborg JB and Goossens E. Primary Human Testicular Cells Self-Organize into Organoids with Testicular Properties. *Stem cell reports* 2017; **8**:30-38.

Pendergraft SS, Sadri-Ardekani H, Atala A and Bishop CE. Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity in vitro. *Biol Reprod* 2017; **96**:720-732.

Stukenborg JB, Jahnukainen K, Hutka M and Mitchell RT. Cancer treatment in childhood and testicular function: the importance of the somatic environment. *Endocr Connect* 2018; **7**:R69-R87.

INVITED SESSION

SESSION 32: ASRM EXCHANGE SESSION - CONTINUING CONTROVERSIES IN ART

Tuesday 25 June 2019 | Haydn 2 | 08:30 - 09:30

O-109 Fresh vs. frozen embryo transfer: Lessons learned - questions remain

O-110 PGT-A in 2019: Costs and benefits

INVITED SESSION

SESSION 33: IMPROVING FEMALE FERTILITY AFTER CANCER

Tuesday 25 June 2019 | Haydn 4 | 08:30 - 09:30

O-111 Fertility preservation in the future – Preventing loss of gametes

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Abstract text

Premature ovarian failure (POI) and infertility are frequent side effects of anticancer therapy, due to the extreme sensitivity of the ovarian follicle reserve to the effects of radiotherapy and chemotherapy. Oocyte and embryo cryopreservation can help some women achieve pregnancy after cancer-induced infertility, but the development of effective methods to protect ovarian function from chemotherapy would have significant advantages.

Cancer patients are commonly treated with combination of multiple drugs, making difficult to determine the mechanism of action of the individual substance on fertility.

Extensive studies have reported the effects of the most commonly used chemiotherapic drugs, such as Doxorubicin, Cisplatin and Cyclophosphamide, on the ovary. These drugs are known to induce follicle depletion, inflammation, stroma and vasculature damage. These adverse effects lead to depletion of the ovarian reserve, therefore to premature ovarian insufficiency, by death and/or accelerated activation of primordial follicles and increased atresia of growing follicles. Kinases have been identified as being central to the action of Cisplatin, Doxorubicin and Cyclophosphamide, and TAp63 as the guardian of primordial/primary oocyte genome integrity through its regulation of DNA damage and apoptosis.

In the last 20 years, attention has focused on the investigation of adjuvant therapies to protect ovarian function against chemotherapy. Mainly in animal models, several potential protective agents have been tested for their action on inhibiting oocyte death, on reducing follicle activation and atresia or reducing oxidative stress.

Understanding the mechanisms underlying the action of chemiotherapic compounds is essential for the development of efficient and targeted pharmacological therapies that could protect and prolong female fertility and ovarian functions.

O-112 Developing networks for effective ovarian tissue cryopreservation

SELECTED ORAL COMMUNICATIONS

SESSION 34: NEW INSIGHTS GAINED THROUGH TIME-LAPSE IMAGING

Tuesday 25 June 2019 | Mozart | 10:00 - 11:30

O-113 Halo characteristics during fertilisation are predictive of human pre-implantation embryo development and pregnancy outcome

K. Ezoe[1], **H. Tasaki**[1], **T. Miki**[1], **C. Hickman**[2], **T. Okimura**[3], **K. Uchiyama**[3], **A. Yabuuchi**[1], **T. Kobayashi**[4], **K. Kato**[4]

[1] *Kato Ladies Clinic, R&D Division, Tokyo, Japan*

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[3]Kato Ladies Clinic, IVF Laboratory, Tokyo, Japan
[4]Kato Ladies Clinic, Department of Gynecology, Tokyo, Japan

Study question:
Is the spatiotemporal halo phenomenon in the cytoplasm of oocytes during the in vitro fertilisation process predictive of embryo viability?

Summary answer:
The presence and time interval (TI) of halo events during fertilisation correlate with cleavage pattern, blastocyst formation, and ongoing pregnancy after single blastocyst transfer.

What is known already:
Embryologists traditionally assess zygotes between fertilisation and the first cell division to grade pronucleates. Using static observations, Stalf et al. (2002) first described the halo effect, a clear half-moon-like zone of the cytoplasm, and its association with improved fertilisation, embryo fragmentation, and clinical pregnancy compared to zygotes not displaying this phenomenon, suggesting halo presence as a potential biomarker for embryo viability. With the flexibility from time-lapse technology, Cottichio et al. (2018) further characterised morphokinetics of halo appearance and disappearance. The association between these stages, in addition to halo centralisation and redistribution, with embryo viability and implantation potential are currently undetermined.

Study design, size, duration:
Time-lapse images from 1,009 zygotes were retrospectively analysed from 560 patients who underwent in vitro fertilisation with minimal stimulation and single vitrified-warmed blastocyst transfer (SVBT) from April 2017 to March 2018. Halo presence, stages, and characteristics were observed in the time between second polar body extrusion and first cell division and compared relative to morphokinetic timing to the blastocyst stage and embryo quality, blastocyst expansion rate, and clinical and ongoing pregnancy rate.

Participants/materials, setting, methods:
Following intracytoplasmic sperm injection, time-lapse images from fertilized oocytes were annotated for halo presence and characteristics including timing of halo appearance (tHa), end of organelle centreing (tHc), initiation of organelle redistribution (tHr), and halo disappearance (tHd). Halo data were compared with morphokinetic data (from first PN appearance to expanded blastocyst), embryo stage of development reached (2-cells to blastocyst), cleavage embryo quality (cleavage symmetry and fragmentation), blastocyst quality (Gardner), and clinical outcome after SVBT.

Main results and the role of chance:
Halo was observed in 88% of fertilised oocytes. Zygotes without halo exhibited significantly higher rates of direct cleavage, reverse cleavage, and asymmetrical division compared with embryos derived from halo-present zygotes. Multivariate logistic regression analysis demonstrated significantly higher developmental rates to the expanded blastocyst stage in embryos with a halo compared to those without, regardless of its distribution (adjusted odds ratio (AOR): 0.435, 95% confidential interval (CI): 0.352–0.717, $P = 0.0004$). Halo presence did not correlate with embryonic morphological grade on days 2 and 5, or the ongoing pregnancy rate (AOR: 0.863, 95% CI: 0.358–2.080, $P = 0.7$). TI from tHa-c, tHc-r, and tHr-d were 4.7 ± 0.1, 7.4 ± 0.1, and 2.3 ± 0.1 h, respectively. TI prolongation from tHc-r ($P = 0.04$) and from tHr-d ($P < 0.0001$) significantly correlated with increased asymmetrical division at the first cell division ($P = 0.006$) and decreased developmental rates to the blastocyst expansion stage ($P = 0.002$). Halo event TIs did not correlate with embryonic morphological grade on days 2 and 5, or the developmental speed to the blastocyst stage. However, extended tHc-r was associated with a decreased ongoing pregnancy rate (AOR: 0.871, 95% CI: 0.788–0.959, $P = 0.006$).

Limitations, reasons for caution:
Although this is the largest and most detailed study of its kind, the uneven distribution between halo-present and halo-absent embryos indicates that there is still insufficient number of embryos in the halo-absent transferred group. Moreover, the data were collected from a single clinic.

Wider implications of the findings:
This is the first report demonstrating a correlation of the spatiotemporal halo phenomenon with blastocyst formation and pregnancy outcome using a time-lapse system. The association between halo characteristics and embryo viability suggests potential applications for diagnosing embryo viability, as a useful tool to aid embryo selection for transfer.

Trial registration number:
not applicable

O-114 Automated time-lapse microscopy for embryo selection: a Phase IV prospective, randomized controlled exploratory trial

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[5]Kinderwunsch Centrum München, IVF-Laboratory, Munich, Germany
[6]Merck KGaA, Global Medical Affairs Fertility, Darmstadt, Germany

Study question:
Are implantation rates for Day 3/5 embryo transfers selected using early embryo viability assessment (Eeva) plus morphology grading (MG) different than transfers selected using MG?

Summary answer:
Implantation rate did not differ between Eeva+MG and MG alone, possibly due to the high rate of non-compliance in the Eeva+MG group.

What is known already:
Eeva is a time-lapse embryo-monitoring system with integrated software that automatically analyses embryo images to define the precise timing of cell divisions and make a recommendation on the likelihood an embryo will develop to the blastocyst stage. Eeva predicts blastocyst formation at the cleavage stage with a specificity of 85%. However, results from several trials of Eeva to date demonstrate substantial heterogeneity (e.g., different embryo rankings, different primary endpoints, lack of generalizability across different centres), meaning the true benefit of Eeva as an adjunct to morphological assessment for embryo prediction has not been determined.

Study design, size, duration:
In this Phase IV, prospective, 2:1 randomized, exploratory clinical trial at 20 sites in eight countries (Canada, Germany, Italy, France, Norway, Sweden, Spain and UK), 970 participants were randomly assigned to Eeva+MG or only MG. 899 completed the trial: 593 (66%) in the Eeva+MG group and 306 (34%) in the MG group. The choice of embryo for transfer was determined by the embryologist. All analyses are descriptive.

Participants/materials, setting, methods:
Women diagnosed with infertility who failed ≤3 previous IVF/ICSI cycles were included. Participant recruitment continued for 9 months, and participants were followed up to Days 12–18 to verify implantation and up to gestational Weeks 5–8 to verify clinical pregnancy. The study duration was 19 months. The original primary endpoint (positive implantation with fetal heartbeat) was amended (implantation with intrauterine gestational sac) after database lock, owing to errors when recording the original endpoint.

Main results and the role of chance:
The implantation rate with intrauterine gestational sac at Week 8 was 30.1% (95% CI 27.2–33.0) in the Eeva+MG group and 35.3% (95% CI 31.1–39.5) in the MG group. Post-hoc supportive analyses were carried out to identify the patients who met the criteria for Eeva compliance (embryo selection for transfer was based on the Eeva score and the Eeva evaluation was applied as per instructions for use). This analysis showed that only 57.7% of patients in the trial met both compliance criteria, implying that almost a half of patients in the Eeva+MG group were assessed on MG alone or had incorrect application of Eeva. Additional post-hoc supportive subgroup analyses of the original endpoint in the Eeva-compliant subgroup showed the implantation rate with fetal heartbeat after Day 3 embryo transfer was 25.7% in the Eeva+MG group (n=163) and 32.7% in the MG group (n=117). For Day 5/6 transfer, the implantation rate was 45.5% in the Eeva+MG group (n=87) and 38.3% in the MG group (n=99). The implantation rate of the Eeva-compliant subgroup was equal or higher than that of the MG group at five of 12 sites (Day 3 transfer) and seven of 14 sites (Day 5/6 transfer).

Limitations, reasons for caution:

The low compliance was based on reliance on MG scores over Eeva scores and the suboptimal use of Eeva, potentially due to insufficient training of embryologists, meaning the true value of Eeva may not have been fully leveraged by these results.

Wider implications of the findings:

The results of this trial are in line with the inconclusive results reported for other trials of Eeva. Our findings also highlight the potential limitations in the conduct of trials of devices to improve embryo assessment and also in the training of embryologists in the use of the Eeva system.

Trial registration number:

NCT02417441

O-115 Quantitative analysis of compaction in human preimplantation embryos with time-lapse imaging and examination of its relevance to implantation

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Study question:

To analyze quantitative parameters of compaction during human embryo development by time-lapse imaging and their impact on blastocyst implantation.

Summary answer:

Dynamics and geometrical parameters were defined in order to describe human embryo compaction and no relation was observed with implantation success.

What is known already:

The shaping of the human embryo results from the action of morphogenetic forces generated by its own blastomeres. Previous studies on mouse embryos mapped the forces shaping the embryo during compaction using non-invasive biophysical methods [Maitre et al., 2015]. Mouse embryos compact at the 8-cell stage by increasing the tension at their surface (γcm) and by reducing the tension at cell-cell contacts (γcc). This changes the ratio of tensions ($\alpha = \gamma cc/2\gamma cm$) and leads to the increase of the contact angle (θ), making the embryo more compact. Very little is known about the forces shaping human embryo during compaction.

Study design, size, duration:

A total of 224 blastocysts obtained from diploid zygotes after IVF or ICSI at Day 5 or Day 6 between February 2016 and April 2017 were included in this retrospective study performed in the Reproduction Biology laboratory in Bichat Hospital (AP-HP, Paris, France). They were cultivated and imaged by time-lapse microscopy (EmbryoScope, Vitrolife). Among them, 117 blastocysts were transferred fresh (n=7) or after freezing-thawing cycles (n=110), corresponding to 75 oocytes retrievals (in 70 couples).

Participants/materials, setting, methods:

Videos were retrospectively analyzed to determine the timing of cell divisions (7-8, 8-9, 15-16 and 16-17cells) and compaction (disappearance of cell limits) and to measure the contact angles at their stages. They were measured using the angle tool from Fiji software through the equatorial plane of two contacting cells. Embryos were classified according to their compaction stage and the relation to implantation success was evaluated. Only embryos reaching the blastocyst stage (Gardner's classification) were considered.

Main results and the role of chance:

During compaction, the contact angle (θ) grew from (103° ± 9°) to (151° ± 9°) (mean ± SEM, n=224), all stages considered. The majority of embryos (64%, n=144) increases their θ angles between the initial 16-cell stage (15 to 16 cells) and the final 16-cell stage (16 to 17 cells). A minority of embryos compacted during the 8-cell stage (36%, n=80). We find no difference in the compaction stage (repartition of compaction stage : 7-8 cells stage 9%. *vs*. 5%, 8-9 cells stage 22% *vs*. 22% and 16 cells stage 69% *vs*. 73%, n=117, P=0.67), in the maximal θ angle (150° ± 8° *vs*. 151° ± 11°, n=117, Student's *t* test P > 0.05) or in the compaction rate between embryos that implant and those which do not (α=0.328 ± 0.095 *vs*. 0.294 ± 0.071, n=117, Student's *t* test P=0.42).

Limitations, reasons for caution:

Further investigations are required to determine whether some differences in culture conditions or fertilization method (IVF or ICSI) could impact the parameters of embryo compaction and to evaluate their interest as a predictor of human embryo development.

Wider implications of the findings:

This study defines objective and physically relevant parameters for the quantitative analysis of compaction of human embryos reaching the blastocyst stage. We find that compaction takes place at the 16-cell stage in the majority of embryos but can also occur at other cleavage stages, without impact on implantation success.

Trial registration number:

Not concerned

O-116 Non-invasive embryo selection: Live birth can be predicted by means of the kinetic analysis of female and male pronuclei formations

J. Otsuki[1,2], T. Iwasaki[2], N. Enatsu[2], Y. Katada[2], K. Furuhashi[2], M. Shiotani[2]

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[2]Hanabusa Women's Clinic, Reproductive Medicine, Kobe- Hyogo, Japan

Study question:

Is there a method of non-invasive embryo evaluation which may exceed the success rates of PGT-A using trophectoderm cells?

Summary answer:

Yes. We have established a non-invasive evaluation of euploid embryos, focusing on kinetic analyses, from the second polar body extrusion to the pronuclear membrane breakdown.

What is known already:

It is generally known that even if embryos are implanted by means of ART procedures, nearly 30 % of them miscarry. Most miscarried embryos are chromosomally abnormal. To select embryos most likely to be normal and result in a successful transfer, the kinetic analysis of embryos using time-lapse observation systems has been employed in many ART laboratories worldwide. Although at present the precise chromosomal abnormalities, such as mosaic or subchromosomal imbalances, cannot be detected without performing pre-implantation genetic testing for aneuploidy (PGT-A), the live-birth rate of embryos transferred after PGT-A is around 50%.

Study design, size, duration:

A retrospective study of 213 frozen-thawed single blastocyst transfers(ICSI:112, IVF:101) between June 2013 and December 2016 was performed. Normal live birth was considered a successful conclusion to this study.

Participants/materials, setting, methods:

Time-lapse recordings were performed and the areas of male and female pronuclei were retrospectively analyzed by measuring their vertical and horizontal diameters. These measurements were taken 4 and 8 hours before the pronuclear membrane breakdown (PNMBD), and immediately before the PNMBD. The difference in microns squared of the 2PNs in embryos resulting in live-births was compared with that of embryos from failed pregnancies.

Main results and the role of chance:

The cut-off values for the differences in area between male and female pronuclei immediately before PNMBD, among embryos resulting in live birth, were 39.3 (AUC: 0.631, CI: 0.518-0.745) for IVF, and 40.0 (AUC: 0.670, CI: 0.568-0.771) for ICSI. (Definition A: the difference in area between the male and female pronuclei immediately before PNMBD should be below the cut-off value). Of the 15 embryos which had female pronuclei larger than male pronuclei 8 hours before PNMBD, none achieved live birth. (Definition B: The size of male pronuclei 8 hours before PNMBD should be larger than female pronuclei). The difference in the sizes of pronuclei in patients with successful births gradually decreased (IVF: $p<0.001$, ICSI: $p=0.012$), while the difference in the size of pronuclei in patients who did not achieve live birth did not decrease.

(Definition C: The difference in size between the male and female pronuclei 8 hours before PNMBD should be larger than the size of immediately prior to PNMBD). When normal embryos were defined as A∪C∩B, the birth rates for IVF and ICSI were 68.1% (47/69) and 50.0% (32/64) respectively. For the remaining embryos, defined as abnormal, birth rates were 9.3% (3/32) for IVF and 4.2% (2/48) for ICSI.

Limitations, reasons for caution:

Birth could have occurred following natural conception, which sometimes occurs during frozen-thawed embryo transfer cycles with hormone therapy. As the detection rate for male and female pronulcei with our current time-lapse system was approximately 50%, this method of evaluation requires further improvement prior to implementation.

Wider implications of the findings:

In this study, we established a method of non-invasive embryo evaluation which may exceed the success rates of PGT-A using trophectoderm cells. Evaluating and transferring embryos at an early stage could simplify laboratory procedures and avoid monozygotic twinning caused by prolonging the use of culture medium to the blastocyst stage.

Trial registration number:

Not applicable

O-117 Impact of reverse cleavage on in vitro development and reproductive outcomes of human embryos

J.M. Capdevila[1], S. Novo[1], J. Massó[1], M. Solans[1], B. Freijomil[1], C. Castelló[1], M. López-Teijón[1]

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Study question:

What are the effects of reverse cleavage (RC) on embryo development and clinical outcomes on in vitro fertilization (IVF) treatments?

Summary answer:

RC has drastic effects on embryo development. However, embryos with RC achieving a good quality blastocyst stage have equal reproductive outcomes as embryos without RC.

What is known already:

Reverse cleavage has gone unnoticed until the implementation of time-lapse technology on IVF labs. The influence of RC is poorly documented, and its effects are unclear. Most of the studies describe a multinucleation increase on embryos with RC. However, there are some discrepancies about the RC effects on the embryo development and clinical outcomes. Several publications have proved no detrimental effects on in vitro development of embryos with RC, but a clear decrease in implantation and pregnancy rates. Conversely, other authors published a lower blastocyst rate but an equivalent euploid rates on embryos with RC.

Study design, size, duration:

This is a retrospective study including 23,340 embryos from 6,340 cycles performed between 2014 and 2018. The sample was split in two groups: RC negative group (RC-) with 23,007 embryos without reverse cleavage in D+2 or D+3 and, RC positive group (RC+) represented for 303 embryos showing reverse cleavage in D+2 and/or D+3.

Participants/materials, setting, methods:

All the cycles were inseminated through intracytoplasmic sperm injection technique and the embryos were cultured in time-lapse incubators until blastocyst stage using one step culture media. Good quality blastocyst, multinucleation, clinical pregnancy and live birth rates were compared between embryos with and without RC. In vitro fertilization cycles inseminated conventionally and/or cultured inside incubators without time-lapse technology were excluded from this study. A Chi-square test has been used to compare the groups.

Main results and the role of chance:

At least one RC event was showed by 1.3% of the embryos on day 2 or 3 of culture (n =303). This RC rate fit with previously published values for others laboratories which ranges between 0.4% and 7%.

The in vitro development rate of embryos RC+ up to day 5/6 (19.1%) was significantly lower than the embryos RC- (54.2%) ($p<0.05$). Also, the presence of some multinucleated cell was more frequent in embryos RC+ (71.5%) than embryos RC- (33.5%) ($p<0.05$). However, embryos RC+ achieving a good quality blastocyst stage showed the same clinical results as blastocysts RC-. In this sense, clinical pregnancy and live birth rates were equivalent between blastocyst RC+ (54.2%; 37.5%) and blastocysts RC- (55,9%; 36,5%). By these findings, the power of the blastocysts culture is enhanced and shows us how embryos can successfully overcome abnormal encounter cleavage patterns, such as reverse cleavage.

Limitations, reasons for caution:

RC happens with a relative low frequency on embryo development. There is a huge difference on the sample number between the compared groups.

Wider implications of the findings:

With the obtained results, we consider that RC could be a part of some cellular error-detection mechanism. Embryos that are able to overcome this checkpoint, repairing or discarding the involved cells to reach a good quality blastocyst, would have the same reproductive potential as blastocyst without RC.

Trial registration number:

Doesn't apply

O-118 Time-lapse imaging acquired nucleation status of 2-cell pre-implantation embryos is predictive of implantation and live births following IVF

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[2]Norwegian National Advisory Unit on Women's Health, Oslo University Hospital, Oslo, Norway

[3]BMP Analytics, Consultancy, Viby J, Denmark

Study question:

May nucleation error phenotypes during the first embryo cleavage as observed by time-lapse imaging be used to predict implantation and live birth following IVF treatment?

Summary answer:

Nucleation errors at the 2-cell embryo stage may be used in selecting embryos for transfer and be predictive of implantation and live birth.

What is known already:

Reliable selection of embryos based on morphology and morphokinetic patterns has been promoted in clinical practice with the establishment of Time-lapse imaging (TLI). One visible nucleus in blastomeres has been indicative of the implantation potential of embryos. Nucleation errors such as binucleation (presence of two nuclei per blastomere), micronucleation (one or two larger nuclei surrounding several smaller nuclei) and multinucleation (more than two similar sized nuclei per blastomere) have been indicative of lower implantation rates and higher miscarriage rates. Self-repair of multinucleated embryos however during early cleavage divisions has been shown to occur, resulting in euploid blastocysts and live births.

Study design, size, duration:

Retrospectively collected TLI data derived from transferred embryos cultured in the EmbryoScope ™ incubator between June 2011 and August 2018 in a private IVF clinical setting was included. Only Known Implantation Data (KID) for implantation and live births were used. Nucleation errors such as micronucleation, binucleation, multinucleation and other minor error groups, were annotated in the time-lapse images which were taken every 10 or 20 minutes for a minimum of 44 hours post insemination.

Participants/materials, setting, methods:

Nucleation error phenotypes (NEP) data was collected from TLI for 2959 transferred embryos. A total of 2082 treatment cycles with exact traceability of transferred embryos were included in the analysis. The association between NEPs and both known implantation and live birth was assessed. The analysis controlled for potential confounding factors by adjusting for maternal age (above and below 36 years) and infertility diagnosis. Fisher's exact test was used to analyse categorical variables.

Main results and the role of chance:

The analyses showed statistically significant lower implantation and live birth rates for embryos with all types of nucleation errors at the 2-cell stage (22.9% of the transferred embryos), compared to embryos not presenting nucleation errors; 14.2% vs. 23,9% for implantation and 11.8% vs. 21.0% for live birth respectively, $P<0.001$. No differences in NEP occurrence were observed when the results were stratified according to type of NEPs and infertility diagnoses.

The occurrence of NEP was significantly ($P<0.02$) higher for women > 36 years old. The woman's age by itself had a significant effect on implantation and live birth rates; 32.9% vs. 10.5% and 29.6% vs. 8.5% respectively for women less than and more than 36 years' old; $P<0.001$. The decline in implantation and live birth by NEP occurrence was 40.7% and 43.8% respectively and was unaffected by age.

Retrospective TLI data analysis of almost 3000 transferred embryos offers a strong indication towards predictive role of NEPs in embryo selection. Given the large data set and high statistical significances in the study, the role of chance in this case may be limited. Hence, confounding factors that can arise due to transfer selection and exclusively analysing embryos with known implantation may be more important.

Limitations, reasons for caution:

Rates are given per KID /embryo, which cannot be compared directly with per treatment rates. Only transferred embryos were analysed retrospectively, so the effect of TLI on other NEP embryos and hence their ability to self-repair is not investigated.

Wider implications of the findings:

Our study provides insight regarding the role of nucleation errors on live births. None of the investigated NEPs completely invalidate the possibility of live birth, but rather lowers the probability of this occurrence. Therefore, the significance of incorporating nuclear status annotations in embryo selection, alongside morphology and morphokinetics, is imperative.

Trial registration number:

INVITED SESSION

SESSION 35: LIVE SESSION

Tuesday 25 June 2019 Haydn 1 10:00 - 13:00

SELECTED ORAL COMMUNICATIONS

SESSION 36: STEM CELLS TO IMPROVE REPRODUCTIVE FUNCTIONS

Tuesday 25 June 2019 Haydn 3 10:00 - 11:30

O-119 Differentiation of embryonic stem cells into germ line cells using decellularized seminiferous tubules bathed in a conditioned medium

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Study question:

We test the suitability of a biochemical and biomechanical microenvironment to allow differentiation of mouse embryonic stem cells into male germ line cells.

Summary answer:

A bioreactor concept approach supported by a biological scaffold allows de novo generation of meiotic male germ cells from murine embryonic stem cells.

What is known already:

In the past decade, tissue engineering has been a rapidly developing field of study that aims at artificially regenerating functional tissues. Extracellular matrix (ECM)-derived scaffold produced by decellularization of different types of connective tissues can provide a microarchitecture suitable for tissue regeneration for prosthetics and transplantation by allowing stem cells to regenerate functional tissue in vitro. In vitro testicular tissue culture can be used to study the process of spermatogenesis. The differentiation process, however, has several limitations due to the complex architectural organization of the seminiferous epithelium and requires a suitable three-dimensional scaffold to progress.

Study design, size, duration:

Eighty embryonic bodies (EBs) constituted mainly by PGCLCs were generated for utilization in the bioreactors, in contact with decellularized seminiferous tubule matrices (DSTMs) and bathed in media conditioned by interstitial cells isolated from adult mouse testes. Ninety 3-mm sections of DSTM, longitudinally sliced open and flattened, were derived from 3 adult B6D2F1 mice; interstitial cells were isolated from the respective contralateral testes. Germ cell stage–specific markers were assessed every 3 days after complete recellularization.

Participants/materials, setting, methods:

Interstitial cells were isolated through differential plating and layered on the interphase of twelve bioreactors. Seminiferous tubules, isolated from the contralateral testicles of these mice following decellularization by exposure to 1% sodium dodecyl sulfate for 24 hours, were placed below the interphase in direct contact with differentiated cells from digested EBs. DAPI and H&E staining were used to confirm complete decellularization. Cell characteristics were analyzed by germ cell stage–specific markers on an H&E-stained background.

Main results and the role of chance:

After culturing mESCs in Activin A, bFGF, and KSR for three days, persistent expression of OCT4 (>90%) and decreased positivity of Nanog (45%) indicated successful progression to EpiLCs. PGCLCs rich in embryonic bodies expressed positive surface SSEA-1 after six days of culture in hanging droplets containing BMP4, BMP8b, SCF, LIF, and EGF. After incubation in the bioreactor, the earliest attachment of PGCLCs onto DSTM occurred on day 3, and complete recellularization occurred approximately at day 10, as observed by optic microscopy. Following complete recellularization, about half of all isolated cells, which were obtained from the enzymatic digestion of recellularized tubules from the bioreactor, displayed decreased expression of OCT4, while about 5% displayed nuclear DAZL positivity at day 10. At around day 16, cytoplasmic VASA staining in 5% of the cells suggested meiotic/post-meiotic germ line differentiation. In 1% of the cells, a perinuclear DAZL confirmed spermatocyte differentiation. The timeline of our bioreactor system was comparable with in vivo spermatogenesis in the mouse, occurring in the course of 21 days.

Limitations, reasons for caution:

Although this culture method is capable of providing a suitable microenvironment to initiate spermatogenesis in vitro, complete in vitro spermato-/spermiogenesis remains challenging. Further refinements of the bioreactor are required to allow complete differentiation into the later post-meiotic stages.

Wider implications of the findings:

The ability of a 3D biocompatible scaffold to induce maturational meiosis will provide insights to overcome human spermatogenic arrest. Neogametogenesis from genotyped stem cells carried out in a scale-down microfluidic device may help to treat men afflicted by Sertoli cell-only syndrome.

Trial registration number:

N/A

O-120 Human embryonic stem cells derived trophoblastic spheroids (BAP-EB) as human embryo surrogate for identification of attachment-related endometrial surface molecules

Y.L. Lee[1], S.W. Fong[1], C.L. Lee[1], C. Yue[1], K.C. Lee[1], A.C.H. Chen[1], K.F. Lee[1], W.S.B. Yeung[2], E.H.Y. Ng[1]

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Study question:

Does BAP-EB induce expression of attachment-related molecules in endometrial epithelial cells?

Summary answer:

BAP-EB produced soluble factor(s) that induced expression of surface proteins on endometrial cells involving in BAP-EB-endometrial cells attachment.

What is known already:

Implantation failure can be caused by impaired endometrial receptivity. However, mechanistic study of the implantation process in humans is limited by lack of an appropriate implantation model. Trophoblastic spheroids (BAP-EB)

derived from human embryonic stem cells by bone morphogenic protein 4, A83-01 and PD173074 treatment possess human blastocoel-like structure and attach specifically onto receptive endometrial epithelial cell lines. The use of BAP-EB as human embryo surrogate for mechanistic study of early implantation is explored in the current report.

Study design, size, duration:

Human embryonic stem cells (H9) were induced to differentiate into BAP-EB for 48h and 72h. Endometrial epithelial cells (EEC) were isolated from endometrial biopsies obtained from infertile women on day 2 (hCG+2, n=20) or day 7 (hCG+7, n=13) post-hCG induction of ovulation. The BAP-EB spent media were collected before (0h) and after BAP differentiation for 48h and 72h.

Participants/materials, setting, methods:

The attachment rates of H9-BAP-EB onto the cultured EEC were compared after 3 hours of coculture. The spent media of BAP-EB-48h (attachment incompetent) or BAP-EB-72h (attachment competent) were used to treat the receptive endometrial Ishikawa cells for 3 hours, and the differentially expressed cell surface proteins were identified by mass spectrometry. The functional roles of the target surface proteins were studied by antibody blocking during the attachment assays.

Main results and the role of chance:

The attachment rates of BAP-EB derived from H9 onto EEC isolated from receptive (hCG+7 day) phase were significantly higher than those of pre-receptive (hCG+2 day) phase (hCG+7: 26.7±14.7% vs hCG+2: 2.3±3.0%, $p<0.001$) endometria. After incubation with the spent media of attachment competent (72h) or incompetent (0h, 48h) BAP-EB for 3 hours, cell surface proteins of treated endometrial Ishikawa cells were biotinylated and identified by liquid chromatography-mass spectrometry. In total, eight cell surface proteins were induced by the spent medium of attachment competent BAP-EB-72h when compared to that of the incompetent BAP-EBs. Five of them namely Catenin alpha-1, Catenin delta-1, Plexin-B2, Extended synaptotagmin-2 and CD98hc were selected for further study. Their expressions were significantly higher in the receptive endometrial cell lines (RL95, Ishikawa) when compared to the non-receptive ones (HEC-1B, AN3CA). The BAP-EB attachment rates were significantly reduced after treating Ishikawa cells with antibodies against CD98hc (42%) and Catenin delta-1 (36%) when compared to normal IgG control (62%, $p<0.01$, chi-square test).

Limitations, reasons for caution:

The soluble factor(s) that secreted from BPA-EB have not been identified. BAP-EB and the endometrial cell lines used may not fully represent the in vivo developed human blastocysts, and the primary endometrial cells, respectively.

Wider implications of the findings:

The number of human embryos donated for research use is limited. The results obtained in this study demonstrated the usefulness of BAP-EB as human embryo surrogate for studying human implantation, and the role of human embryos in inducing attachment related endometrial epithelial cell surface molecules.

Trial registration number:

nil

O-121 Improving three-dimensional in vitro culture methods of human endometrial stem cells: bioengineering tissue-specific hydrogels

S. López Martínez[1], H. Campo[1], L. De Miguel-Gómez[1], A. Faus[1], H. Ferrero[1], I. Cervelló[1]

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Study question:

Can tissue-specific extracellular matrix (ECM) hydrogels derived from decellularized (DC) porcine endometrium improve three-dimensional (3D) culture of human endometrial stem cells (ICE6-7, Cervelló et al.,2011)?

Summary answer:

ECM hydrogels from DC porcine endometrium are biocompatible and improve ICE6-7 proliferation compared to standard 3D culture, showing much potential for future regenerative medicine purposes.

What is known already:

The use of hydrogels made of tissue-specific ECM from DC organs represents a revolution in regenerative medicine due to its therapeutic potential. This is attributable to the presence of bioactive components that mimic the natural tissue-specific environment, together with low immunogenicity. Recently, our group has established a method to decellularize whole pig uteri (Campo et al., 2017). Here we furthered this line of investigation using the DC organ to create endometrial ECM hydrogels. These were used in novel *in vitro* approaches and will be tested in the near future *in vivo* as a novel solution for untreatable endometrial pathologies.

Study design, size, duration:

Pig uteri (n=4) were collected for whole organ decellularization, endometrium isolation and hydrogel creation. Physicochemical features were determined and compared with hydrogels from a non-DC endometrium and DC myometrium. Biocompatibility of DC endometrial hydrogels (at 3, 6 and 8 mg ECM/ml) were studied using stem cell lines from epithelial (ICE6) and stromal (ICE7) origins (n=3) during 24 and 72hrs in different conditions. Standard matrix for 3D culture, collagen and Matrigel, were used as controls.

Participants/materials, setting, methods:

To create hydrogels, uteri were decellularized by perfusion and endometrium was isolated by microdissection. Subsequently, DC endometrial tissue was milled, lyophilized and enzymatically digested. The characterization was carried out by turbidimetry, scanning electron microscopy (SEM), quantification of ECM proteins and proteomic analysis. ICE6 and ICE7 cell lines were cultured on top of (2.5D) or embedded in (3D) hydrogels and cell proliferation was measured by MTS Assay. Two-way Anova was used for statistical analysis.

Main results and the role of chance:

While decellularization removes much of the protein fraction (81%), a significant amount of elastin (18%) and glycosaminoglycans (18%) was retained. Moreover, a significant enrichment of collagen (50%) was observed. SEM showed that endometrial ECM hydrogels are porous scaffolds with a homogenous, randomly interlocking fibrillar structure. No significant differences in fiber thickness (0.10 µm) and ultrastructure were found between non-DC endometrial, DC myometrial hydrogels and different concentrations. Turbidimetry showed that stable endometrial ECM hydrogels quickly formed after incubation at standard culture conditions (12.97±1.46 min at 37°C), independently of the concentration. After 72hrs, cell proliferation of both cell lines was improved in 3D culture system with endometrial ECM hydrogel (all concentrations) compared to collagen and Matrigel controls. A fold change of up to 2.13 and 2.11 (6 mg/ml $p<0.0001$) compared to collagen, for ICE7 and ICE6 respectively was measured. In 2.5D culture, collagen and Matrigel poorly retained the cells compared to the endometrial hydrogels. Here, a fold change of up to 1.84 and 1.83 (8mg/ml, $p<0.0001$) for ICE7 and ICE6 respectively was observed. These data showed that porcine endometrial ECM hydrogels are biocompatible and improve *in vitro* proliferation of human endometrial stem cells.

Limitations, reasons for caution:

This study was only performed with endometrial stem cell lines, *In vivo* studies will be required to confirm the regenerative potential of endometrial ECM hydrogels under pathological conditions.

Wider implications of the findings:

These findings support the hypothesis that porcine endometrial ECM hydrogels are biomimetic to the native endometrium and stem cell niche. This study represents a first step to the use of this biomaterial to improve 3D *in vitro* culture, opening a window towards *in vivo* studies and treatment of endometrial pathologies.

Trial registration number:

not applicable

O-122 Single cell sequencing reveals gene expression signatures and key transcriptional regulators during human folliculogenesis.

Y. Zhang [1], Z. Yan [1], Q. Qin [1], J. Yan [1], L. Yan [1], J. Qiao [2]

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[2]peking university third hospital, medical center for human reporduction, beijing, China

Study question:

Can single-cell resolution RNA-seq analysis on a cohort of women showing the dynamic transcriptional regulation and interactions of human germlines and surrounding somatic cells during folliculogenesis?

Summary answer:

This approach is effective to clarify the transcriptome datasets of oocytes and granulosa cells and to reveal signature genes and candidate markers for ovarian reserve.

What is known already:

Folliculogenesis is a complex and highly regulated process that involves bidirectional interactions of the oocytes and surrounding granulosa cells. Follicle growth and oocyte maturation are associated with dynamic transcriptional regulation in both oocyte and GC compartments of the follicle. The selection of primordial follicles in the ovarian reserve, established early in life, provides all growing follicles and ovulated oocytes. The transition from the primordial to the growing follicle through the antral follicle, which culminates in dominance was achieved in waves by one or more follicles.

Study design, size, duration:

To explore the mechanism of transcriptional regulation of human follicle development and oocyte-granulosa interactions, the whole transcriptome datasets of human oocytes and granulosa cells at each stage of folliculogenesis were obtained.

Participants/materials, setting, methods:

We collected adult ovarian tissues, digested them by mechanical and enzymatic digestion, isolated and obtained follicles and oocyte-granulosa cell complex in five developmental stage (primordial follicle, primary follicle, secondary follicle, antral follicle and preovulatory follicle). Furthermore, follicles or oocyte-granulosa cell complexes were digested to obtain oocytes and granulosa cells. The single cell cDNA libraries of oocytes and granulosa cells were obtained by single cell transcriptome amplification, and transcriptome sequencing was performed.

Main results and the role of chance:

According to the characteristics of transcriptomic profiles, oocytes and granulosa cells can be divided into five groups, which are coincident with the follicular development stage (primordial follicle, primary follicle, secondary follicle, antral follicle and preovulatory follicle). The stage-specific expressed genes of oocytes and granulosa cells at different stages are identified, as well as secretory protein coding genes that are candidate biomarkers for ovarian reserve. During the activation of primordial follicles, Insulin, GnRH, Neurotrophin and mTOR pathways may play a co-regulatory role in oocytes and granulosa cells, while JAK-STAT pathway may play a regulatory role in granulosa cells. The analysis of the interaction between oocytes and granulosa cells during follicular development indicated that NOTCH and TGF-β signaling pathways exhibited stage-specific interaction during follicular development.

Limitations, reasons for caution:

Despite rigorous experimental approach and robust analyses, our findings are not sufficient to confirm the functional characteristics of the identified transcripts. Our methods relied on the mechanical or enzymatic dissociation of follicular compartments, thus the information on temporal and spatial regulation of gene expression in GCs was not reflected.

Wider implications of the findings:

This provides key insights into the crucial features of the transcriptional regulation in the stepwise folliculogenesis and offers important clues for improving follicle recruitment *in-vivo* and restoring fully competent oocytes *in-vitro*.

Trial registration number:

not applicable

O-123 Human Adipose Tissue-Derived Stem Cells improve ovarian function in a mouse model of chemotherapy-induced partial Premature Ovarian Failure

G. Salvatore[1], A. Camaioni[1], M. De Felici[1], S. Dolci[1], F.G. Klinger[1]

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Study question:

Are human Adipose Tissue-Derived Stem Cells (hADSCs) able to counteract the impairment of ovarian function induced by chemotherapy?

Summary answer:

hADSC transplantation alleviates the damage induced in the ovary by busulfan (BU) and cyclophosphamide (CY) reducing follicle depletion and improving the oocyte quality.

What is known already:

Mesenchymal Stromal Cells (MSCs) hold great promise in the field of regenerative medicine because of their ability to home to the injury site, where they repair tissues mainly secreting various growth factors, cytokines and chemokines that decrease local inflammation. Several studies have recently shown that the *in situ* or systemic injection of MSCs of various animal species and from different anatomical sources are able to restore impaired ovarian functions in animal models of Premature Ovarian Failure (POF), but no information exists whether hADSCs, which are the least invasive MSCs to harvest, possess such capability.

Study design, size, duration:

Thirty-seven 11 weeks old female CD1 mice with regular oestrous cycle were randomly assigned to four groups: vehicle treated control group (CTR), chemotherapy group (CH), *in situ* (IS) and intravenous (IV) hADSC transplanted groups. Chemotherapy and transplanted groups received a single injection of BU+CY to induce POF. A week later (day0) IS and IV received cell transplant. At day28 mice were sacrificed to collect ovaries, or superovulated and mated to recover 2-cell embryos.

Participants/materials, setting, methods:

12mg/kg BU and 120mg/kg CY were intraperitoneally administered. hADSCs were isolated from female patients and injected into one of the two ovaries for IS group (75.000 cells) or through the tail vein for IV group ($1x10^6$cells). Body weight was recorded at day0 and day28. Ovaries were weighted at day28 and sectioned for follicle counting (H&E staining). After superovulation and harem mating, 2-cell embryos were isolated and developed *in vitro* up to blastocysts.

Main results and the role of chance:

At day28, IS and IV females showed a significant body weight increase compared to day0 (IS: 36.1±2.0g vs 31.0±1.7g, $p<0.001$; IV: 32.8±1.9g vs 29.3±3.1g, $p<0.01$; n=3), while such increment did not occur in CH or CTR groups. Moreover, ovarian weight, normalized for the animal weight, was significantly higher in the same groups compared to CH group (IS: 0.97±0.07mg/g and IV: 1.02±0.03mg/g vs CH: 0.64±0.12mg/g, $p<0.05$ and $p<0.01$, n=2). Interestingly, for IS group, ovarian weight significantly increased also when compared to the untreated controlateral ovary (0.97±0.07mg/g vs 0.65±0.05mg/g, $p<0.05$, n=2). Follicle number in CH ovaries was significantly lower than in CTR (CH: 572.2±138.1 vs CTR: 2688.0±65.9, $p<0.01$, n=2), while such depletion was reduced in IV (CH: 572.2±138.1 vs IV: 1399.0±145.8, $p<0.05$, n=2). If such difference occurs also in IS ovaries is under investigation. Despite the reduced number of follicles, superovulated CH females did not show reduced fertilization rate or number of 2-cell embryos compared to the other groups. However, the percentage of 2-cell embryos able to develop into blastocyst was significantly higher in IV compared to CH (65.8±6.0% vs 36.8±3.3%, n=2, $p<0.05$), but still slightly lower than CTR group (CTR: 90.9±1.7%, p=0.051, n=2).

Limitations, reasons for caution:

Results were obtained from low number of animals. The chemotherapy protocol used, although caused a marked depletion of follicles, did not result in loss of fertility. Different chemotherapy and mating protocols must be chosen to reproduce POF. The transfer of the results obtained in mice to human requires caution.

Wider implications of the findings:

The results suggest that hADSCs transplantation might be useful to alleviate follicle depletion and improve the quality of oocytes in chemotherapy treatments of human patients. In this regard, hADSCs would provide a new, abundant, easy to harvest and minimally invasive alternative source of MSCs for clinical treatment of chemotherapy-induced POF.

Trial registration number:

Not applicable

O-124 Autologous bone marrow derived CD34+ stem cell therapy for refractory Asherman syndrome (AS) and endometrial atrophy (EA): 5 year follow up study

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Study question:
Can bone marrow derived stem cells (BMSCs) regenerate functional endometrium in cases of endometrial atrophy (EA), or Asherman Syndrome (AS) and improve fertility outcome?

Summary answer:
Autologous bone marrow derived stem cell treatment is promising novel approach for refractory cases of Asherman syndrome (AS) and endometrial atrophy (EA) and improves fertility.

What is known already:
Endometrium has high capacity for self renewal- 400-500 cycles in reproductive life, regulated by Hormones. Endometrium derived stem cells (EDRSCs) present in niche basal layer are responsible for regeneration of endometrium after complete shedding. It is the absence of functional endometrium, which leads to endometrial atrophy (EA) or intrauterine adhesion (AS) and infertility. BMSCs are CD34+ adult stem cells with characteristics of self-renewal, multi potential differentiation, and auto transplantation without immune rejection. Since there is no effective treatment available for refractory AS and EA, based on these observations, the role of BMSCs for endometrial regeneration was explored

Study design, size, duration:
Prospective, single arm longitudinal study, 25 patients (12 with AS and 13 with EA)

5 year follow up study. Patients underwent stem cell implantation between September 2013 and January 2014 and each patient was followed up at 3 months, 6 months, 9 months and 5 year interval.

approved by the Institutional Ethics Committee and National Human Ethics Committee for Stem Cell therapy & Research. Untreated patients were internal controls

Participants/materials, setting, methods:
25 women (24 - 37 years) with AS and/or EA with infertility (16 primary and 9 secondary)

19 =scanty menses, 6 = amenorrhea. all cases had failed to respond to standard treatment (Hysteroscopic adhesiolysis and extended estrogen treatment).

Tertiary care University hospital

Bone marrow aspirated from iliac crest of cases,Stem cells harvested, CD 34+ cell count done

2-3 ml injected sub-endometrial zone at 2-3 sites once under trans-vaginal ultrasound (TVS)guidance by vaginal route using oocyte aspiration needle

Main results and the role of chance:
Statistical analysis using STATA version 9.0. Changes in ET values due to treatment at 3, 6, 9 months and 5 years were compared using paired *t*-test. For all the statistical tests $P < 0.05$ was considered for statistical significance. Menstrual flow and endometrial thickness were assessed at 3, 6, 9 months and 5 years. Mean ET (mm) prior to treatment was 3.3 + 1.0 which increased to 5.1+ 1.9 (P = 0.001), 5.6 + 1.5 (P = 0.001) and 6.1 + 1.7 (P = 0.001) at 3, 6 and 9 months respectively. 6 out of 7 cases presenting with secondary amenorrhea resumed menses 6 months after treatment. At the 5-year visit, only 15 patients (7 with AS and 8 with EA) showed up. EA subgroup showed improvement in Endometrial thickness in 6 of 8 cases. All 15 cases showed improvement in the endometrial cavity on 4D USG and hysteroscopy. Fertility outcomes (n=15) 2 conceived spontaneously 3 years after treatment, 1 conceived 4 years post treatment by in-vitro fertilization (IVF) with successful pregnancy outcomes (20%). 1 had an ectopic pregnancy following IVF which required salpingectomy. There were no pregnancies among untreated controls.

Limitations, reasons for caution: Small sample size (but largest amongst published literature till date) and high loss to follow up (due to a long follow up period).

No reason for caution. No adverse effects of therapy noted in any of the patients.

Wider implications of the findings:
Stem cell treatment may give new hope for infertile women with refractory Asherman syndrome and endometrial atrophy who may otherwise require surrogacy, which is facing scrutiny world over. However more robust multi centric studies are needed to validate the results.

Trial registration number: Trial was registered with CTRI - CTRI/2013/08/003896

SELECTED ORAL COMMUNICATIONS

SESSION 37: RECURRENT PREGNANCY LOSS AND IMPLANTATION FAILURE

Tuesday 25 June 2019 Haydn 2 10:00 - 11:30

O-125 Artificial Intelligence (AI) based-method applied in recurrent pregnancy loss (RPL) patients diagnostic work-up and classification: a potential innovation in common clinical practice.

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[9]Tor Vergata University Hospital, Medical Engineering Service, Rome, Italy
[10]Tor Vergata University Hospital, Surgical Sciences Department for Integrated Activities- Section of Gynecology, Rome, Italy

Study question:
To classify RPL patients in different risk class by AI *algorithm*, through a diagnostic work-up to validate it for the appropriate prognosis and therapeutic approach.

Summary answer:
AI approach provides a *Support Decision System*tool to stratify RPL patients and address them in an objective way to the proper clinical management.

What is known already:
RPL is a very debated and controversial field for researchers and clinicians. RPL definition, etiological factors to investigate, therapies to apply are not shared by the several research groups in this field: this leads to the inability of reaching an evidence-based medicine level, through systematic reviews or metanalysis. In this line, *biomedical engineering* and AI could lead the clinicians to reach an objectiveness in RPL access to care. Furthermore, although there are several known causes for RPL, 40-50% of the cases remains unexplained, and the clinical approach to this subgroup of patients is debated.

Study design, size, duration:
Retrospective study on data collected from the "Tor Vergata University Hospital" Gynecologic Section, and analyzed by Biomedical Engineering Team of the same hospital. 677 patients were enrolled: 579 cases (RPL) and 98 controls, according to the ESHRE guidelines (2017) RPL definition. Patients were divided into 4 risk class, according to the numbers of miscarriages (I class: 0 miscarriage; II class: 1 miscarriage; III class: 2-3 miscarriages; IV class $\geq$ 4 miscarriages).

Participants/materials, setting, methods:

A *bootstrap* procedure was performed to balance the dataset risk class size: to deal with the problem of low size of control study population (since it does not undergo the specific RPL diagnostic work-up), we introduce the *bootstrap method* to balance the numbers of each class patients. This procedure was essential to apply the AI method, called *Support Vector Machine*(SVM).

Main results and the role of chance:

The *training set* is made of 42 *features*(diagnostic assays) and their corresponding *labels*(risk class), which in combination generate a model, by an *algorithm*, to use in the *testing set*. In the *testing set* each patient's *features* are introduced in input to obtain the predicted *label* in output, by using the model created. In the *testing set* each patient's result is represented as a percentage of belonging to the different risk classes and the higher percentage is assumed as the *predicted class*. By comparing the *predicted class* versus the *real class* of all patients, we have obtained a correct classification in 81,5% of cases. By applying the same method, introducing the only *features* recommended by ESHRE guidelines 2017, we obtain a correct classification in 56,5%. The method used in this study is in line with the scientific trend drawing RPL as a multifactorial disease, which could be represented by a *threshold model*. This model could help to find the correlation between different factors that cannot cause itself the disease, but that taken together lead to a *classification score* for the appropriate clinical management. The diagnostic standardization could be reinforced by the application of AI to this clinical model.

Limitations, reasons for caution:

SVM method applied prospectively in a larger population could lead to a higher percentage of correct classification. On the other, to obtain this, the diagnostic work-up applied in this study, should be extended to both sporadic and recurrent pregnancy loss couples, weighed down with the national health system finance.

Wider implications of the findings:

The AI application through a validated diagnostic-therapeutic algorithm could guarantee: an objective clinical management of RPL patients; a more appropriate communication closer to real prognosis to patients, in terms of successful subsequent pregnancy; an ultra-stratification of RPL patients in risk subclasses, generated from different combinations of etiological features groups.

Trial registration number:

Not applicable.

*Valentina Bruno, Matteo Biasiotti and Michele D'Orazio equally contributed to this study

†Emilio Piccione and Nicola Rosato equally contributed to this study

O-126 GM-CSF TREATMENT IN RECURRENT IMPLANTATION FAILURE WOMEN AFTER PGS: A RANDOMIZED CONTROLLED TRIAL

F. Scarpellini[1], M. Sbracia[2]

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[2]*CERM, IVF Dept., Rome, Italy*

Study question:

Is GM-CSF (sargramostim) a possible treatment for recurrent implantation failure in women undergoing IVF?

Summary answer:

The clinical use of G-CSF in women experienced implantation failure may be useful

What is known already:

The GM-CSF is a cytokine promoting leukocyte growth as well as trophoblast development. We described that this cytokine may be used in the treatment of recurrent abortion.

Study design, size, duration:

The study is a randomized controlled trial conducted on 73 women with recurrent implantation failure after IVF cycles. Patients were randomly divided in two groups: one (36 women) treated with subcutaneous GM-CSF 1.5mg/kg/daily (60-100) from the day of embryo transfer to the day of b-hcg day : the control group (37 women) was treated with subcutaneous saline solution infusion in the same way of the study group.

Participants/materials, setting, methods:

The study was conducted to the CERM, Rome, Italy,, on 73 women with recurrent implantation failure after IVF cycles. Inclusion criteria were: at least 9 good embryos previously transferred, women less than 38 years old, absence of systemic diseases. These women underwent IVF cycle and PGT-A on blastocysts obtained. Single healthy blastocyst transfer was performed in the next cycle. Primary outcome was the pregnancy rate.

Main results and the role of chance:

Epidemiological data of the two groups did not show statistically significant differences. Pregnancy rate in the group treated with GM-CSF was 75.0% (27/36) whereas in the control group was 43.2% (16/37), P= 0.0087. No side effects were observed.

Limitations, reasons for caution:

The number of patients weras low and more patients are needed for definitive conclusions.

Wider implications of the findings:

These results when confirmed can offer to clinicians a valid treatment to improve IVF outcome.

Trial registration number:

NCT01718210

O-127 Does uterine natural killer (uNK) cells density predict euploid miscarriage in women with a history of recurrent miscarriage?

X. Chen[1], Y. Liu[1], W.C. Cheung[1], J.P.W. Chung[1], T. Zhang[1], Y. Zhao[1], C.C. Wang[1], T.C. Li[1]

[1]*The Chinese University of Hong Kong, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong*

Study question:

Does uterine natural killer (uNK) cells density in the peri-implantation endometrium predict a subsequent miscarriage with euploid product of conception in women with a history of recurrent miscarriage (RM)?

Summary answer:

Uterine NK cells density is of prognostic value for a subsequent euploid miscarriage in women with a history of RM.

What is known already:

Previous studies have shown an increase percentage of uNK cells in the peri-implantation endometrium of non-conception cycles in women with RM. However, the predictive value of uNK cells density in the maintenance of pregnancy is controversial.

Study design, size, duration:

It is a prospective cohort study. A total of 134 women with a history of RM participated in the study from January 2015 to August 2018, of whom 75 women became pregnant following biopsy. Forty-five women had an ongoing pregnancy and the other 30 women miscarried in the subsequent pregnancy and karyotyping analysis for the product of conception was performed.

Participants/materials, setting, methods:

All the endometrial biopsies were obtained precisely 7 days after luteinizing hormone surge in non-conception natural cycles. Endometrial sections were immunostained for CD56 to identify uNK cells and cell counting was performed based on a standardized protocol. Results were expressed as percentage of CD56+ uNK cells/ total stromal cells.

Main results and the role of chance:

The median uNK cells density in women who miscarried (n=30, median 2.80%, range 1.17-8.77%) was significantly higher than those who had ongoing pregnancy (n=45, mean 1.97%, range 0.16-6.97%). The median uNK cells density in women with euploid miscarriage (n=19, median 3.57%, range 1.25-8.77%) was significantly higher than those with aneuploid miscarriage (n=11, median 2.31%, range 1.17-7.52%). In addition, the median uNK cells density in women with euploid miscarriage was significantly higher than those with ongoing pregnancy. However, there was no significant difference in uNK cells density between aneuploid miscarriage group and ongoing pregnancy group.

Limitations, reasons for caution:

The association between uNK cells density and the definitive outcome of live birth in the subsequent pregnancy has yet to be confirmed.

Wider implications of the findings:

Embryonic factor should be taken into account when investigating the association between endometrial factor and implantation failure or miscarriage in the future studies.

Trial registration number:
None.

O-128 In patients with repeated implantation failures, endometrial receptivity window turns on gradually under HRT compared to natural cycle: highlights by genomic testing

F. Entezami[1,2], D. Haouzi[2,3], C. Innocenti[3], A. Ferrieres-Hoa[2,3], C. Baron[2], C. Vincens[3], S. Bringer-Deutsch[3], E. Vintejoux[3], A. Torre[4], S. Hamamah[2,3]

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[4]University of Nottingham, Ob-Gyn, Nottingham, United Kingdom

Study question:
To identify the receptivity window in patients with repeated implantation failure (RIF) prepared for frozen embryo transfer under hormone replacement therapy (HRT) treatment or natural cycle.
Summary answer:
The acquisition of the endometrial receptivity phenotype is more progressive under HRT compared to natural cycle. Personalized embryo transfer improves pregnancy outcome for RIF patients.
What is known already:
Many approaches for human endometrial receptivity testing including microarray have been previously reported. Accordingly, identification of a delay of the receptivity window has been reported in repeated implantation failure patients under hormone replacement therapy. How far a delay of the receptivity window can be observed in RIF patients under natural cycle remains debated.
Study design, size, duration:
Endometrial biopsies were performed during the implantation window 7-9 days after the LH surge in natural cycle or 5-9 days after progesterone administration under HRT respectively. According to genomic testing result, the transfer strategy was: blastocysts transferred at the specific day where endometrium is identified as 'receptive' and D2/D3 cleavage stage embryos transferred 72/48 hours before the specific cycle day where endometrium is identified as 'receptive'.
Participants/materials, setting, methods:
141 RIF patients with several unsuccessful fresh and/or frozen embryo transfers were included. The number of previous failed attempts and non-implanted embryos were 4.5±2.1 and 6.6±4 respectively. Genomic testing of endometrial biopsies was performed under natural cycle or HRT. RNAs from biopsies were extracted and mRNA expression levels of specific genes predictive of endometrial receptivity were established using RT-qPCR. Clinical pregnancy was defined by visualization of a gestational sac with a positive fetal heartbeat.
Main results and the role of chance:
Analyses of endometrial receptivity status in 141 RIF patients (age 37.9± 3.8 years) revealed a strong inter-patient variability in the occurrence of the receptivity window with mostly a delay between 1 to 3 days. More precisely, biopsies were evaluated under natural cycle (n=29), natural cycle with recombinant human chorionic gonadotropin (hCG, Ovitrelle) (n=7) HRT (n=68) and HRT with GnRH analogue (Decapeptyl) (n=37). In patients evaluated under plain natural cycle, the majority were receptive at LH+8 (52%). The remaining 48% displayed receptivity equally at LH+6/+7 (24%) and LH+9 (24%). Under natural cycle with recombinant hCG, 72 % of RIF patients were receptive at hCG+9 while 14% were at hCG+6/+7 and 14 % at hCG+8. In patients under HRT, 38% and 41% were receptive at Pg+7 and Pg+8, respectively, whereas the remaining 21% were receptive before (Pg+5/+6 for 18%) or after (Pg+9 for 3%). Under HRT with GnRH analogue, the majority of RIF patients were receptive at Pg+8 (67%). Others were receptive at Pg+5/+6 (22%), Pg+7 (5%) and Pg+9 (8%). After personalized embryo transfer using the genomic testing strategy, the clinical pregnancy and live birth rates were 36.2 % and 28.4% respectively.
Limitations, reasons for caution:
The benefit of this innovative strategy should be analysed with respect to the genetic status of the transferred embryos after PGT for aneuploidy.
Wider implications of the findings:
The majority of RIF patients displayed a delay in occurrence of their receptivity window revealing a potential cause for the previous implantation failures. Personalized embryo transfer according to the specific cycle day where endometrium is 'receptive' improves clinical pregnancy and LBR in RIF patients under both HRT and natural cycle.
Trial registration number:
Not Applicable

O-129 The impact of previous live births on peripheral and uterine natural killer cells in patients with primary and secondary recurrent miscarriage

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Study question:
To evaluate alterations in peripheral and uterine natural killer cells (pNK and uNK) in patients with primary and secondary recurrent miscarriage (pRM and sRM).
Summary answer:
The immune profile of pRM and sRM patients shows distinct differences in pNK as well as uNK cell numbers.
What is known already:
PNK and uNK cells are key players in the establishment and maintenance of pregnancy and are disturbed in patients with RM. Different immunologic risk factors have been proposed for patients with pRM (no previous live birth) and sRM (≥ 1 previous live birth). However, so far, study populations were small and results controversial. Therefore, we aimed to analyse pNK and uNK cells in a large, well defined study population.
Study design, size, duration:
Within the outpatients clinic of the University Hospital Heidelberg n=575 couples with ≥3 consecutive RM were included in this single-center prospective study between March 2012 and October 2018. Analyses were performed in the mid-luteal phase at least 3 months after the last pregnancy.
Participants/materials, setting, methods:
Subgroups consisted of n=245 pRM and n=101 sRM patients. After screening for established risk factors, n=156 idiopathic RM (iRM) were identified, including n=102 primary iRM (piRM) and n=54 secondary iRM (siRM) patients. Differences between CD45+CD3-CD56+CD16+ NK cells (analysed by FACS /µl and percentages of total lymphocytes) and CD 56+ uNK cells (analysed by immunohistochemistry; absolute numbers per mm^2) were defined as primary outcome measures.
Main results and the role of chance:
Patients with pRM and piRM showed significant higher absolute numbers and percentages of pNK cells compared to sRM and siRM patients (pRM/piRM vs sRM/siRM, mean±SD /µl: 239.1±118.7/244.9±112.9 vs 205.1±107.9/206.0±105.6, p=0.004/ p=0.009; mean±SD %: 12.4±5.5/ 12.8±5.4 vs 11.1±4.6/11.1±4.3, p=0.001; p=0.002). Only patients with siRM showed significantly higher uNK levels compared to patients with piRM (mean±SD /mm^2 288.4±239.3 vs 218.2±184.5, p=0.044). Our large cohort revealed a positive correlation between CD56+ uNK cells and CD45+CD3-CD56+CD16+ pNK cells in piRM patients (/µl: r=0.393, n=102, p<0.001; percentages: r=0.331, n=102, p<0.001). Number of miscarriages and luteal phase progesterone levels did not differ between the subgroups of RM patients. Patients with siRM had a significant higher BMI than patients with piRM. Mean age, gravidity and parity of patients were significantly higher in sRM and siRM versus pRM and piRM patients respectively.
Limitations, reasons for caution:
Differences in pNK and uNK cell numbers were analysed in patients, not in controls. However, most recently we showed differences in pNK and uNK cells between RM patients and controls. This study was therefore designed to show the specific differences of pRM and sRM patients.

Wider implications of the findings:

Differences in pNK and uNK cells in RM patients depending on previous live births might indicate distinct pathways in NK cell recruitment and potentially different underlying immune disorders between pRM and sRM.

Trial registration number:

not applicable

O-130 Treatment strategies to increase the live birth rate in patients with KIR-HLA-C mismatch: a retrospective cohort study

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Study question:

Does the selection of HLA-C1C1 oocyte donor or the administration of granulocyte colony-stimulating factor (G-CSF) improve the live birth rate/cycle in couples with KIR-HLA-C mismatch?

Summary answer:

The selection of HLA-C1C1 oocyte donors improves live birth rate (LBR) compared to random HLA-C donors or to G-CSF administration in couples with KIR-HLA-C mismatch.

What is known already:

Increased risk of recurrent miscarriage (RM), preeclampsia, and fetal growth restriction has described in KIR AA mothers when the fetus has more HLA-C2 genes than the mother, and this HLA-C2 are paternally or egg donor inherited. In ART oocyte donor cycles, oocyte HLA-C behaves as the paternal HLA-C and KIR-HLA-C combination is not currently taken into consideration on donors' selection. KIRAA women have lower live birth rates (LBR) after double embryo transfer (DET) in egg-donation ART cycles especially when the embryo carries HLA-C2. G-CSF administration seems to improve the LBR in patients with recurrent miscarriages lacking activating KIR.

Study design, size, duration:

Between January 2017 and December 2018, we performed a retrospective study that included 72 women whose RM/RIF were of unknown etiology and 261 embryo transfers (ET). All couples had KIR-HLA-C mismatch: maternal KIR AA and paternal HLA-C2. All the patients underwent egg donation. Forty-five couples (group 1) had 135 ETs (70% SET and 30% DET) and 27 couples (group 2) had 126 ETs (83% SET and 27% DET).

Participants/materials, setting, methods:

All the patients were selected from IVI RMA Clinics. Group 1 had 90 ETs with random HLA-C egg donor and 45 SET with HLA-C1C1 egg donor. Group 2 had 99 ETs with random HLA-C egg donor and 27 SET with HLA-C1C2/C2C2 egg donors and G-CSF administration. We performed genetic typing for maternal KIR and paternal and oocyte donors HLA-C. Pregnancy, miscarriage and LBR/transfer have studied by groups and cycles. Fisher test has used.

Main results and the role of chance:

The median age of our patients was 40 years, and 25 years for oocyte donors. In our cohort, all women had KIR AA and their partners HLA-C2.

A higher LBR/cycle was observed in group 1 when their HLA-C1C1 egg donor cycle (48.89%) was compared to the previous random HLA-C egg donor cycles (5.77%) (OR 42.82).

A higher LBR/cycle was observed in the group 2 when compared the cycles using G-CSF administration (14.81%) and their previous random HLA-C egg donor cycles (6.38%) (OR 6.86).

Higher LBR/cycle was observed in HLA-C1C1 egg donor cycles - group 1 (48.89%) when compared to HLA-C1C2/C2C2 egg donors and G-CSF administration cycles -group 2 (14.29%) (OR 6.82, $p<0.002$).

A higher pregnancy rate was observed in group 1 when compared their HLA-C1C1 egg donor cycles (80%) to HLA-C1C2/C2C2 egg donors and G-CSF administration cycles -group 2 (24.44%) (OR 3.8, $p<0.01$).

We did not observe any differences on miscarriage rates between both groups (C1C1 egg donor 13.33% and G-CSF 17.86%).

Limitations, reasons for caution:

Our sample was small and this is the first report to observe differences in LBR by oocyte donor/embryo HLA-C or C-GSF administration in KIR AA mothers with embryos HLA-C2 and egg donation. However, apart from statistical significance, the association strength was noticeably high, which confers our findings more confidence.

Wider implications of the findings:

We speculate that completing a normal pregnancy is posible only for those KIR AA mothers who carry a baby with a least one non-self HLA-C1. Therefore, selecting HLA-C1C1 amongst oocyte donors for KIR-HLA-C mismatch couples could improve the LBR compared to random HLA-C egg donors or the G-CSF administration.

Trial registration number:

1812-MAD-101-DA

SELECTED ORAL COMMUNICATIONS

SESSION 38: FERTILITY PRESERVATION 1

Tuesday 25 June 2019 Haydn 4 10:00 - 11:30

O-131 Fertility in female cancer survivors: a systematic review and meta-analysis

A. Busnelli [1], A. Vitagliano [2], L. Mensi [3], F. Filippi [3], E. Somigliana [3]

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[2]University of Padua- Padua- Italy, Obstetrics and Gynaecology Unit- Department of Women and Children's Health-, Padua, Italy
[3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Obstet Gynecol, Milan, Italy

Study question:

What are the chances of female survivors of different types of cancer to fulfil their reproductive desire?

Summary answer:

Bone, breast, brain and kidney cancer survivors have reduced chances of childbirth. Women with thyroid cancer, melanoma and Non Hodgkin lymphoma can be reassured.

What is known already:

Several epidemiological studies investigated the effects of cancer therapies on fertility considering the chances of pregnancy and childbirth as primary outcomes. However, conflicting data have been reported for the different cancer sites and a global interpretation of the results is not possible due to the different weight of the individual studies. In the absence of a data synthesis, at present, it is therefore not possible to provide an accurate counselling to patients on the fertility damage of oncological treatments and to share decisions regarding fertility preservation options.

Study design, size, duration:

The present systematic review and meta-analysis was restricted to published research articles that investigated the chances of pregnancy or live birth in women after treatment of different types of cancer (i.e. leukaemia, breast cancer, Hodgkin lymphoma, Non Hodgkin lymphoma, brain cancer, soft tissue cancer, liver cancer, digestive tract cancer, kidney cancer, thyroid cancer). We systematically searched Pubmed, MEDLINE, Embase and Scopus, from database inception to January 15, 2019. The study is registered with PROSPERO (CRD42019119786).

Participants/materials, setting, methods:

The literature overview was reported according to the PRISMA guidelines. Published cohort, case-control and cross-sectional studies were eligible for inclusion. All pertinent articles were retrieved, and the relative reference lists were reviewed. Studies were excluded if: (i) crude or adjusted effect estimates with corresponding 95%CIs or results allowing their calculation were not reported, (ii) control population was not clearly defined. The quality of case-control and cohort studies was evaluated by means of the Newcastle-Ottawa scale.

Main results and the role of chance:

Our searches identified 41 non-duplicate records, of which 18 relevant studies were included in the qualitative and quantitative analysis. Childbirth chances resulted significantly reduced in women with a history of bone cancer (HR 0.86, 95%CI[0.77-0.97]; I^2=0%; p=0.02; RaR 0.76, 95%CI[0.61-0.95]; I^2=69%; p=0.01), breast cancer (HR 0.74, 95%CI[0.61-0.91]; RaR 0.51, 95%CI[0.47-0.57]; I^2=0%; p<0.00001), brain cancer (HR 0.61, 95%CI[0.53-0.70]; I^2=14%; p<0.00001; RaR 0.44, 95%CI[0.33-0.60]; I^2=95%; p<0.00001; OR 0.49, 95%[0.40-0.60; RR 0.62; 95%CI [0.42-0.91]; p=0.02) and kidney cancer (RaR 0.69, 95%CI[0.61-0.78]; p<0.00001; RR 0.66; 95%CI [0.43-0.98]; p=0.04). Data pooling showed conflicting results in soft tissue cancer, Hodgkin lymphoma, and leukaemia patients. Reproductive chances in women survived from Non Hodgkin lymphoma, melanoma and thyroid cancer resulted unaffected.

Limitations, reasons for caution:

Most of the studies did not report detailed information on the characteristics of the oncologic treatments. Possible lurking variables should also be considered. In fact, physical and psychological sequelae of such aggressive therapies may affect the relationship life confounding the association between cancer or cancer treatment and fertility.

Wider implications of the findings:

WomNNen are currently informed providing data on the chemotherapy effects on poorly reliable fertility markers and on the reproductive chances cumulatively calculated for many cancer sites. Estimates by type of cancer are therefore of utmost importance to counsel patients and to assess the risk-benefit ratio before starting fertility preservation programs.

Trial registration number:

N.A.

O-132 In Vitro Maturation Rates in Young Pre-Menarche Patients

N. Schachter - Safrai[1], G. Karavani[1], A. Revel[1], D. Bauman[1], T. Imbar[1]

[1]Hadassah Medical Center- Jerusalem, Obstetrics and Gynecology, Jerusalem, Israel

Study question:

To evaluate oocyte retrieval rates and in vitro maturation (IVM) efficacy following ovarian tissue cryopreservation (OTC) in young pre-menarche girls facing chemo-radiotherapy.

Summary answer:

IVM performed following OTC in pre-menarche girls and specifically in very young girls (under 5 years) yields substantially decreased maturation rates compared with post-menarche patients.

What is known already:

As childhood cancer survival rates improve, ovarian cortex harvesting and cryopreservation became an ancillary standard procedure of fertility preservation prior to gonadotoxic treatment. Immature oocyte can be found during ovarian tissue handling and the ability to mature them in vitro and cryopreserve them as mature oocytes, provides additional option of fertility preservation. Recent studies showing outcomes of IVM in children demonstrated retrieval and maturation of oocytes even in the pre- pubertal age. However, these studies investigated only a small group of pre-pubertal patients and included mixed population of both chemotherapy naive and exposed patients.

Study design, size, duration:

This is a retrospective cohort study which included a total of 84 chemotherapy naïve patients aged 0-18 years referred for fertility preservation between 2004 and 2017. Of them, 33 were pre-menarche and 51 were post-menarche.

Participants/materials, setting, methods:

A comparison was made between the pre- menarche and post-menarche patients with regard to OTC and IVM outcomes. Additionally, a correlation between age and OTC and IVM outcomes was performed in the pre- menarche group. This group was later divided into two age groups: 0-4 years and 5-10 years. A further analysis was aimed to evaluate the differences between the very young patients and the older pre-menarche patients regarding IVM outcomes.

Main results and the role of chance:

Number of oocytes retrieved did not significantly differ between the post-menarche and pre-menarche groups (10.8±8.5 and 8.1±6.8, respectively). However, the overall in vitro maturation rate was significantly higher in the post-menarche group (28.2% vs. 15.5%, respectively; p<0.001, OR=0.47). A separate analysis for patients up to 4 years of age demonstrated significantly less oocyte yield compared with the older (5-10 years) pre-menarche girls (4.7±5.2 vs.10.3±7.0 oocytes, respectively; p=0.02) and much lower IVM rates (4.9% and 18.2% respectively, p=0.02). Correlation of age with number of retrieved and matured oocytes showed a positive significant correlation (r=0.45 and r=0.64, respectively; p<0.05 for both).

Limitations, reasons for caution:

This is a retrospective study with a limited sample size, as such, it is more prone to selection bias. No routine assessment of the ovarian reserve before or after OTC was performed. Finally, the clinical utility of the IVM procedure in the pre- menarche group is yet unknown.

Wider implications of the findings:

Our cohort of chemotherapy naïve pre-menarche patients undergoing IVM is the largest published so far. As previously described on smaller groups of pre-menarche patients, we demonstrated that IVM is feasible in these patients although in a significantly lower yield of retrieved oocytes and in vitro maturation rate of these oocytes.

Trial registration number:

not applicable

O-133 Short exposure to low-dose rapamycin reduces the negative impact of PTEN inhibition on DNA damage response but maintains follicle growth activation in bovine cortical strips

M. Maidarti[1,2,3], Y.L. Clarkson[2], M. McLaughlin[2], R.A. Anderson[1], E.E. Telfer[2]

[1]MRC Centre for Reproductive Health- Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom

[2]Institute of Cell Biology and Genes and Development Group- CDBS- Hugh Robson Building, University of Edinburgh, Edinburgh, United Kingdom

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Study question:

Does pharmacological inhibition of mTOR (mammalian target of rapamycin) by rapamycin, modify the defective DNA damage response following PTEN inhibition whilst promoting follicle growth activation?

Summary answer:

Ovarian strips exposed to bpv(HOpic) and rapamycin show increased follicle activation and significantly reduced negative effects on DNA damage response observed with bpv(Hopic) alone.

What is known already:

Modulation of phosphoinositide 3-kinase (PI3K)/Akt/mTOR signalling pathway determines the balance between activation and inhibition of primordial follicle growth. Inhibition of phosphatase and tensin homolog (PTEN) is known to activate primordial follicles but may compromise further developmental potential, with oocytes showing increased DNA damage and reduced DNA repair functions. A recent study showed that modulation of the activation rate by inhibiting mTOR1 may improve the quality of *in vitro* activated follicles. We hypothesised that combined inhibition of PTEN and mTOR to modulate *in vitro* activation of follicles may improve DNA damage response of follicles *in vitro*.

Study design, size, duration:

Bovine ovarian fragments (4x2x1mm) were cultured for 24 hours in medium containing either: 1) PTEN inhibitor, 1μM bpv(HOpic), 2) 1μM bpv(HOpic)+0.1nM rapamycin or 3) vehicle control (0.001% DMSO), then incubated for a further 5 days in control medium. Cultured fragments were fixed and analysed by immunohistochemistry and immunofluorescence to determine follicle activation and expression of markers reflecting DNA damage and repair mechanisms. 116 ovarian cortical strips and 12,242 follicles from four independent experiments were analysed.

Participants/materials, setting, methods:

Cortical fragments of bovine ovaries were prepared for culture. After a total of 6 days in culture, fragments were fixed and analysed histologically. Immunostaining for: 1) nuclear exclusion of Forkhead Box O3 (FOXO3) as a marker of follicle activation 2) γH2AX as a marker of DNA damage; 3) DNA repair factors: meiotic recombination 11 (MRE11), Ataxia telangiectasia

mutated (ATM), Breast cancer susceptibility gene 1 (BRCA1), Breast cancer susceptibility gene 2 (BRCA2) and Rad51.

Main results and the role of chance:

Tissue exposed to 1μM bpv(HOpic) or 1μM bpv(HOpic)+0.1nM rapamycin contained a higher proportion of growing follicles (81.9% and 70.2%) compared to control (57%) ($p \leq 0.001$). In the bpv(HOpic) only group, increased growth was associated with increased nuclear exclusion of FOXO3 and increased γH2AX expression in oocytes of non-growing and primary follicles (80.0% and 64.5%) compared to control (20% and 17.0%)($p<0.05$). γH2AX expression in oocytes was reduced with the addition of rapamycin (23.0% and 29.5%) ($p \leq 0.001$). The mean percentage of granulosa cells per follicle expressing γH2AX was higher in bpv(HOpic) treated growing follicles (16.0±1.1%) compared to control (0.48±0.19%)($p=0.03$) and was significantly reduced with the addition of rapamycin (1.4±1.3%)($p=0.01$). MRE11, ATM and Rad51 expression was lower in oocytes of primary follicles in bpv(HOpic) (69.5%, 30.1% and 31.3% respectively) compared to control (88.0%,$p \leq 0.001$; 65.0%,$p=0.014$; and 74.0%,$p \leq 0.001$) but increased in rapamycin+bpv(HOpic) (85.7%,$p=0.033$; 65.3%,$p \leq 0.001$; 49.3%,$p=0.007$). Neither bpv(HOpic) (13.5%;63.0%) or bpv(HOpic)+rapamycin (8.0%;56%) affected BRCA1 and BRCA2 expression in oocytes of primary follicles compared to control (5.7%, 44.0%)($p>0.05$). Expression of MRE11, BRCA1, BRCA2 and Rad51 in granulosa cells of growing follicles showed no significant differences between any treatments ($p>0.05$). ATM expression decreased in granulosa cells exposed to bpv(HOpic) (72.1±4.6%) compared to control (91.8±2.6%) and rapamycin+bpv(HOpic) group (90.4±2.4)($p<0.05$).

Limitations, reasons for caution:

This study is limited to primordial follicle activation and implications for later stages of follicle development have not been assessed. While the bovine ovary is a good large mammalian mono-ovulatory model for the human, it may not recapitulate all aspects.

Wider implications of the findings:

A more controlled increase in follicle activation may preserve bidirectional signalling between oocyte and granulosa cells. This model may be useful to provide additional insights into DNA damage response prior to their use in human ovaries, which may subsequently lead to improvements in systems that support *in vitro* follicle growth.

Trial registration number:

Not applicable.

O-134 In vivo imaging of human primordial follicles and growing follicles by new optical coherence tomography (OCT)

R. Zhang[1], T.S.M. Law[2], J.P.W. Chung[2], T.C. Li[2], C.C. Wang[2]

[1]*Chinese University of Hong Kong, Obstetrics and Gynecology, Hong Kong, Hong Kong*

[2]*Prince of Wales Hospital, Obstetrics and Gynecology, Hong Kong, Hong Kong*

Study question:

Can primordial follicles and growing follicles be identified and measured by OCT in vivo?

Summary answer:

Human primordial follicles and growing follicles can be identified and measured by the new OCT imaging system in cooperate with endoscopy in vivo.

What is known already:

Optical Coherence Tomography (OCT) is a non-invasive imaging technique with resolution 100 times higher than ultrasound. OCT can detect tissue structure down to micrometer scale and the depth of detection is up to 3mm. OCT technology has been widely used mainly in/through surface organs, such as ophthalmology and dermatology. Recently a report used OCT to assess localization of primordial follicles in ovarian tissue using non-fixed ex vivo human ovary sections that had been cryopreserved for fertility preservation due to premature ovarian insufficiency for treatment of hematological disease or cancer. In vivo OCT imaging of ovary has never been reported.

Study design, size, duration:

New OCT imaging system (OCTISTM) with newly designed catheter-based rotational and pullback optical probes and improved OCT technologies were used in animal experiment in vitro and also in in human study incorporate with laparoscopy in vivo.Prospective observation study of animal experiments in vitro and human studies in vivo. 5 animals and 10 human subjects between Oct 2018 and Oct 2019 will be recruited for the study.

Participants/materials, setting, methods:

To test the detection and accuracy of OCTISTM, pig ovaries were excised and examined by OCTISTM. 3D reconstruction was employed to examine the spatial distribution of follicles in ovary. To evaluate the accessibility and sensitivity of OCTISTM, human ovaries undergoing laparoscopic ovarian surgery (such as ovarian drilling, cystectomy, oophorectomy) were examined by OCT before the surgery. The corresponding tissue sections were prepared for histological examination and comparison.

Main results and the role of chance:

Follicles at different size were clearly identified and accurately measured by the OCTISTM in pig ovaries in vitro. Size and distribution of follicles in the ovary can be reconstructed in 3D imaging. In human subjects, primordial follicles and growing follicles can be observed in vivo through laparoscopic surgery and after laparoscopic ovarian drilling. The OCTISTM probe can be immobilized inside the ovary to obtain full images closer to the ovarian cortex and closer to the primordial follicle in vivo. Distribution of the primitive follicles can be imaged easily in the cortex of the ovary. However, the OCTISTM probe is not easily fixed on the surface of ovary in vivo, so quality of the OCT image is not as good as reported in vitro.

Limitations, reasons for caution:

OCT penetration is still limited. OCTISTM imaging depth is up to 3mm, so it is suitable for superficial imaging of organs. As human ovarian capsule and cortex are thick, we can only detect follicles close to ovary surface, but cannot detect the follicles in the deeper part of the cortex.

Wider implications of the findings:

We can assess the location of primordial follicles by minimally/non-invasive, real-time, high-resolution, cross-sectional, and potentially microscopic structural OCTISTM imaging in vivo through laparoscopic surgery. Our experiment indicated that OCT may help select the most reproductive ovarian tissue for in-vitro activation (IVA) and ovarian tissue transplantation, thus potentially better pregnancy outcome.

Trial registration number:

CREC 2016.160-T

O-135 Outcomes from heterotopic and orthotopic grafting of human cryopreserved ovarian tissue.

D. Gook[1], L. Hale[2], A. Polyokov[2], K. Stern[1]

[1]*The Women's Hospital, Reproductive Services /Melbourne IVF, Parkville- Victoria, Australia*

[2]*Melbourne IVF, Melbourne IVF, East Melbourne, Australia*

Study question:

Is the outcome from ovarian tissue grafted at a heterotopic site similar to that grafted at an orthotopic site?

Summary answer:

Similar outcomes in terms of number of oocytes recovered, number mature, proportion of fertilized oocytes which cleave (day 2), was observed with both graft sites.

What is known already:

Over 120 live births have been reported from cryopreservation of ovarian tissue and subsequent grafting. In the majority of these, tissue was grafted into the remaining ovary (orthotopic site). Although heterotopic grafting to a non ovarian, non pelvic site (e.g. abdominal) has also resulted in births, it has been suggested that compromised outcomes may be expected from a non-pelvic site.

Study design, size, duration:

The aim of the study was to assess the outcome from cryopreserved ovarian tissue grafted to heterotopic (abdominal) or orthotopic (pelvic) sites. Ovarian tissue was slow frozen using the cryoprotectants propanediol and sucrose (n=17 women) or using DMSO and sucrose (n=1 women). Tissue was thawed appropriately and prepared on 6.0 vicryl sutures for grafting. Tissue was placed laparoscopically into an abdominal pocket and a pelvic side wall pocket close to the ovary.

Participants/materials, setting, methods:

Following resumption of cycling, gonadotrophin stimulation commenced with FSH, LH and antagonist and a trigger was given when one follicle was >13 mm in diameter. Abdominal follicles (heterotopic) were aspirated under abdominal

ultrasound guidance; ovarian and pelvic follicles were aspirated transvaginally. Due to an inability to distinguish under ultrasound between pelvic and ovarian follicles, both were classified as orthotopic.

Main results and the role of chance:

Age at time of grafting was generally 6 years older than at time of cryopreservation. Resumption of cycling occurred on average 3.5 months post grafting regardless of graft site. Mean follicle diameter was 14 mm for both sites. Aspiration failed to retrieve an oocyte in 29.8% (31/104) of heterotopic and 33.3% (48/144) of orthotopic follicles. A similar proportion of retrieved oocytes were mature in both sites [66.7% (44/66) heterotopic, 63.5% (54/85) orthotopic]. The proportion of embryos which developed on day 2 from those fertilized was also similar in both groups [88.9% (32/36) heterotopic, 86.5% (32/36) orthotopic]. This is the first reported in house comparison of outcomes from ovarian tissue grafted to heterotopic (abdominal) and orthotopic (pelvic) sites.

Limitations, reasons for caution:

Study reports outcomes from a small number of women following grafting. Follicle density and amount of tissue grafted varies between patients. Outcomes from orthotopic grafting may be biased by the inclusion of non-ovarian grafts ie pelvic side wall.

Wider implications of the findings:

Preliminary data suggests that the outcomes assessed are similar from the heterotopic and orthotopic graft sites and that an abdominal site is a suitable site for ovarian grafting.

Trial registration number:

not applicable

O-136 Improving Autologous Ovarian Transplantation Outcomes with Robotic Surgery and the Utility of a Neovascularizing Human Extracellular Matrix (ECM) Scaffold

K.H. Oktay[1,2], G. Bedoschi[3], T. Kawahara[1,4], L. Marin[1,5], Y. Sugisihita[1,4], E. Taylan[1], L. Ursillo[2]

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[5]*University of Padua School of Medicine, Department of Woman's and Child's Health, Padua, Italy*

Study question:

Can Autologous Cryopreserved Ovarian Tissue Transplantation (ACOTT) outcomes be improved with the utility of robotic surgery and neovascularizing ECM scaffold combined with peri-operative pharmacological support?

Summary answer:

The robotic ACOTT technique with the use of ECM scaffold and peri-operative pharmacological support leads to higher rates of ovarian function restoration then previously reported.

What is known already:

First successful case of ACOTT was reported in 2000. The procedure has resulted in over hundred livebirths since then. However, a major rate-limiting factor for this procedure is the initial ischemia following grafting, resulting in significant primordial follicle loss. As a result, according to a recent meta-analysis, only about one out of three women has had a child after ACOTT and the average longevity of the grafts is approximately 3 years. We developed a robot-assisted technique combined with the use of a neovascularizing ECM and peri-operative pharmacological support to improve ACOTT outcomes.

Study design, size, duration:

Six women underwent laparoscopic ovarian cortical tissue cryopreservation before or shortly after initiating gonadotoxic treatments in a prospective study. These women then underwent robot-assisted laparoscopic ACOTT 7-12 years following ovarian tissue cryopreservation.

Participants/materials, setting, methods:

Inclusion criteria were: cryopreservation with slow freezing, ovarian failure caused by gonadotoxic treatments (chemo and/or radiation therapy), peri-operative pharmacological support (transdermal estrogen 4 weeks prior and post-op and baby aspirin 10 days prior), robot assisted ACOTT by single surgeon, utility of ECM scaffold (Alloderm, regenerated human cadaver skin with neovascularizing effects), and a minimum of 1 year follow up. Receiving gonadotoxic treatment before tissue harvesting was not an exclusion criterion.

Main results and the role of chance:

The amount of transplanted tissue was based on the pre-transplant follicle density assessment and varied from 50-70 % of cortex from one ovary. Patients were perioperatively treated with transdermal estrogen and low dose aspirin to enhance recipient site vascularization. Tissues were sutured on the ECM prior to grafting onto the bivalved remaining ovary in 4 cases, onto mesosalpinx in one and retroperitoneally in lower abdomen in the other. Ovarian function returned 13.9±2.7 weeks (range 11-16.2) after transplantation with a mean duration of function 34.2±17.7 months (14-67) at the time of this report. Endocrine function and follicle development rates were 100%. Oocytes were retrieved from all patients. One patient dropped out after cryopreserving one oocyte. In remaining 4 patients, multiple embryos were generated (8.2± 3.4 per case, range 3-12). Two of those women had two livebirths each, while remaining two had two (+1 pending PGT-A result) and six euploid embryos cryopreserved for future embryo transfer. In the case of heterotopic ovarian transplantation, embryo quality was consistently poor, not resulting in blastocyst formation.

Limitations, reasons for caution:

Although our findings suggest that robotic assisted orthotopic ACOTT technique with the use of ECM scaffold and perioperative pharmacological support leads to high fertility and endocrine function restoration rates than previously reported, larger studies are needed to strengthen this conclusion.

Wider implications of the findings:

Robot-assisted orthotopic ACOTT results in 100% success based on endocrine function, follicle development, livebirths and/or euploid embryo development even when ovarian tissue was cryopreserved after chemotherapy exposure. On the other hand, egg quality seems to be impaired with heterotopic ACOTT which has also been an observation with previous techniques.

Trial registration number:

not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 39: CLINICAL RESEARCH IN ENDOMETRIOSIS AND ENDOMETRIUM: FROM DIAGNOSIS TO TREATMENT AND PREVENTION

Tuesday 25 June 2019 Strauss 1+2 10:00 - 11:30

O-137 Bidimensional rectal-water contrast-transvaginal ultrasonography (2D-RWC-TVS) versus 3D-RWC-TVS in the diagnosis of rectosigmoid endometriosis: a pilot prospective comparative study

F. Barra[1], C. Scala[1], V.G. Vellone[2], S. Ferrero[1]

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[2]*IRCCS Ospedale Policlinico San Martino- University of Genoa, Department of Surgical and Diagnostic Sciences, Genoa, Italy*

Study question:

This study aims to compare the performance of bidimensional rectal-water contrast-transvaginal ultrasonography (2D-RWC-TVS) with 3D-RWC-TVS for diagnosing the presence of rectosigmoid endometriosis.

Summary answer:

Despite of being both feasible and well tolerated, 3D acquisitions do not seem to improve the performance of RWC-TVS for the diagnosis of rectosigmoid endometriosis.

What is known already:

An accurate diagnosis of the presence, location and extent of the rectosigmoid endometriosis is of paramount importance for the clinicians in order to inform the patients on the potential surgical or medical treatments. It is well established that TVS is the first-line investigation in patients with suspicion of

deep infiltrating endometriosis and improvement in the performance of TVS in diagnosing rectosigmoid endometriosis may be obtained by RWC-TVS.

Study design, size, duration:

This pilot prospective comparative study was performed between January 2018 and December 2018, including 36 women of reproductive age who were referred to our institution because pain symptoms and intestinal complaints suggestive of rectosigmoid endometriosis. Exclusion criteria for the study were previous surgical or radiological diagnosis of rectosigmoid endometriosis.

Participants/materials, setting, methods:

2D-RWC-TVS was performed at the time of the first consultation at our institution. A flexible catheter was introduced into the rectal lumen in order to injected up to 300 mL of sterile saline solution. Patients underwent a second evaluation by 3D-TVS in the same consultation by a physician blinded to the findings of the first exam. The results of the two exams were compared with surgical findings and histology.

Main results and the role of chance:

The mean (± SD) age of the study population was 33.6±5.2 years. At the time of the study, 20 women (55.5%) were under hormonal therapies. 21 patients (61.1%) had rectosigmoid endometriosis at surgery. The McNemar's test demonstrated that 3D-RWC-TVS and 2D-RWC-TVS had similar performance in the diagnosis of rectosigmoid endometriosis (p=0.50). The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of 2D-RWC-TVS and 3D-RWC-TVS were respectively 90.1% (95% CI, 70.8%-98.9%), 78.6% (49.2%-95.3%), 87.0% (70.8%-94.8%), 84.6% (58.8%-95.5%), 4.2 (1.5-11.7), 0.1 (0.0-0.4) and 86.4% (65.1%-97.1%), 85.7% (57.2%-98.2%), 90.5% (72.3%-97.1%), 80.0% (57.8%-92.1%), 6.1 (1.7-22.1), 0.2 (0.1-0.5). Overall, there was no significant difference in the accuracy of 2D-RWC-TVS and 3D-RWC-TVS in estimating the volume of the largest intestinal nodule (P=0.524) and in estimating the distance between the lower endometriotic bowel nodule and the anus (P=0.860).

Limitations, reasons for caution:

The limitations of this study are the small sample size and that the surgeons were aware of the findings of 2D and RWC-TVS.

Wider implications of the findings:

Despite of being both feasible and well tolerated, 3D acquisitions do not seem to improve the performance of RWC-TVS for diagnosing rectosigmoid endometriosis.

Trial registration number:

Not applicable

O-138 The IDEAL study: MRI for suspected deep endometriosis assessment prior to laparoscopy is equally reliable as radiological imaging as a complement to transvaginal ultrasonography

D. Bielen[1], L. De Wever[1], D. Vanbeckevoort[1], D. Van Schoubrouck[2], C. Tomassetti[2]

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[2]University Hospitals Leuven, Gynaecology, Leuven, Belgium

Study question:

Can a 'one-stop' abdominal MRI (magnetic resonance imaging) replace ionizing radiological imaging (urography plus barium-enema) in the preoperative work-up of suspected deep endometriosis (DE)?

Summary answer:

'One-stop' MRI plus transvaginal ultrasound (TV-US) predicts intra-operative findings equally well as compared to standard radiological imaging plus TV-US.

What is known already:

When planning surgery for suspected DE, guidelines emphasize the importance of assessing the extent of involvement of ureter, bladder and bowel. Whereas several techniques are available (e.g. double-contrast barium-enema (DCBE), TV-US, trans-rectal ultrasonography, MRI, intra-venous pyelography (IVU), there is no current guidance on which type of technique is more adequate. In our institution, standard work-up has since long included the combination of TV-US, IVU and DCBE. Although the radiological techniques clearly have the disadvantage of ionizing radiation exposure, so far it is not well established whether they can be replaced reliably by an MRI for (pre-)operative patient-counselling and planning.

Study design, size, duration:

Single-centre prospective study, between 20/2/2014 and 23/11/2015, including 120 women who underwent TV-US, IVU, DCBE and MRI. The sample size was based on the presumed accuracy for DCBE (75%) and MRI (85%). Both standard (TV-US with IVU/DCBE) and MRI-based imaging (TV-US with MRI) were compared with the laparoscopic findings and the extent of the surgery (multidisciplinary or not). Radiologists performing the DCBE, IVU and MRI were blinded; MRI-results were unblinded at the end of surgery.

Participants/materials, setting, methods:

120 women with suspected DE in a tertiary referral centre, with an presumed indication for excisional surgery, received standardized hormonal treatment (nomegestrol acetate) for at least 4 weeks prior to their work-up which was planned in a 2-week timeframe. Work-up included TV-US, DCBE, IVU and contrast-enhanced abdominal MRI.

All MRI scans were performed on a 1.5 Tesla scanner with vaginal and rectal opacification to obtain adequate distention and delineation of the vaginal and rectal wall.

Main results and the role of chance:

Imaging and surgical data of 74/120 women were available for analysis. Both the standard work-up as the MRI-based work-up predicted correctly whether surgery eventually was mono- or multidisciplinary in 90.5% of the women (67/74).

In both approaches, the severity of the rectal involvement was underestimated in 2.7% (2/74), whereas it was overestimated in 6.8% (5/74) of the patients; in 2 different patients with obvious DIE lesions findings were false negative due to a perceptual error on DCBE, and incomplete bowel filling on MRI. Both lesions were located at a distance > 15 cm from the internal anal orifice.

The overall assessment of the involvement of the bowel wall, gynaecological structures, bladder and ureters was correct in 50/74 patients (67,6%) for the standard preoperative imaging, as compared to in 64/74 patients (86,5%) for the MRI-based work-up (absolute difference between of 18,9% (3,2% to 33,7% CI 95%) (p-value 0,02)).

In one patient, a deep lesion on the diaphragm was only detected by MRI-based work-up and confirmed laparoscopically. Four lesions were not detected on any preoperative work-up, but only during surgery: involvement of the ischioanal fossa (n=1), small intestine (n=2), and abdominal wall (n=1).

Limitations, reasons for caution:

Due to symptom improvements, or failure to comply with the study prerequisites, 46/120 patients didn't undergo all exams and/or surgery.

Significant delay between preoperative work-up and time of surgery (mean 327 days) may have an negative effect, but this is applicable to all techniques.

Wider implications of the findings:

MRI shows the direct DIE signs and its consequences on the surrounding tissues, especially the involvement of the organs that tend to escape routine evaluation, e.g. the small bowel, diaphragm or abdominal wall, without any ionizing radiation. Better bowel filling and MR hydrogram without contrast may further improve the technique.

Trial registration number:

The study was registered on ClinicalTrials.gov, a service of the U.S. National Institutes of Health, with identifier NCT01939535 'Preoperative staging of endometriosis with MRI (IDEAL)'.

O-139 Association between disease extent and pain symptoms in patients with deep infiltrating endometriosis (DIE)

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Study question:

The aim of this study was to assess associations between preoperative symptoms in patients with deep infiltrating endometriosis (DIE) and the intraoperatively determined disease extent described by the ENZIAN score.

Summary answer:

Disease localization and extent as described by the ENZIAN score correlated with presence and severity of different preoperative symptoms.

What is known already:

The revised American Society for Reproductive Medicine (rASRM) score is the currently most widely used classification system for endometriotic lesions. However, deep infiltrating endometriosis (DIE) is not fully taken into account and disease extent shows only a poor correlation with pain and sterility.

In order to describe DIE more accurately, the ENZIAN classification was developed in addition to the rASRM score and was revised in 2010 and 2011. Up to now, there were only very limited data available on the association between anatomical extent of DIE as described by the ENZIAN score and symptoms such as dysmenorrhea, dyspareunia, dysuria or dyschezia.

Study design, size, duration:

This study was a retrospective data analysis of prospectively collected data of 245 women who underwent surgery for deep infiltrating endometriosis between 2014 and 2018 at a tertiary referral center for endometriosis.

Participants/materials, setting, methods:

Data of women who underwent surgery for deep infiltrating endometriosis between 2014 and 2018 at a tertiary referral center for endometriosis were analysed. Possible associations between presence and severity of different preoperative symptoms such as dysmenorrhea, dyspareunia, dysuria and dyschezia and the intraoperatively determined disease extent as described by the ENZIAN score were assessed.

Main results and the role of chance:

Data of 245 women were analysed. Statistically significant associations between involvement of ENZIAN compartment B (uterosacral ligaments, parametrium) and presence of dyspareunia (p=0.002), ENZIAN compartment C (rectum, sigmoid colon) and dyschezia (p <0.001) and ENZIAN compartment FB (urinary bladder) and dysuria (p <0.001, Fisher's exact test) were found. Statistically significant correlations were also detected between symptom severity of dyschezia and lesion size in ENZIAN compartment C (Spearman's correlation coefficient: 0.334, p <0.001) as well as dyspareunia and lesion size in ENZIAN compartment B (correlation coefficient: 0.127, p=0.046). Severity of dysmenorrhea correlated with lesion size in ENZIAN compartment A (Spearman's correlation coefficient: 0.244, p <0.001) and with presence of adenomyosis (ENZIAN compartment FA, p=0.005, Mann-Whitney-U-test). Additionally, the number of affected compartments (A, B, C and FA) significantly correlated with the severity of dysmenorrhea (Spearman's correlation coefficient: 0.256, p <0.001) and dyschezia (correlation coefficient: 0.161, p=0.012).

Limitations, reasons for caution:

Symptom presence and severity may not only depend on involvement of a single anatomical site but rather represent a cumulative effect of single DIE lesions. Resulting symptom severity may therefore be greater or lesser than expected. However, the number of affected ENZIAN compartments correlated with severity of dysmenorrhea and dyschezia.

Wider implications of the findings:

In contrast to previous studies evaluating disease extent based on the rASRM score, disease localization and extent described by the ENZIAN score correlated with presence and severity of different preoperative symptoms, which underlines the importance of DIE extent evaluation using the ENZIAN score in addition to the rASRM score.

Trial registration number:

not applicable

O-140 The effect of post-operative medical interventions on endometrioma recurrence: A systematic review and meta-analysis

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Study question:

Does post-operative medical intervention prevent the risk of recurrent endometrioma? What is the medical intervention of choice in preventing recurrence?

Summary answer:

In women who had surgery for endometrioma, post-operative medical intervention reduces the endometrioma recurrence compared to no intervention. All medical interventions are found equally effective.

What is known already:

Surgery for endometrioma is often difficult as they are more adherent. The primary surgery is important to remove as much disease as possible to prevent recurrence as repeated surgeries are more difficult and cause more damage to the normal ovarian tissues. Post-operative medical intervention is given to delay or prevent endometrioma recurrence although the effectiveness of these interventions is not clear. Several systematic review and meta-analysis have described the effects of oral contraceptive (OC), gonadotrophin releasing hormone (GnRH) analogue and levonogestrel intrauterine system (LNG-IUS) in reducing endometrioma recurrence. However, these reviews are dated and none have combined all the interventions.

Study design, size, duration:

We searched Pubmed, MEDLINE and Cochrane Library for relevant studies published online up to December 2018 in English language using predefined keywords. Published randomised controlled trials (RCT) and non-RCTs were included. We included all medical interventions using OC, GnRH-analogue, all kind of oral progestogens and LNG-IUS. The disease recurrence was separated where possible into disease recurrent (reoccurrence of endometriotic cyst) and pain symptoms at any time post operatively.

Participants/materials, setting, methods:

From the initial search we found 426 studies, out of which 405 were excluded after reviewing the abstracts. Further, after retrieving the full-text 2 review authors agreed to include 9 RCTs (N=1245 patients) and 12 Non-RCTs (N = 2381 patients). We calculated Mantel-Haenszel odds ratio (OR) and presented 95% confidence interval for all outcomes. Statistical heterogeneity was measured by I^2 with random effect model was used in substantial heterogeneity (I^2 more than 50%).

Main results and the role of chance:

Compared to placebo, medical interventions reduced the overall risk of recurrence (OR 0.26, 95% CI 0.15-0.45, P=<0.00001, 16 studies, 2721 patients, I^2=81%) with GnRH analogue (OR 0.66, 95% CI 0.45-0.96, P=0.59, 6 studies, 716 patients, I^2=0%); OC (OR 0.19, 95% CI 0.09-0.40, P=0.002, 6 studies, 1097 patients, I^2=73%), Dienogest (OR 0.08, 95% CI 0.02-0.30, P=0.002, 5 studies, 1027 patients, I^2=77%) and LNG IUS (OR 0.35, 95% CI 0.10-1.81, P=0.19, 2 studies, 135 patients, I^2=43%).

Head to head comparison between each medical interventions showed that GnRH has no advantage against OC (OR 0.98, 95% CI 0.33-2.92, P=0.23, 2 studies, 179 patients, I^2 32%) and Dienogest (OR 1.86, 95% CI 0.71-4.83, P=0.58, 3 studies, 212 patients, I^2=0%). GnRH analogue also shows no significant reduction in post-operative endometriosis related pain when compared to placebo (OR 0.73, 95% CI 0.41-1.33, P=0.69).

Pooling the result comparing the effectiveness of LNG IUS against OC showed that LNG IUS was not superior to OC in preventing recurrent endometrioma (OR 1.52 95% CI 0.15-14.86, P=0.02, 2 studies, 191 patients). However, the result has significant heterogeneity I^2=83%.

Limitations, reasons for caution:

We included cohort studies in this meta-analysis, as there is no enough data from RCTs. Therefore, it is difficult to control for confounding and selection bias which may attribute to tight confidence interval around spurious result. This meta-analysis also exhibits high heterogeneity, hence the result should be interpreted with cautions.

Wider implications of the findings:

The result of this meta-analysis suggests that it is reasonable to prevent endometrioma recurrence with medical interventions rather than by conservative management. However, we cannot conclude which agent has higher superiority. Therefore, more large scale RCTs are needed to compare the effectiveness of various post-operative medical interventions.

Trial registration number:
Not applicable

O-141 Linzagolix for treating endometriosis-associated pain: efficacy and safety results from a randomized, placebo-controlled, multinational, phase 2b dose-ranging trial

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Study question:
What are the effects of once daily oral doses of 50, 75, 100 and 200 mg taken for 24 weeks in women with endometriosis-associated pain (EAP)?
Summary answer:
Linzagolix significantly reduced EAP compared to placebo with once daily oral doses of 75 mg to 200 mg. All doses were well tolerated.
What is known already:
Suppression of estradiol (E2) using GnRH analogues has been shown to be an effective treatment for EAP. However, suppression of E2 leads to menopausal symptoms including vasomotor symptoms and bone mineral density (BMD) loss. It has been postulated that partial suppression of E2 could provide reduction in endometriosis pain symptoms without significant long-term BMD loss. Linzagolix is a new, non-peptide, GnRH receptor antagonist being developed to treat EAP. It has previously been shown to suppress E2 and decrease EAP in Japanese patients.
Study design, size, duration:
Study treatments were taken once daily for 24 weeks. The primary endpoint was the proportion of subjects with 30% or more reduction in pelvic pain over 28 days, assessed on a Verbal Rating Scale using an electronic diary. Secondary endpoints included dysmenorrhea, non-menstrual pelvic pain (NMPP), dyspareunia, dyschezia, serum E2, difficulty of doing daily activities, Patient Global Impression of Change/Severity (PGIC/S), Endometriosis Health Profile-30 (EHP-30), amenorrhea, BMD with Dual-energy X-ray Absorptiometry (DXA) and adverse events.
Participants/materials, setting, methods:
327 women aged 18 to 45 years with moderate-to-severe EAP were recruited in USA and Europe in 2016 to 2018. Participants had to have surgically confirmed endometriosis. Women with a history of osteoporosis or other metabolic bone disease were excluded. BMD of the femoral neck, total hip and lumbar spine was assessed by DXA at baseline and after 12 and 24 weeks of treatment. Reading of DXA scans and monitoring of scan quality was centralized.
Main results and the role of chance:
Compared to placebo, doses ≥ 75 mg resulted in a significant increase in the proportion of responders at 12 weeks (34.5%, 49.5% (P=.155), and 61.5% (P=.003), 56.4% (P=.039) and 56.3% (P=.034) for placebo, 50, 75, 100 and 200 mg, respectively). A similar pattern was seen for dysmenorrhea and NMPP. The effects were maintained or increased at 24 weeks. Median E2 levels were 35 to 47 and 12.5 to 13 pg/mL with 75 mg and 200 mg, respectively. Side effects, such as hot flashes, were consistent with E2 levels.

Doses ≥ 75 mg also consistently and significantly (P<.05) improved dyschezia and patient well-being as assessed by EHP-30, PGIC, PGIS and difficulty of doing daily activities. Dyspareunia was also improved, reaching statistical significance at 200 mg (P=.02). The incidence of amenorrhea increased with increasing dose up to about 75% of women at 12 weeks for 200 mg.

Mean percent (95% CI *P* value) BMD changes for lumbar spine from baseline to week 24 in the 50, 75, 100 and 200 mg dose groups were 0.137% (-0.83, +1.11 *P*=.777), -0.798% (-1.57, -0.03 *P*=.042), -1.365% (-2.14, -0.59, *P*<.001), -2.602% (-3.56, -1.65, *P*<.001), respectively. BMD of femoral neck and total hip showed a similar pattern.
Limitations, reasons for caution:
This was a dose-ranging study with about 55 patients per treatment group treated for 6 months. The findings of this study need to be confirmed in larger confirmatory trials.
Wider implications of the findings:
Phase 3 confirmatory trials will include a 75 mg (partial E2 suppression) daily dose without need for concomitant estrogen/progestin add-back therapy (ABT), and a 200 mg (full E2 suppression) daily dose in combination with low dose ABT (1 mg E2 / 0.5 mg norethisterone acetate) to mitigate BMD loss.
Trial registration number:
ClinicalTrials.gov: NCT02778399

O-142 The long term costs and effects of tubal flushing: a follow-up study of a randomized clinical trial comparing oil- versus water-based contrast at hysterosalpingography

B.W. Mol[1], N. Van Welie[2], C. Pham[3], J. Van Rijswijk[2], K. Dreyer[2], H. Verhoeve[4], A. Hoek[5], J.P. De Bruin[6], A. Nap[7], M. Van Hooff[8], M. Goddijn[9], A. Hooker[10], C. Lambalk[2], J. Karnon[3], V. Mijatovic[2]

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Study question:
What are the long term costs and effects of oil- versus water-based contrast in infertile women undergoing hysterosalpingography (HSG)?
Summary answer:
Over a 3-5 year follow-up period, HSG with oil-based contrast results in higher live birth rates than HSG with water-based contrast for a comparable cost.
What is known already:
We recently showed in a randomized clinical trial (RCT) that HSG with oil-based contrast (Lipiodol®, Guerbet, France) results in higher 6-month ongoing pregnancy and live birth rates compared to water-based contrast (Telebrix®) (Dreyer et al., 2017). Within a time horizon of 6 months, the use of oil-based contrast costed US$8,198 for an additional ongoing pregnancy as compared to water-based contrast. A follow-up study of this RCT showed shorter time to pregnancy after tubal flushing during HSG with the use of oil-based contrast compared to water-based contrast. However, the long-term costs and effects of oil-based contrast have not been studied.
Study design, size, duration:
We performed an economic evaluation of the long-term follow-up of the H2Oil trial. The H2Oil trial was a multi-center RCT in the Netherlands in which 1,119 women were included and randomized to hysterosalpingography with oil-based contrast (n= 557) or water-based contrast (n= 562). Here, we present the long-term cost-effectiveness of the use of oil- versus water-based contrast in infertile women undergoing HSG.
Participants/materials, setting, methods:
The economic evaluation took a health care system perspective, and compared costs related to all treatment in the 3-5 years since randomization. The effectiveness outcome was ongoing pregnancy leading to live birth up to 5years. We calculated incremental cost-effectiveness ratios (ICERs) (differences

in costs versus differences in ongoing pregnancy rates) between the two groups. Bootstrapping was used to estimate the level of uncertainty around the ICERs. Data were analyzed using the intention to treat principle.

Main results and the role of chance:

Between February 2012 and October 2014, we randomized 1,119 women to receive an HSG with oil-based (n=557) or water-based (n=562) contrast medium. Of the women in the oil-based contrast group, 40% had no other treatment, 35% had intrauterine insemination (IUI), and 25% had in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) in the 5 years following HSG. Comparatively, of the women in the water-based contrast group, 35% had no other treatment, 34% had IUI, and 31% had IVF/ICSI in the 5 years following HSG (p=0.11).

In the oil-based contrast group, 445 women (80%) had an ongoing pregnancy leading to live birth within the 5 years of follow-up compared with 420 women (75%) in the water-based contrast group (HRR 1.27; 95% CI 1.10-1.46).

The mean costs per couple were €5,341 for oil-based contrast and €5,441 for water-based contrast. After 5 years of follow-up, HSG using an oil-based contrast resulted in comparable costs (-€100; 95% CI -€652 - +€402, p=0.642) for a 5% higher cumulative ongoing pregnancy rate compared with HSG using water-based contrast, making it the dominant strategy.

Limitations, reasons for caution:

This study was limited to women at low risk for tubal pathology, <39 years of age and without known endocrinological diseases. The findings should therefore not be generalized to other groups of infertile women.

Wider implications of the findings:

Tubal flushing with oil-based contrast resulted in higher cumulative live-birth rates. The higher price of oil-based contrast was compensated by lower IVF-uptake. Since HSG with oil-based contrast gives better ongoing pregnancy rates and shorter time to pregnancy for similar costs, we recommend it as preferred strategy for tubal testing.

Trial registration number:

Dutch Trial Register, NTR 6577

INVITED SESSION

SESSION 40: EUROPEAN AND GLOBAL ART MONITORING

Tuesday 25 June 2019 Haydn 3 11:45 - 12:45

O-143 European IVF-monitoring of ART

O-144 ICMART preliminary world report 2015

G.D. Adamson[1], S. Dyer[2], G. Chambers[3], O. Ishihara[4], R. Mansour[5], M. Banker[6], J. De Mouzon[7], F. Zegers-Hochschild[8]

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Abstract text

ABSTRACT TITLE

International Committee for Monitoring Assisted Reproductive Technologies (ICMART) Preliminary World Report on ART, 2015

STUDY QUESTION

In 2015 what was global utilization, effectiveness and safety of ART?

SUMMARY ANSWER

Globally, ART utilization and data collection continue to increase but with wide variations in utilization, effectiveness and safety.

WHAT IS KNOWN ALREADY

ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower multiple birth rates, the latter as a result of transfer of fewer embryos. Frozen embryo transfer (FET) and donor egg cycles continue to increase. However, wide variations in practice and outcomes exist globally. Approximately 8 million ART babies have been born. ICMART has helped develop registries internationally, notably with the African Network and Registry for Assisted Reproductive Technology (ANARA). A new electronic data collection platform is being developed; nevertheless, data collection and quality remain challenging.

STUDY DESIGN, SIZE, DURATION

Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested these data from all known global sources for 2015 and reviewed them for missing or incorrect data. The dataset was corrected and then analyzed utilizing standardized definitions from The ICMART/WHO Revised Glossary on ART Terminology, 2009 which was current at the time but is now replaced by The International Glossary on Infertility and Fertility Care, 2017, and previously developed methods. Preliminary results are presented.

PARTICIPANTS/MATERIALS, SETTING, METHODS

The European IVF Monitoring Consortium (EIM), Latin American Network of Assisted Reproduction (REDLARA), Australian/ New Zealand Registry and ANARA submitted regional data, and other countries contributed national data, through standardized formats to ICMART. A few individual clinics with no registry access also contributed. Data were reviewed, corrected, validated to the extent possible, analyzed and summarized by ICMART using descriptive statistics.

MAIN RESULTS AND THE ROLE OF CHANCE

Data collection and analysis are ongoing, so presented results are preliminary. The number of ART cycles continues to increase, but utilization is still highly variable among countries and regions. Regional and country differences persist in the age of the population treated, number of embryos transferred, rate of multiple births, use of ICSI, cryopreservation cycles and other factors.

The role of chance is minimal. Actual global ART results are limited to reporting countries and clinics representing approximately 2/3 of global cycles. However, this is a very large sample size from which imputation of total global results is performed.

LIMITATIONS, REASONS FOR CAUTION

Some countries have limited data and many countries have limited data validation. ICMART can perform only minimal verification of submitted data. Widespread adherence to consensus definitions provided in the Glossary takes time and requires translation into multiple languages.

WIDER IMPLICATIONS OF THE FINDINGS

ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART quality. Better understanding of ART increases societal acceptance and support for equitable access and ART research.

O-145 Access to ART: Concepts, indicators, impact

S.J. Dyer[1], G. Chambers[2], F. Zegers-Hochschild[3], G.D. Adamson[4]

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Abstract text

This presentation has three objectives: to address the importance of ART utilization, present new terminology pertaining to it, and explore relevant correlations between drivers of ART utilisation and access to quality care.

The global drive towards reaching health-related Sustainable Development Goals (SDG) requires the identification of key indicators and subsequent monitoring of change over time. In addition, the recognized need to reduce

inequality requires data and analyses that identify inequities in access to care and possible drivers.

According to the World Health Organization, the right to access health care comprises four domains: availability, accessibility, acceptability, and quality. Utilization of health services lies downstream from these domains and is an indicator of realized access. The association between all these indicators applied to ART may provide insight into barriers and facilitators of access to fertility care.

There is as yet no universally accepted key indicator for access to infertility care. ART utilization can be considered a proxy indicator for realized access to ART as well as infertility care *per se*. ICMART proposes that ART utilization be adopted for this purpose. This in turn requires more accurate reporting systems pertaining to ART utilization. ICMART proposes new terminology and an approach to deal with missing data.

Using ICMART data and global indicators of health and welfare, correlations will be shown between ART utilization and facilitators or barriers, as well as ART practices and outcomes. These possible associations further emphasise the importance of ART utilization in the global context of the right to infertility health care; and ICMARTs role in reporting ART data at a global level.

INVITED SESSION

SESSION 41: LUTEAL PHASE SUPPORT – HAVE WE GOT IT RIGHT?

Tuesday 25 June 2019 Haydn 2 11:45 - 12:45

O-146 The artificial endometrial preparation for embryo transfer: The litmus test for a sex steroid regimen?

E. Labarta Demur[1]

[1] *IVI RMA Valencia, Human Reproduction, Valencia, Spain*

Abstract text

The artificial endometrial preparation for embryo transfer: The litmus test for a sex steroid regimen?

The administration of exogenous progesterone (P) is needed for the luteal phase support in artificial endometrial preparation cycles with hormone replacement therapy (HRT) for embryo transfer. By promoting endometrial maturation, is essential for the embryo implantation process. The transformation of the endometrium from a proliferative to a receptive state depends on an adequate exposure to P, which prepares the uterus for implantation and the maintenance of pregnancy.

Progesterone has also non-hormonal actions such as allowing immune tolerance during pregnancy, especially affecting the synthesis of cytokines and the function of Natural Killers cells. Low exposure to P at the time of implantation or during early pregnancy may result in an implantation failure or spontaneous miscarriage.

Surprisingly, there is no individualization in the best route, dose or time of exogenous P administration, as published studies do not demonstrate that there is a clearly superior protocol. There are different administration routes of P such as vaginal, subcutaneous, intramuscular, oral or rectal. Until now, the vaginal route has been the most used in Europe. It has been shown that although serum P levels appear to be lower after vaginal than intramuscular administration, vaginal route results in an adequate secretory transformation and good pregnancy rates. This discrepancy suggests that the bioactivity of vaginal P in the uterus is higher, due to the effect of the first step of the uterus. For this reason, no measurement of serum P levels have been conducted to analyse if the absorption was adequate.

The best context to analyse the net impact of exogenous P is the artificial cycle, in which there is no endogenous production, in comparison with the stimulated or natural cycle. In this scenario, recent studies have suggested that serum P levels have a correlation with the success rate in artificial cycles when using the vaginal route. The only published prospective study analysing this topic was carried out in oocyte donation cycles performed in our centre, showing that there is a serum P threshold (9.2 ng/ml) below which ongoing pregnancy rates significantly decrease (32.7% vs 52,8%, p=0.016). These results have been confirmed and validated in a large prospective study of almost 1200 unselected patients undergoing an embryo transfer in an artificial cycle, regardless the origin of the oocytes and after adjusting for any confounding factors (submitted to this ESHRE meeting).

Once demonstrated that these results can be extrapolated to the general infertile population, new research is focused on finding the best way to increase serum P levels and improve the live birth rate. Moreover, data about the reproducibility of this situation in a subsequent cycle when using the same doses of P or even the ability to predict at the beginning of the luteal phase that serum P levels will be low in the mid luteal phase will be also presented.

This unexpected finding has opened a line of research on the role of serum P on endometrial receptivity and the possibility of reversing the cases with low serum P levels, which could imply a change in our daily clinical practice and management of the luteal phase.

O-147 Luteal phase support: Old habits and new avenues

INVITED SESSION

SESSION 42: MITOCHONDRIA IN HEALTH AND AGEING

Tuesday 25 June 2019 Haydn 4 11:45 - 12:45

O-148 Origin and long-term consequences of transmission of oocytes' damaged mitochondria in obesity

O-149 Mitochondrial aging in stem cells- intriguing stories

SELECTED ORAL COMMUNICATIONS

SESSION 43: BARRIERS AND BOUNDARIES IN INNOVATIVE ASSISTED REPRODUCTION TECHNOLOGIES

Tuesday 25 June 2019 Strauss 1+2 11:45 - 12:45

O-150 Regulating human germline gene editing: should we be concerned about human dignity?

S. Segers[1], **H. Mertes**[1]

[1] *Bioethics Institute Ghent Ghent University, Department of Philosophy and Moral Sciences Ghent University, Ghent, Belgium*

Study question:

Is the violation of human dignity a convincing argument against human germline gene editing?

Summary answer:

Dignity concerns cannot underpin the current ban on heritable genome editing, but they can inform the implementation of side constraints.

What is known already:

Fast developments in the field of genome editing put pressure on legislators and ethicists to investigate whether – and if so: how – this research can be translated into clinical practice in an acceptable way. In 2018, the first genome-edited human babies were reportedly born, despite widespread moral concerns about the safety risks of these techniques. Official international documents (like the so-called Oviedo Convention) mainly oppose germline gene editing on the basis of concerns about human dignity. It is, however, unclear whether dignity concerns can serve as a basis to hold on to a ban on human germline gene editing.

Study design, size, duration:

A literature study of the scientific literature was performed to inventory the state-of-the-art of gene editing techniques. Next, official international and legal documents were analysed and claims about how germline gene editing

assumedly breaches human dignity were inventoried. This was evaluated against the backdrop of ethical analyses of the concept 'dignity' and contextualized by referring to recent reports by international committees that call for an update of current regulations in the light of recent developments.

Participants/materials, setting, methods:

Literature study, conceptual analysis, normative analysis.

Main results and the role of chance:

Human germline gene editing is opposed by both the Council of Europe and UNESCO. Their plea for banning heritable gene editing is based on the argument that this would breach human dignity. However, it is highly dubious whether germline gene editing leads to infractions against human dignity, especially when germline interventions are aimed at avoiding diseases. It is argued in this study that the concept of 'human dignity' can also be invoked to plead *for* germline gene editing, at least for non-trivial interventions. This is not to deny that a focus on dignity may signal risks of breaching people's rights, but it is argued that if 'dignity' is left unspecified and if it is invoked in a way like it is now by current European and international documents, it has to be concluded that the argument is self-defeating. This is problematic in the light of fast technical developments in gene editing and challenges the legislative and ethical aim of responsible innovation. We show that this underpins recent calls – like those by The European Society of Human Genetics (ESHG) and the European Society of Human Reproduction and Embryology (ESHRE) – to rekindle the debate about the regulation of gene editing.

Limitations, reasons for caution:

The feasibility of germline gene editing is hypothetical. Except for the reported birth of the genome-edited babies in China (November, 2018), the use of these techniques has been limited to research contexts. In addition, 'human dignity' is an ambiguous concept, and there is no general consensus on its meaning.

Wider implications of the findings:

The argumentative link between germline gene editing and human dignity is weak which might undermine current international regulations. This underpins recent calls for a public debate about genome editing and how it should be regulated.

Trial registration number:

Not applicable

O-151 Between innovation and precaution: the role of offspring safety considerations in strategies of introducing new reproductive techniques

V. Jans[1], W. Dondorp[1], G. De Wert[1]

[1]*Maastricht University, Health- Ethics and Society, Maastricht, The Netherlands*

Study question:

How did offspring safety considerations play a role in strategies of introducing new reproductive techniques and what can we learn from this?

Summary answer:

Responsible strategies of introducing new ARTs require explicit accountability with regard to dealing with offspring safety risks.

What is known already:

As several commentators have pointed out, many assisted reproductive technologies (ARTs) have been introduced without systematic research on possible offspring safety risks. However, the lack of safety studies does not mean that safety considerations were absent from earlier debates about innovation in this field. Explicitly or implicitly, professionals and policy-makers have translated their views about the relative importance of these considerations into concrete policy choices.

Study design, size, duration:

In this study, we describe how offspring safety considerations have played a role in selected case-studies of the introduction of potentially risky new ARTs: Intracytoplasmic Sperm Injection (ICSI), Pre-implantation Genetic Diagnosis (PGD) and Mitochondrial Replacement Therapy (MRT). As a framework for analyzing these cases we use Per Sandin's account of the four dimensions of dealing with risks (threat, uncertainty, action, command) that are central to debates about the possible role of the so-called 'precautionary principle'.

Participants/materials, setting, methods:

This combined empirical/ normative ethics study has three parts. 1) Literature study (ethics of technological innovation, precautionary principle, history of case studies: debates about introducing ICSI, PGD, and MRT). 2) Interviews with key figures: professionals who were actively involved in one of those case studies. 3) Ethical analysis of the findings with an eye to defining 'lessons for the future'. Our aim here is to contribute to guidance for the responsible introduction of new ARTs.

Main results and the role of chance:

In our case studies, we found that possible offspring safety effects were clearly part of the debate about whether and how to introduce the relevant technology ('threat-dimension'). However, especially the lack of evidence about the chances of those effects materializing ('uncertainty-dimension') led to divergent answers as to what (if any) measures aimed at reducing possible risks were desirable ('action-dimension') and whether or not those measures should somehow be imposed ('command-dimension'). On one end of the spectrum, a tendency in the field has always been to deal with such uncertainty in the light of the so-called 'innovation principle', stating that the benefits of innovation should only be weighed against *known* harms. On the other end, we found some examples of relatively strong precautionary measures such as the legally imposed moratorium on ICSI using surgically obtained sperm that was in place in the Netherlands around the turn of the century. Positions in between focus on trying at least to reduce the level of uncertainty through preclinical and/or follow-up studies. We conclude that if both 'anything goes' and forgoing important benefits are problematic, transparency and explicit accountability are essential elements of a responsible approach to dealing with offspring health risks of new ARTs.

Limitations, reasons for caution:

Our reconstruction of the three case studies is partly based on interviews, which may introduce bias. Moreover, as in all contributions to normative ethics, our conclusions are based on arguments that we have tried to make as strong as possible, but about which reasonable people may still disagree.

Wider implications of the findings:

With new promising and potentially risky new ARTs on the horizon (e.g. reproductive use of stem cell derived gametes, gene-editing in a reproductive context) our analysis and conclusions can enrich the necessary debate about how to responsibly introduce those technologies.

Trial registration number:

not applicable

O-152 Guarding the bounds of a morally sensitive practice. The role and verdicts of the Dutch National Indications Committee for preimplantation genetic diagnosis (PGT-M/SR).

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[2]*Maastricht University Medical Centre MUMC+, Clinical genetics, Maastricht, The Netherlands*

Study question:

How did the Dutch National Indications Committee (LIC) fulfill its task of assessing new indications for preimplantation genetic diagnosis (PGT-M/SR)?

Summary answer:

Instead of prescribing the boundaries of PGT-M/SR, the LIC's verdicts ultimately refer to the consultation room as the place to decide about treatment requests

What is known already:

Many European countries have legislation limiting the scope of acceptable PGT-M/SR indications to conditions entailing a 'high risk of serious genetic disorder'. Of the countries upholding this standard, most leave its application to individual centres and practitioners. Some countries (Germany and Norway) require that each PGT-M/SR case must have the prior approval of a multidisciplinary ethics committee. An in between position is taken in two other countries (the Netherlands and the U.K.), who have a national committee or authority determining on a more general level which 'new' conditions are sufficiently 'high risk' and 'serious' to be acceptable as PGT-M/SR indications.

Study design, size, duration:

Since 2009, the Netherlands has a National Indications Committee (LIC) for PGT-M/SR, tasked with ensuring that PGT is only done for couples at a 'high risk' of a child with a 'serious hereditary disorder'. The LIC's green light is

needed for any new conditions, i. e. conditions for which PGT-M/SR has not as yet been done in the Netherlands. We have analysed all LIC-verdicts In order to assess how this system has functioned in practice.

Participants/materials, setting, methods:

The LIC has issued >100 verdicts. Its remit requires it to use a decisional framework defined in the Statutory Regulation of 2009, referring to relevant co-determinants of risk/seriousness. In addition to medical criteria, the framework also acknowledges 'psychosocial or moral factors' that however are only allowed to weigh in for conditions considered acceptable on medical grounds. This is comparable to the framework used by the Human Fertilisation & Embryology Authority (HFEA) in the UK.

Main results and the role of chance:

The LIC has been given a difficult task: to guard responsible indications-setting on the basis of criteria that can only partly be operationalized in general terms. Taking a different approach than the HFEA (which assesses new indications on the basis of a 'worst case analysis'), the LIC presents its verdicts as general assessments that allow for divergence in concrete cases. None of the verdicts we found was a flat 'yes' or 'no'; all were a qualified 'yes, provided that. . ..', or no, unless. . .'. Interestingly, the scope for making exceptions was not only linked to medical criteria (e.g. allowing the centre to take account of the specific phenotype of affected family-members), but also to the subjective perspective of the applicants. For instance, with regard to a condition where the LIC considered the impact to be psychosocial rather than strictly medical, the verdict was still 'no unless', allowing the centre to consider 'highly special circumstances and motives of the applicants'. By taking this line, the LIC seems to deviate from the policing task envisaged for her by Dutch politics. However, we argue that precisely by taking a more advisory role she has contributed to the responsible development of PGT practice in the Netherlands.

Limitations, reasons for caution:

This study concerns the regulation of PGT-M/SR practice in one specific European country.

Wider implications of the findings:

As the issues at stake (e.g. how to deal with the applicants' subjective perspective of risk/seriousness in debates about indications setting) are of a general nature, our observations about the role and verdicts of the Dutch National Indications Committee may be relevant for policy discussions in other countries as well.

Trial registration number:

not-applicable

O-153 International consensus: Ovarian tissue cryopreservation in young Turner syndrome patients

M.J. Schleedoorn[1], B.H. Mulder[1], D.D.M. Braat[1], C.C.M. Beerendonk[1], R. Peek[1], W.L.D.M. Nelen[1], E. Van Leeuwen[2], J.A.E.M. Van Alfen - van der Velden[3], K. Fleischer[4]

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Study question:

What is the standpoint of an international expert panel on ovarian tissue cryopreservation (OTC) in young females with Turner syndrome (TS)?

Summary answer:

The majority of the expert panel states that OTC should be offered to young females with TS.

What is known already:

OTC is a valid option to preserve the fertility of young females at risk of iatrogenic premature ovarian insufficiency (POI). Offering OTC to females with a genetic cause of POI seems a logical next step. One of the most common genetic disorders related to POI is TS. Due to an early depletion of the ovarian reserve, most females with TS are confronted with infertility before reaching adulthood. However, before offering OTC as an experimental fertility preservation option to young females with TS, the medical and ethical concerns need to be addressed first.

Study design, size, duration:

A three-stage ethical[Z2] Delphi study was conducted to discuss the pros and cons of OTC in young females with TS in a systematic manner. The aim of this study was to reach group consensus and to form an international standpoint based on selected key statements. The study was conducted between February and December 2018.

Participants/materials, setting, methods:

A mixed panel of 12 gynaecologists, 13 (paediatric) endocrinologists, 10 medical ethicists and 20 patient representatives participated in this international Delphi study. In the first two rounds, all experts were asked to rate and rank 38 statements and 155 supporting arguments regarding OTC in females with TS. Opinions were swayed via repetitive feedback after each round. The selection of key statements was based on strict inclusion criteria.

Main results and the role of chance:

A total number of 46 participants from 16 different countries completed the first Delphi-round (response rate 84%). Based on strict selection criteria, 6 key statements were selected and 13 statements were discarded. The remaining 19 statements and 2 additional statements submitted by participants were re-evaluated in the second round by 41 participants (response rate 76%). The analysis of the second survey resulted in the inclusion of 2 additional key statements. The final selection of key statements was approved by 96% of the participants. After the pros and cons were discussed and the main arguments were selected, the majority of our expert panel (75%) believed that OTC should be offered to young females with TS in a safe and controlled research setting. Arguments that focused on beneficence, autonomy, and justice outweighed statements regarding non-maleficence. The remaining participants (25%) did not object, but chose to remain neutral.

Limitations, reasons for caution:

The anonymous nature of this study might have led to lack of accountability. The selection of experts based on their willingness to participate, and the fact that not all panellists took part in all rounds, might have resulted in selection bias

Wider implications of the findings:

This international standpoint is the first step in the global acceptance of OTC in females with TS. Future collaborative research with focus on efficacy and safety and long-term follow-up are urgently needed. Furthermore, we recommend an international registrar for fertility preservation procedures in females with TS.

Trial registration number:

not applicable

INVITED SESSION

SESSION 44: MHR SYMPOSIUM: DYNAMIC INTERACTION BETWEEN THE MALE GAMETE AND ITS ENVIRONMENT

Tuesday 25 June 2019 Haydn 1 14:00 - 15:00

O-154 Epididymis contribution to male fertility

O-155 A step by step development of a bovine oviduct-on-a-chip model for better biomimicking in vivo early embryo development

B.M. Gadella[1]

[1]Utrecht University - Institute of Biomembranes Facultity of Veterinary Medicine, Dept of Biochemistry and Cell Biology, Utrecht, The Netherlands

Study question:

Can a double perfusion microfluidic oviduct on a chip culture system provide a more in vivo like in vitro fertilization environment and does this improve embryo production?

Summary answer:

Yes as it allows exclusive monomeric fertilization while conventional IVF results also in polyspermic fertilization and parthenotes. Moreover, the (epi)genome is more in vivo like.

What is known already:

ART only focuses on increasing the low efficiency of obtaining and maintaining pregnancy or live birth, whereas the long-term impact on the health of the resulting child has been relatively neglected. Animal models have highlighted

that *in vitro* embryo culture is not exactly mimicking embryo development in the female genital tract and also has effects after embryo transfer on subsequent offspring development and health. *In vivo*, the oviduct hosts the first embryo developmental period coinciding with a complete reprogramming of its (epi)genome in preparation for the reacquisition of (epi)genetic marks.

Study design, size, duration:

A 3D-printed oviduct-on-a-chip model using a stereo-lithographic technique was used in which bovine oviduct epithelial cells were cultured and regained and maintained their ciliated and cuboidal to columnar epithelium for a period of at least 6 weeks, with a mixed population of ciliated and secretory cells comparable to that in the in vivo oviduct epithelium. This was used to condition the apical medium and to study both bovine IVF and embryo production.

Participants/materials, setting, methods:

The oviduct on a chip was made from PDMS material and a polycarbonate porous filter. The culture was followed on an live imaging inverted confocal microscope at zygote stage individual embryos were sequences to monitor their (epi)genome. these were compared with routine IVF zygotes and with zygotes flushed from cow oviducts in vivo also the (de)methylation of DNA was followed at fertilization until the two cells stage.

Main results and the role of chance:

A non-toxic material (PDMS) to create an improved bovine oviduct-on-a-chip. Cultured oviduct epithelial cells were responsive to steroid hormone stimulation, mimicking the luteal- and pre-ovulatory phases. Moreover, the oviduct-on-a-chip supported not only exclusive monomeric fertilization, but also embryo development up to the blastocyst stage. The zygotes resulting from on chip (CH) culture were more similar to their in vivo counterparts (VV) than to conventional in vitro (VT) zygotes, in terms of their global DNA methylation level and transcriptome. Interestingly, VV and CH zygotes exhibited lower global DNA methylation levels than VT zygotes, which is presumably a factor of the up-regulation of genes related to DNA demethylation in 80% of the VV and 50% of the CH zygotes (G2). This reduced level of DNA methylation seems to be essential to the minor embryonic genome activation event, since an up-regulation of genes related to transcription and translation initiation was observed in G2 zygotes. Suggesting that standard IVP conditions delay zygote minor transcriptome activation, but that the delay can be partially ameliorated using our oviduct-on-a-chip platform. Finally, our results highlight the importance of using a more in vivo-like environment to study pathways related to normal fertilization and embryo development in vitro.

Limitations, reasons for caution:

(i) Biofabricated materials may release endocrine disrupting agents. We have used non-toxic materials but other 3D-printed materials used released such components and blocked embryo development after the second cleavage. (ii) Although (epi)genetic changes have been noted at zygotes it is not clear whether is influences later (epi)genetic remodeling.

Wider implications of the findings:

Our approach permits live imaging for tracking cell migration and/or specific molecular pathways. Thus it opens avenues for interrogating pathways associated with tubal derived ovarian cancers and thereby for the identification of biomarkers for the early diagnosis of this lethal disease. This could be extremely valuable for personalized medicine purposes.

Trial registration number:

No trial registration number

INVITED SESSION

SESSION 45: PRIORITIES FOR FUTURE INFERTILITY RESEARCH

Tuesday 25 June 2019 Haydn 3 14:00 - 15:00

O-156 Prioritising future infertility research using the James Lind Alliance methodology

O-157 Top ten research priorities for female and unexplained infertility

O-159 Top ten research priorities for medically assisted reproduction

O-158 Top ten research priorities for male infertility

O-160 Top ten research priorities for ethics, access, and organisation of care

INVITED SESSION

SESSION 46: GENETICS OF MALE INFERTILITY: DAD'S CONTRIBUTION

Tuesday 25 June 2019 Haydn 2 14:00 - 15:00

O-161 De novo mutations affecting male infertility

J. Veltman[1]

[1]*Newcastle University, Institute of Genetic Medicine, Newcastle upon Tyne, United Kingdom*

Abstract text

During spermatogenesis and oogenesis the 6 billion letters of our diploid genome need to be accurately copied in order for the gametes to successfully pass on our genetic information. By studying the genomes of children and their parents, we now know that each human is born with 50-100 *de novo* mutations. These *de novo* mutations, by affecting important genes during development, can result in severe neurodevelopmental disorders such as intellectual disability or epilepsy. In this research we test the hypothesis that these *de novo* mutations may explain a significant proportion of the most severe forms of male infertility and may point us to many new male infertility genes. I will discuss our first studies in which we studied the DNA of 100 patients with azoospermia and severe oligospermia and compared this DNA to that of their unaffected parents. We identified not only de novo point mutations but also de novo deletions in the genome, determined the parent of origin and are currently replicating this work as well as performing functional studies within the framework of the International Male Infertility Genomics Consortium (www.IMIGC.org). This work is aimed at furthering our understanding of the genetic causes of male infertility with the goal to improve diagnostics, provide more insight into the success of assisted reproductive technologies, as well as the health of future offspring.

O-162 Genes involved in sperm functional impairment

M.K. O'Bryan[1]

[1]*Monash University, School of Biological Sciences, Clayton- Victoria, Australia*

Abstract text

The presence of large numbers of functionally competent spermatozoa is a primary determinant of male fertility in both humans and animals, and for animals wherein polyandrous mating is the norm, a primary determinant of evolution. We are interested in how sperm are built and the relationship between sperm form and function. A greater understanding of these processes will improve our understanding of the normal processes of male fertility, and the aetiology of human male infertility in the large number of patients who present with teratozoospermia and/or asthenozoospermia. To define these processes, we are using both forward genetic (clinical phenotype to underlying gene) and reverse genetic (gene to phenotype) approaches, and in doing so have uncovered many novel regulators of male fertility. Within this presentation I will focus on the validation of causal genes in human male infertility and the definition of their mechanism within normal spermatogenesis.

INVITED SESSION

SESSION 47: PATIENT SESSION - COMMUNICATION DURING THE INFERTILITY JOURNEY

Tuesday 25 June 2019 Haydn 4 14:00 - 15:00

O-163 First visit - problems in communication with fertility specialists

S. Jovanovic[1]

[1]*Association Chance for parenthood, Association Chance for parenthood, Belgrade, Serbia*

Abstract text

The First Visit – Problems in Communication with Fertility Specialists

Research goal:

The first part of the research is aimed at presenting the basic problems in communication between patients and infertility specialists on the first visit – the first consultations at an IVF clinic. Problems in communication between doctors and patients may influence the quality of the treatment, the establishment of a diagnose, the very course of the IVF procedure, an increased fear from the continuation of the treatment, as well as the quality of patients' life. Also, problems in the first communication, in the first consultations, may substantially influence patients' very choice where they will continue the treatment, which means they directly influence the very choice of a clinic and a doctor.

The second part of the research is focused on questioning specialists. This part of the research includes the aspect and perception of communication problems in the first consultations by infertility specialists. The accent is placed on doctors' point of view, the problems that doctors possibly notice, as well as the possibilities of solving the same.

Both the first and the second parts of the research are aimed at improving the quality of the services provided by clinics and doctors towards patients.

Methods: The research was conducted during one month. About 700 patients and more than 50 specialists were questioned. The questionnaire was fully anonymous, displayed online, and the respondents were not time-limited in completing it. The research covers patients and doctors from throughout Europe, and the largest number of the questioned patients and doctors were from Serbia – the country the researcher herself comes from. The research (survey) contained 25 short questions with the offered answers for the patients and 11 questions with the offered answers for the infertility specialists. The survey was only possible to complete once.

Based on the obtained answers, we wanted to demonstrate:

- the current and realistic situation showing the manner of patient-doctor communication in the first consultations;
- patients' assessment and first impression after the first IVF consultations;
- the expectations patients come to an IVF expert with and whether their expectations are met;
- whether patients receive all necessary information about the IVF process and the very course of the procedure;
- what patients expect from the IVF team – which, what and how big patients' expectations are;
- if patients inform themselves on the Internet after the first consultations, and why;
- based on what they chose the doctor and the clinic for the first consultations;
- how long it took them from becoming aware of the need for consultations with an IVF expert to the first consultations;
- whether doctors think patients are sufficiently informed;
- whether, in doctors' opinions, patients ask clear and direct questions regarding their treatment;
- whether, in doctors' opinions, patients come to an infertility specialist late;
- what doctors expect from patients in the first consultations – what their expectations are;
- if doctors consider themselves to be sufficiently supportive already in the first consultations.

The general goal of this research was to assess the quality of the service and care from patients' and doctors' perspectives in the first consultations regarding the IVF treatment, and also to show the realistic condition, for which reason the questionnaires were absolutely anonymous.

General conclusion: The first consultations and communication with an IVF specialist are of vital importance to patients when the continuation of the procedure, the quality of further treatment and life, too, are concerned.

O-164 Dear Doctor - can you hear me? Improving communication between professionals and patients

O-165 When there's a big disappointment, we don't know if that's the end of the story

E. Van der Valk[1]

[1]*Fertility Europe, n/a, Naaldwijk, The Netherlands*

Abstract text

Communications during the infertility journey

When there is a big disappointment, we don't know if that is the end of the story . . .

Ellen van der Valk tells her vulnerable story about deciding to stop further fertility treatments and how her life went after that.

By sharing her experiences and those of other women in this situation, she wants to give professionals in the field of fertility health care insight in:

- the impact of fertility treatments on your patients' daily lives
- the internal struggle between the wish to have a child versus the wish to enjoy life again
- the pressure of the idyllic 'happy familiy' picture on your patients' decisions
- how to help your patients in their decission making process

As an experience expert she feels that fertility doctors should not only talk about the next steps for treatment, but also consider no (further) treatment as an option to be discussed.

INVITED SESSION

SESSION 48: IMPACT OF PELVIC PATHOLOGY ON PREGNANCY OUTCOMES

Tuesday 25 June 2019 Strauss 1+2 14:00 - 15:00

O-166 Endometriosis and obstetric outcomes

O-167 The effect of fibroids on reproductive outcomes

M. Metwally[1]

[1]*Sheffield teaching hospitals, Reproductive medicine, Sheffield, United Kingdom*

Abstract text

Uterine fibroids are the most common benign uterine tumours in women and hence it is not surprising that they are commonly encountered in woman with reproductive problems. It is estimated that uterine fibroids may be found in 5-10% of women presenting with infertility[1]. In addition, fibroids have been associated with other reproductive problems such as miscarriage, preterm labour, foetal malpresentation, increased risk of caesarean section, low birth weight and postpartum haemorrhage.

Since fibroids do not represent a single entity, their effect on reproductive performance is variable according to their number, size and position. The general consensus is that the closer the fibroid is to the endometrium, the more adverse effect it can have on fertility. Submucous fibroids therefore are commonly thought of as having the greatest effect. This is supported by a number of primary and secondary studies showing that women with submucous fibroids have significantly lower fertility rates, clinical pregnancy rates, ongoing pregnancies and higher miscarriage rates[2]. However, despite the

wide practice of hysteroscopic myomectomy, there is a paucity of evidence from well-designed randomised controlled studies investigating the value of surgical removal for the sole purpose of improving fertility and further evidence from well-designed trials is needed, balancing the gains against any potential harm from surgical intervention[3].

At the other end of the spectrum lie subserous fibroids, where the evidence has shown them to have no direct adverse effect on fertility[2]and there is currently no evidence that their removal improves fertility. However, surgery may be considered in large subserous fibroids that distort the pelvic anatomy and may lead to difficult access to the ovaries for oocyte retrieval or cause problems such as pain during pregnancy or interfere with labour and delivery.

The most controversial fibroids however are of the intramural type. An early meta-analysis had initially suggested that they had no effect on fertility while a follow up meta-analysis from the same group suggested that they may indeed have a negative effect[2,4]and this was supported by a later meta-analysis from another group[5]. However, a later study highlighted the heterogeneity and therefore the unreliability of the evidence and hence the urgent need for good quality randomised controlled studies[6]. Until such studies are available, the management of intramural fibroids needs to be considered on a case to case basis.

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SELECTED ORAL COMMUNICATIONS

SESSION 49: INTELLIGENT AUTOMATION IN THE EMBRYOLOGY LABORATORY

Tuesday 25 June 2019 Mozart 15:15 - 16:30

O-168 Automatic morphological grading of human blastocysts with time-lapse imaging and artificial intelligence

M.F. Kragh[1], J. Rimestad[2], J. Berntsen[2], H. Karstoft[3]

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[2]*Vitrolife A/S, Research & development, Aarhus S, Denmark*
[3]*Aarhus University, Department of Engineering, Aarhus N, Denmark*

Study question:

Can human blastocyst evaluation be fully automated with time-lapse imaging and artificial intelligence (AI) to provide similar or improved morphological grades compared to experienced embryologists?

Summary answer:

AI performed better than embryologists on inner cell mass (ICM) and trophectoderm (TE) grading and provided better implantation rates for the highest graded embryo groups.

What is known already:

Blastocyst morphology grading is the most commonly used criteria for embryo selection, although subjective and prone to both inter- and intra-observer variances. Different automated approaches have been proposed to predict blastocyst quality from static images, acquired at a fixed time after fertilisation. These approaches are deterministic and thus ensure consistent annotations. However, due to the dynamic nature of embryo development, evaluation at a fixed time may easily be affected by slow blastocyst formation, collapses, and hatching. Today, no automated methods for morphological blastocyst grading have been proposed to address this issue by leveraging the temporal information available with time-lapse.

Study design, size, duration:

7438 human embryos from 4 different fertility clinics were recorded with time-lapse imaging. ICM and TE were graded by experienced embryologists according to the Gardner blastocyst scoring system. For AI development, the dataset was split into training (80%), validation (10%), and testing (10%). Furthermore, a second, independent test set of 55 embryos was graded by multiple experienced embryologists, allowing comparison of the embryologist´s majority votes vs. AI performance.

Participants/materials, setting, methods:

A convolutional neural network (CNN) was trained to jointly predict ICM and TE grades (A,B, or C) from static blastocyst images with 3 focal planes (fixed method). A recurrent neural network (RNN) was then trained on the same task using the CNN outputs from a sequence of time-lapse images ranging from time of blastocyst formation to time of maximum blastocyst size (dynamic method).

Main results and the role of chance:

On static images acquired at 110 hours after fertilisation, the fixed method achieved accuracies of 59.2% and 56.8% on ICM and TE predictions, respectively, when evaluating against single embryologist annotations in the first test set. Using sequences of time-lapse images, on the other hand, the dynamic method achieved higher accuracies of 66.9% and 70.8% on ICM and TE predictions, respectively. Furthermore, only 1.9% and 1.5% of the examples had A-C or C-A disagreements with the embryologists on ICM and TE, respectively. On the second test set with multiple annotators, leave-one-annotator-out cross-validation was applied to allow comparison of embryologist vs. AI performance. Here, AI achieved average ICM and TE accuracies of 72.7% and 75.6%, whereas average embryologist accuracies were 65.1% and 73.8%. When evaluating the AI against outcome data (fetal heartbeat) on the first test set, the AI had an implantation rate of 46.0% for top quality blastocysts (ICM/TE: A/A; n = 100) and 34.4% for good quality blastocysts (ICM/TE: A/B, B/A, B/B; n = 131). For embryologists, the same implantation rates were 44.6% (n = 112) and 30.7% (n = 127).

Limitations, reasons for caution:

Our results suggest the AI to be at least on level with individual embryologist annotations of both ICM and TE. However, more data from multiple annotators are needed to determine superiority.

Wider implications of the findings:

Automated blastocyst grading using AI is consistent, faster, labour saving, and potentially more accurate than the equivalent annotations from human embryologists. The proposed AI applies the widely used Gardner scoring system and can therefore help embryologists in their daily annotations without introducing new parameters or requiring new implantation models.

Trial registration number:

Not applicable.

O-169 Metabolic activity of human preimplantation embryos relates to their morphokinetics, morphological grade, KIDScore and artificial intelligence selection

L. Ferrick[1], Y.S.L. Lee[2], D.K. Gardner[1]

[1]*University of Melbourne, Biosciences, Melbourne, Australia*
[2]*Melbourne IVF, IVF Lab, Melbourne, Australia*

Study question:

Is there a relationship between preimplantation embryo metabolic activity and other biomarkers of embryo viability?

Summary answer:

Embryos consuming higher levels of glucose exhibit faster blastocoel formation, and higher morphological grades, KIDScores, and EmbryoScores generated by artificial intelligence / deep learning (Ivy).

What is known already:

Glucose is the key metabolite utilised by the embryo post-compaction to support blastocyst development and has been reported as a biomarker of day 4 and 5 human embryo viability. Morphology, morphokinetics, KIDScore and more recently EmbryoScore (a measure of the chance an embryo will result

in a fetal heartbeat), have been correlated to transfer outcomes and used as biomarkers of embryo viability to aid embryo selection.

Study design, size, duration:

110 human preimplantation embryos from 21 patients were cultured in a time-lapse incubator system in both freeze all and fresh transfer cycles. A retrospective analysis of morphokinetics, morphology, KIDScore, EmbryoScore and day 5 glucose metabolism was conducted.

Participants/materials, setting, methods:

ICSI was conducted in patients, aged ≤37 years, who previously had no more than two oocyte collections, and no more than four embryo transfers. Embryos were cultured in a time-lapse incubator system, and those reaching the blastocyst stage had their morphokinetics annotated and were each assigned a morphology grade, KIDScore and EmbryoScore. Glucose concentration in spent media samples were analysed using ultramicroflurescence and compared to control media.

Main results and the role of chance:

Analysis of glucose metabolism of embryos that reached blastocyst stage revealed embryos with top morphological scores (AA) consumed significantly more glucose than lower scoring embryos (<AA) (126.0 ± 17.5 pmol/embryo/h vs 69.2 ± 8.6 pmol/embryo/h, $p<0.01$). Assessment of embryo morphokinetics revealed blastocoel formation was delayed in embryos exhibiting significantly lower glucose uptake on day 5 of preimplantation period (53.1 ± 13.9 vs 94.9 ± 9.5 pmol/embryo/h, $p<0.05$). Additionally, embryos assigned a KIDScore higher than the median score exhibited a significantly higher glucose uptake on day 5 of preimplantation development than embryos with a lower KIDScore (104.6 ± 11.0 vs 54.9 ± 8.9 pmol/embryo/, $p<0.001$). Similarly, embryos that were assigned an EmbryoScore above the median score consumed significantly more glucose than embryos that were assigned a lower EmbryoScore (95.0 ± 10.4 vs 64.8 ± 10.9 pmol/embryo/h, $p<0.05$).

Limitations, reasons for caution:

Samples were collected from one clinic.

Wider implications of the findings:

These results demonstrate a direct link between embryo glucose metabolism and specific morphokinetic events. Additionally, these results provide further evidence that glucose is a valid biomarker of embryo viability and, therefore could be used in conjunction with other biomarkers to aid embryo selection methods.

Trial registration number:

N/A

O-170 Clinical validation of an automatic time-lapse algorithm classification system for blastocyst selection

B. Aparicio-Ruiz[1], S. Perez[1], L. Romany[1], T. Viloria[1], L. Alegre[1], J. Remohi[2], M. Meseguer[1]

[1]IVIRMA Global, IVF Laboratory, Valencia, Spain
[2]IVIRMA Global, Gynecologist Department. IVI President, Valencia, Spain

Study question:

Is there a correlation between an automatic time-lapse algorithm classification(Xtend) with blastocyst implantation potential? If so, is standard morphology compatible or combinable with Xtend analysis?

Summary answer:

There is a direct correlation between Xtend-categories with implantation potential of blastocysts. This relationship is even more evident in good-quality embryos according to standard morphology.

What is known already:

Aparicio-Ruiz et al. 2016 validated an automatic algorithm by which embryos were classified based on the values of P2 (t3-t2; second cell cycle) and P3 (t4-t3; synchrony). This algorithm was improved including more parameters (number of cells on day 3, egg age and changes of the texture of the embryo from 42 to 60 hours after ICSI) in order to classify embryos in 5 different categories (1-5).

Study design, size, duration:

Retrospective cohort study (egg donation program) performed in a University-affiliated infertility clinic on 1031 embryos analyzed from April 2016 to October 2018.

Participants/materials, setting, methods:

The study includes 362 patients and 1031 embryos generated by ICSI in the egg donation program and incubated in a GERI Time-Lapse Incubator (Genea, Australia) with especially designed scopes that used dark field microscope and an automatic cell-tracking software (Eeva, Xtend).

Main results and the role of chance:

A total of 1031 embryos were incubated in the Geri-system. Without distinguishing if embryos transferred were fresh or previously thawed, implantation rates of KID blastocysts(n=514) were directly correlated with the Xtend classification(1:53.91%; 2: 52.63%; 3:45.68 %; 4:39.02%; 5:24%). These differences were statistically significant p=0.017.

We assembled different Xtend categories and studied implantation rates in two groups: 1-3(52%) or 4-5(32.90%), showing significant differences between both groups (p= 0.003).

To check if algorithm selection properties were conditioned by morphology of blastocysts, we separated good quality(A/B according to ASEBIR morphology classification) from those with worse quality(no A/B).When Xtend categories were grouped, important differences were observed in percentage of A/B blastocyst between 1-3(83.9%) and 4-5(16.10%) that were less evident for not good quality blastocysts: 1-3(54.1%) and 4-5(45.9%)($p<0.0001$).

Even though no significant differences were observed, the algorithm seemed to define better implantation potential in good quality blastocysts(1:56.7%; 2:55.9%; 3:51.6%; 4:39.35; 5:27.8%) than in those not classified as A/B(1:33.3%; 2:36.4%; 3:23.5%; 4:41.7%; 5:18.2%). We also performed a logistic regression model for implantation, in which BMI, number of MII donated and standard morphology were included. The model assembled Xtend(1-3 vs 4-5) presented an OddsRatio(OR) of 1.917(CI95%1.40-3-77) and blastocyst morphology(A/B vs not A/B) OR of 2.30 (CI95% 1.11-3.30).

Limitations, reasons for caution:

Retrospective nature of this study may be a reason for caution; nevertheless, is the largest sample size reported with this test, based in blastocyst transfer with>90% of single-embryo-transfer, additionally a multivariable-analyisis confirmed the magnitude of the results. The classification system has some errors due to difficulties in cell-tracking generating "none-result".

Wider implications of the findings:

To our knowledge, this is the biggest set of data using Xtend-algorithm. Results obtained validate the utility of this classification and showed higher accuracy in good-morphology blastocysts. It confirms that morphology and time-lapse classifications are independent and combinable showing that not all embryos classified morphologically asA/B correspond with better Xtend-categories.

Trial registration number:

Not applicable

O-171 Embryo development detection by automated software vs. embryologist team.

R. Del Gallego Bonilla[1], L. Alegre[1], L. Bori[2], T. Peura[3], S. Azaña[2], M. Meseguer[1]

[1]IVI Valencia- SL, IVF Laboratory, Valencia, Spain
[2]IVI Valencia- SL, Research, Valencia, Spain
[3]Genea, Research, Sydney, Australia

Study question:

To compare outcomes and accuracy of embryo morphokinetic event annotations performed in a routine clinical practice to those performed by an automated embryo assessment software.

Summary answer:

High detection rate accordance was found between embryologist and embryo assessment software. This novel technology is a useful tool under a qualified embryologist supervision.

What is known already:

Time-lapse technology has helped to elucidate key events of embryo development. The relevance of this methodology has increased in the last years, since it reduces subjectivity and makes possible to perform deeper and more accurate analyses of embryo development. The use of deep learning algorithms to analyse developmental events automatically is a step towards implementation of artificial intelligence into embryo assessment, which is becoming a significant trend in the future.

Study design, size, duration:

This is a retrospective study with data collected between December 2017 and August 2018 in IVI Valencia clinic from embryos analysed manually by

IVI team and automatically by GeriÒ Assess 2.0 automated annotation software for nine embryo development events. Percentage of events detected by either IVI team, the software or both was calculated, as well as the accuracy of event timings between the two.

Participants/materials, setting, methods:

A busy embryologist team at IVIRMA clinics annotated embryos for nine developmental events as per normal clinical practice using GeriÒ Assess 1.3 software. The same videos were then analysed retrospectively with a stand-alone GeriÒ Assess 2.0 software, applying the same pre-determined filtering time-ranges for the events as are incorporated in the full GeriÒ Assess 2.0 system. Finally, the event detection rates, and event timings as mean and standard deviation between the two were compared.

Main results and the role of chance:

From 12,618 possible developmental events, IVI detected 86% (10,857), GeriÒ Assess 2.0 software 81% (10,191), and both concurrently 75% (9,470).The mean and standard deviation of the event timings between manual and automated annotations were as follows (hrs): PN appearance 0.3 (±4.1), PN disappearance 0.6 (±1.1), 2-cell 0.6 (±1.3), 3-cell 0.5 (±2.1), 4-cell 0.7 (±4.1), 5-cell 1.1 (±4.3), 6-cell 0.1 (±4.4), morula 4.1 (±7.4) and early blastocyst 1.1 (±5.4). Time-ranges for filtering of automatic annotations were: PN disappearance 17-30 h, 2-cell 20-40 h, 3-cell 30-48 h, 4-cell 32-54 h, 5-cell 38-68 h, 6-cell 46-78 h, morula 64-100 h and early blastocyst 86-126 h. Thus excluded data points represented 12% of all automatically annotated events.

The lowest accordance rates in detection were found in the more subjective events: PN disappearance, morula or expanded blastocyst stage, and especially after the events were filtered according to time-ranges. Differences in timings varied according to the event, the largest differences detected in the later events. However, in the great majority of the events, especially early cleavage divisions, the match rate was very high.

Limitations, reasons for caution:

Prospective validation is needed. Chaotic embryos with aberrant divisions and variability between annotators makes exact annotations difficult for both manual and automated annotations.

Wider implications of the findings:

Automated annotations ease the embryologists' workload and especially early events can be annotated with high accuracy. Furthermore, non-annotated events can still be annotated manually, increasing the accuracy of the data. Move into the use of automated annotations is a natural progression for a clinic utilising timelapse systems, such as ours.

Trial registration number:

Not applicable

O-172 Continuous flow of pre-mixed gas is not required to maintain culture medium pH in a benchtop incubator.

D. Mortimer[1], S. Geelhood[2], S. Olds[2]

[1]Oozoa Biomedical Inc., President, West Vancouver- BC, Canada
[2]Blood Cell Storage Inc, SAFE Sens, Seattle, U.S.A.

Study question:

Do benchtop incubators require continuous gassing with pre-mixed gas to maintain culture medium pH?

Summary answer:

Intermittent gassing can be used to maintain culture medium pH.

What is known already:

The equilibration and maintenance of culture medium pH depends on providing the correct partial pressure of carbon dioxide (pCO_2) inside the incubator. In compact benchtop incubators this achieved using a continuous supply of pre-mixed gas: 6.0% CO_2 / 5.0% O_2 / balance N_2 when working with typical culture media containing 25 mM bicarbonate and at 37°C at sea level (atmospheric pressure 760 mmHg), i.e. pCO_2 = 45 mmHg. However, the explicit need for a continuous supply of this gas has not been previously established.

Study design, size, duration:

This was an observational experimental study using the SAFE Sens real-time pH monitoring system (BCSI, Seattle, WA, USA) installed into a Planer/Origio BT37 compact benchtop incubator (Cooper Surgical, Trumbull, CT, USA).

Participants/materials, setting, methods:

Planer's BT37 Toolbox software was used to program 10-minute gas flow events (15ml/min/chamber) every 30, 60 or 90 minutes, abbreviated as 10:20, 10:50 and 10:80 respectively. Sensors were loaded with 125µl of Universal IVF Medium and covered with 75µl of Oil for Tissue Culture (both Cooper Surgical); pH readings were logged every minute for up to 72h per experiment. Control pH values were measured using a Siemens RapidLab 348 blood gas analyzer.

Main results and the role of chance:

Stability of pH measurements over a 4-hour steady state period (SDs of >200 measurements) were very similar: 0.0036, 0.0042, 0.0044 and 0.0039 (control, 10:20, 10:50 and 10:80 intermittent gassing respectively). Stable pH readings, averaged at 24–25h, were 7.35, 7.36, 7.42 and 7.44, respectively), all with 95% ranges of ±0.01. Given the stable medium pH levels achieved, and their low variability over time, these results do not support the necessity for a continuous supply of pre-mixed gas. However, periods of >60 minutes between gas flows should be avoided because the data indicate a lower effective pCO_2 beyond 1h, as indicated by the higher pH levels. In the 10:80 cycle it was also observed that a lid opening followed by the incubator's normal gas purge cycle caused a noticeable pH dip from 7.44 to 7.41 over a 4h period followed by a slow rise back to the stable level.

Limitations, reasons for caution:

Intermittent gas flow control is not available in all benchtop incubators that use pre-mixed gas. Surrogate sensors replicate microdroplet culture, open culture systems are expected to have differing results. Sensor cups monitor medium separate from embryo culture dishes, and CO_2 absorption in the sensors might differ from culture drops.

Wider implications of the findings:

After allowing for essential post-opening purges, the adoption of an intermittent flow rate pattern reduces pre-mixed gas usage: the 10:20 and 10:50 intermittent flow protocols can reduce gas consumption by ~68% and ~79% respectively, optimizing culture conditions while providing significant operational savings.

Trial registration number:

Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 50: THE LUTEAL PHASE

Tuesday 25 June 2019 Haydn 1 15:15 - 16:30

O-173 A large prospective trial in unselected population confirms that low serum progesterone on the day of embryo transfer impairs pregnancy outcome in artificial cycles.

E. Labarta Demur[1], G. Mariani[2], S. Paolelli[2], C. Vidal[2], J. Giles[2], J. Bellver[2], F. Cruz[2], A. Marzal[2], P. Celada[2], I. Olmo[2], P. Alamá[2], J. Remohí[2], E. Bosch[2]

[1]IVI RMA Valencia, Human Reproduction, Valencia, Spain
[2]IVI-RMA Valencia, Human Reproduction, Valencia, Spain

Study question:

Which is the threshold of serum Progesterone the day of embryo transfer in artificial endometrium preparation cycles that significantly reduces the chances of ongoing pregnancy?

Summary answer:

Patients with serum Progesterone levels <8.8 ng/mL the day of embryo transfer had significantly lower ongoing pregnancy rate than the rest of patients.

What is known already:

Recent retrospective studies have suggested a relationship between serum Progesterone (P) levels on the mid-luteal phase and pregnancy outcome in artificial cycles when using vaginal progesterone. Our group published the first prospective trial analyzing this topic and found that those patients with serum P levels <9.2ng/ml on the day of embryo transfer had 20% less chances of ongoing pregnancy ($p<0.05$) in a sample of 211 oocyte donation recipients' patients with strict inclusion criteria. Our aim is to analyze if these results are applicable to the general population under same preparation protocol, due to the significant clinical impact of the results.

Study design, size, duration:

Prospective cohort study including 1166 patients undergoing embryo transfer after an artificial endometrial preparation cycle with estradiol valerianate and vaginal micronized progesterone (400 mg/12 hours).

The study was performed between September 2017 and November 2018.

Sample size was calculated to detect a 10% difference (40-50%) between 2 groups according to serum P levels, in a two-sided test with a statistical power of 80% and a confidence level of 95%.

Participants/materials, setting, methods:

Patients undergoing a blastocyst transfer with an artificial cycle with their own or donated eggs, age <50, BMI <30 Kg/m^2, with a triple layer endometrium >6.5 mm, and being transferred 1-2 good quality blastocysts; in a private infertility centre.

Serum P determination was performed immediately before the embryo transfer. Primary endpoint was ongoing pregnancy rate beyond the 12thweek of pregnancy. The timeframe between last dose of exogenous progesterone and the embryo transfer was recorded.

Main results and the role of chance:

A total of 1155 patients could finally be analysed (pregnancy outcome is not available in 11 patients). The mean age was 39.6±4.6; BMI: 23.7±4.2;Endometrial thickness: 8.8±1.6 mm. A mean of 1.2±0.3 blastocysts were transferred. Ongoing pregnancy rate (OPR) was 53.1±5.0% in the whole population.

Mean serum P the day of ET was 12.0±5.8 ng/mL. The OPR according to the 10 percentiles (p) of serum P levels were: <p10: 34.2%; p10-20: 41.3%; p20-30: 43.0%; p30-40: 56.1%; p40-50: 53.8%; p50-60: 63.4%; p60-70: 59.8%; p70-80: 59.8%; p80-90: 51.7%, >p90: 61.8% (p=0.000). Women with serum P levels <8.8 ng/mL (percentile 30) had a significantly lower OPR compared to the rest of patients: 39.8% vs. 58.0%, RR (95% CI): 0.69 (0.59-0.79); p<0.0001.

After adjusting for all potential confounders (age, BMI, endometrial thickness, serum E2, number of embryos transferred, origin of oocytes), having a serum P<8.8 ng/mL remained as a significant negative factor for ongoing pregnancy; OR (95% CI)= 0.39 (0.28-0.53), p=0.000.

The timeframe between the last dose of vaginal progesterone and embryo transfer was not correlated neither with the serum P levels nor the ongoing pregnancy rate.

Limitations, reasons for caution:

Only women with micronized vaginal progesterone were included in the study, thus, extrapolation to other ways of progesterone administration needs to be validated.

Wider implications of the findings:

This large study has found the optimal threshold of serum P values the day of embryo transfer that needs to be reached in artificial endometrial preparation cycles to optimize the outcome.

Trial registration number:

NCT03272412

O-174 Cabergoline versus calcium gluconate infusion in the prevention of ovarian hyperstimulation syndrome. A randomized controlled trial

H. El-Shaer[1,2], U. Fouda[1,2], G. Youssef[1], A. Hanafy[1], H. Nabil[1], W. Mehrem[3]

[1] *Cairo University- Faculty of medicine, Obstetrics and Gynecology Department, Giza, Egypt*

[2] *Riyadh fertility and reproductive health center, Assisted conception unit, Giza, Egypt*

[3] *Al Gazeera hospital, Assisted conception unit, Giza, Egypt*

Study question:

Is calcium gluconate infusion more effective than cabergoline in the prevention of ovarian hyperstimulation syndrome (OHSS) in IVF patients at risk for developing OHSS ?

Summary answer:

Calcium gluconate infusion is as effective as cabergoline in the prevention of OHSS in IVF patients at risk for developing OHSS.

What is known already:

Several animal and human studies highlighted the role of vascular endothelial growth factor (VEGF) in the pathogenesis of OHSS. The use of cabergoline (which inhibits VEGF receptor 2 phosphorylation and signaling) and calcium gluconate infusion (which inhibits VEGF synthesis) in the prevention of OHSS has been investigated in several studies. A recent Cochrane review revealed that cabergoline was associated with a lower incidence of moderate/severe OHSS compared with placebo (11.83% Vs 33.88%). A randomized controlled study revealed that calcium gluconate infusion was associated with a lower incidence of moderate/severe OHSS compared with placebo (1% Vs 12%).

Study design, size, duration:

Between October 2016 and December 2018, this randomized controlled study recruited 170 patients who were stimulated using the long luteal GnRH agonist protocol and at high risk for developing OHSS. Patients with more than 18 follicles (> 11mm) and serum estradiol ≥ 3000 pg/ml on the day of HCG administration were considered at risk for OHSS. The study was conducted at Riyadh fertility and reproductive health center, Giza, Egypt and Al Gazeera hospital, Giza, Egypt.

Participants/materials, setting, methods:

Patients were randomized in a 1:1 ratio to cabergoline group and calcium gluconate group. In cabergoline group , 0.5 mg of cabergoline was administered once daily p.o for 8 days starting on the day of HCG administration. In calcium gluconate group, intravenous calcium gluconate (10%,10 ml in 200 ml of physiologic saline) was administered daily for 4 days starting on the day of ovum pickup.

Main results and the role of chance:

Six patients in cabergoline group and eight patients in calcium gluconate group developed moderate OHSS. One patient in each group developed severe OHSS. The incidence of moderate/severe OHSS was comparable between both groups (8.24% Vs 10.59%, P value = 0.599, OR= 0.76, 95% CI [0.269 - 2.138]). Both groups did not differ statistically in the age, body mass index, basal FSH, antral follicle count, anti-Müllerian hormone level, cause of infertility and duration of infertility. Both drugs were well tolerated and there were no adverse effects occurring in both groups. The number of retrieved oocytes , fertilization rate, quality of embryos transferred and number of embryos transferred were comparable between both groups. The implantation, clinical pregnancy and ongoing pregnancy rates were similar in the two groups (16.91% Vs. 15.84%, P = 0.771, 35.29% Vs. 32.94%, P = 0.746, 30.59% Vs. 28.24% , P = 0.736, respectively).

Limitations, reasons for caution:

The main limitations of the current study are the open label design and lack of measurement of serum levels of calcium, renin, angiotensin II and VEGF before and after calcium gluconate infusion.

Wider implications of the findings:

Calcium gluconate infusion and cabergoline are equally effective in minimizing the risk of OHSS. Both drugs are well tolerated, cheap and have no adverse effects on the reproductive outcomes of IVF cycle.

Trial registration number:

ClinicalTrials.gov: NCT02875587

O-175 Clinical pregnancy rates among anovulatory and oligoovulatory women after letrozole versus hormone replacement therapy in frozen-thawed embryo transfer cycles

M. Sharon - weiner[1], S. Farladansky-Gershnabel[1], A. Berkovitch[1]

[1] *Meir Medical Center, Obstetrics and Gynecology, Kfar Saba, Israel*

Study question:

Does letrozole versus HRT result in higher clinical pregnancy rate among anovulatory and oligoovulatory women in frozen-thawed embryo transfer (FET) cycles?

Summary answer:

Letrozole use among anovulatory or oligoovulatory women was associated with significantly higher clinical pregnancy rate, as compared to HRT in FET cycles.

What is known already:

The use of letrozole in FET cycles is associated with higher clinical pregnancy rates. However, the advantage of letrozole compared to HRT in FET cycles has not been evaluated specifically among anovulatory and oligoovulatory women for whom the alternative of a natural cycle without endometrial preparation is not an option.

Study design, size, duration:

This prospective study of 213 FET cycles among anovulatory and oligoovulatory women was conducted from 1/2017 through 12/2018. Since letrozole has recently become an effective alternative to HRT cycles, all patients with ovulation disorders undergoing FET, were given letrozole beginning in 1/2018, instead of HRT.

Participants/materials, setting, methods:

Letrozole was given in 86 cycles and HRT was given in 127 cycles of anovulatory and oligoovulatory women undergoing FET. The primary outcome measure was clinical pregnancy (fetal heartbeat) rate. Secondary outcome measures were rates of multiple pregnancies and miscarriages.

Main results and the role of chance:

Clinical pregnancy (fetal heartbeat) rate was significantly higher in the letrozole group as compared to the HRT group. The adjusted risk ratio for clinical pregnancy (fetal heartbeat) for letrozole as compared with HRT cycles was 1.98 (95% CI: 1.07-3.67). Miscarriage and multiple pregnancy rates did not differ significantly between groups. The adjusted risk ratio for maternal smoking for letrozole as compared with HRT cycles was 3.59 (95% CI: 1.07-12.07). No significant difference was found between groups regarding maternal age, BMI, indication for fertility treatment (male versus nonmale), peak estradiol levels, number of oocytes retrieved, number of cleavage/blastocyst embryos, number of frozen embryos and number of transferred embryos.

Limitations, reasons for caution:

A randomized prospective study design was not used to prescribe letrozole versus HRT to the study participants.

Wider implications of the findings:

These results suggest that letrozole as compared to HRT may improve clinical pregnancy rates among anovulatory and oligoovulatory women undergoing FET cycles.

Trial registration number:

not applicable

O-176 The luteal phase of double ovarian stimulation treatment (DuoStim) provides higher oocyte and blastocyst yield in unselected infertile patients: a retrospective, same-patient, cohort study

D. Bodri[1], J.C. Castillo[1], B. Moliner[1], J. Llácer[1], A. Fuentes[1], P. Cirillo[1], Á. Llaneza[2], J. Ten[1], A. Bernabeu[1], R. Bernabeu[1]

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[2] *Instituto Bernabeu, Assisted Reproduction Unit, Madrid, Spain*

Study question:

What are the differences in stimulation and laboratory outcome variables between the follicular and luteal phases of double ovarian stimulation (DuoStim) in unselected infertile patients?

Summary answer:

Luteal phase of Duostim achieved higher follicular recruitment, oocyte and blastocyst yield. Fresh oocytes from aged infertile patients had better developmental competence than vitrified ones.

What is known already:

After it was first presented in the reproductive literature (Kuang, 2014), double ovarian stimulation has gradually gained in popularity, but was only applied in low responders or for fertility preservation in cancer patients. So far only few, small-sized studies have evaluated DuoStim and observations regarding laboratory and clinical outcomes are still preliminary. Furthermore, cost-sparing strategies including accumulation by oocyte vitrification were still not investigated in conjunction with DuoStim treatment.

Study design, size, duration:

All consecutive patients (n=69) who initiated DuoStim treatment during January - December 2018 were included in this retrospective analysis. Stimulation characteristics and laboratory outcomes variables were compared between the follicular (first) and subsequent luteal (second) stimulation phases. Clinical outcomes were evaluated for those patients who underwent (n=23) subsequent frozen-thawed embryos replacement (FER).

Participants/materials, setting, methods:

Recombinant and/or urinary gonadotropins (up to 300 IU daily) and GnRH antagonists were used in both stimulations with 0-6 (mainly 2-3) days gap between phases. Oocyte maturation was triggered with a GnRH agonist (triptorelin 0.2 mg) in the first, and rhCG or dual trigger in the second phase. Mature eggs were frozen during the egg accumulation phase(s) and fertilized together with fresh retrieved eggs at the final stimulation; cultured to until blastocyst-stage and electively vitrified.

Main results and the role of chance:

Sixty-nine patients underwent 73 treatment cycles. Mean female age was 38.1±3.5 (range: 24-43 years) with 67% having low ovarian reserve. Main indications were oocyte accumulation/embryo freezing in low (n=46) or normo-responder (n=14) patients, PGT-A (n=18) or fertility preservation (n=5). There were no significant differences in stimulation duration (9.9±2.3 versus 10.3±2.6, p=0.3) or total gonadotropin dose (2851±1025 versus 3047±1035, p=0.25). Total DuoStim treatment duration was 26.3±4.3 (range: 16-38 days). Follicular (>13 mm) recruitment (5±3 vs 6.7±4.1, p=0.003), the number of retrieved total (3±2.7 vs 5.3±4.3, p=0.0002) and mature (2.6±2,1 vs 4.7±3.5, p=0.0001) oocytes and blastocysts vitrified (0.7±0.9 vs 2±1.7, p=0.0003) was significantly higher in the luteal phase. More cases of no-oocytes retrieved occurred in the follicular-phase stimulation (15% vs 5%, p=0.05). The average survival rate of frozen oocytes from the first stimulation phase was 90%. Fertilization (43% vs 66%, p=0.002) and blastocyst formation rates per fertilized eggs (33% vs 55%, p=0.03) were higher for fresh oocytes. So far 10 clinical/ongoing pregnancies (43%) were achieved in 23 patients who have undergone subsequent FER. A 38-year-old, low-responder patient has undergone three consecutive stimulation attempts ("TripleStim") lasting a total of 49 days with increasing oocyte yield in each phase (0, 5 and 9 eggs).

Limitations, reasons for caution:

Due to the retrospective nature of this study patients were not included according to prospectively established inclusion criteria resulting in a heterogenous cohort. Due to the recent study period, not all frozen oocytes and/or blastocysts were utilized limiting conclusions on clinical outcomes.

Wider implications of the findings:

Although so far DuoStim was mainly used in aged low-responder patients, its indications could be extended to other groups who could benefit from an increased oocyte yield: intermediate-aged (36-39 years) patients with suboptimal ovarian response, PGT-A candidates or used as "cycle-rescue" following unexpected low oocyte yield or no oocytes retrieved.

Trial registration number:

not applicable

O-177 Luteal serum progesterone levels cannot be predicted in IVF patients - A prospective study of 432 IVF/ICSI cycles

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[2] *Department of Clinical Medicine- University of Copenhagen- Denmark., The fertility Clinic- Herlev Hospital- Denmark., Herlev, Denmark*

Study question:

Is it possible to predict the early luteal serum progesterone (P4) level of IVF patients receiving a fresh embryo transfer?

Summary answer:

The corpus luteum (CL) function after trigger is highly individual and the luteal serum P4 level cannot be predicted based on general patient characteristics.

What is known already:

Luteal P4 levels seem to influence the reproductive outcome following fresh embryo transfer (ET). A recent study has shown that both very low (<60 nmol/l) and very high (>400 nmol/l) serum P4 levels in the early luteal phase reduce the chance of pregnancy. Monitoring of luteal P4 levels and an individualization of luteal phase treatment according to serum P4 levels may improve the chance of live birth. To avoid monitoring the total patient cohort, it would be beneficial if patients with the risk of suboptimal luteal P4 levels could be identified prior to ET based on known patient characteristics.

Study design, size, duration:

Prospective study of 432 patients undergoing IVF treatment and fresh ET. Patients were recruited from Danish fertility centers from May 2014 to June

2017. Patients were co-treated in a GnRH-antagonist protocol or a long GnRH-agonist protocol and triggered for final oocyte maturation with either a bolus of hCG or GnRH-agonist. Identical luteal phase support regimens were used in all patients. Serum P4 levels were measured during the early luteal phase (2-3 days following oocyte retrieval).

Participants/materials, setting, methods:

Maternal age, maternal BMI, number of final follicles, type of ovulation trigger and the day of P4 monitoring were included in a linear regression model to explore whether these parameters could predict the subsequent luteal serum P4 level with sufficient accuracy for clinical usage. In a second model, additional information in terms of protocol type, duration of stimulation, smoking, total gonadotropin dose and type of gonadotropin was added.

Main results and the role of chance:

Significant individual differences in CL function were seen, also in patients with similar characteristics. In patients with the same number of final follicles (11), the same protocol (GnRH antagonist) and the same regimen for final oocyte maturation (GnRH agonist), the inter-patient luteal P4 level varied from 68 to 566 nmol/l. Using a linear regression model, including maternal age, BMI, day of P4 monitoring and type of ovulation trigger as independent variables, the adjusted R-squared was 0.48. Adding protocol type, duration of stimulation, smoking, total gonadotropin dose and type of gonadotropin only increased the R-squared to 0.49. Thus, even though a wide range of patient parameters are known, the statistical model only explains approximately 50% of the random variation seen in the data. The remaining variation is caused by biological differences in corpus luteum function between patients. Using the regression model and the present data set, the prediction interval (PI) for early luteal P4 levels (nmol/l) in a patient with known age, BMI, final number of follicles and type of trigger was 95% PI [45;421]. This clearly demonstrates that the prediction of luteal serum P4 levels based on patient characteristics is troublesome and has no clinical relevance.

Limitations, reasons for caution:

The findings of this study should be confirmed in future larger trials with the possibility to include additional significant covariates in the statistical model.

Wider implications of the findings:

If patients with suboptimal corpus luteum function should be identified for the individualization of treatment, the circulating serum P4 level must be measured in the total patient cohort as the corpus luteal function is highly individual and cannot be predicted by patient characteristics nor by responses to treatment.

Trial registration number:

NCT02129998 (Clinicaltrials.gov).

SELECTED ORAL COMMUNICATIONS

SESSION 51: MALE FERTILITY, EPIGENETICS, THE ENVIRONMENT AND LIFESTYLE

Tuesday 25 June 2019 Haydn 3 15:15 - 16:30

O-178 Dietary patterns and testicular function in young men

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Study question:

Is adherence to specific dietary patterns associated with testicular function in young men?

Summary answer:

Adherence to high fat unhealthy Western dietary pattern was associated with impaired testicular function while prudent diet high in fruits and vegetables was positively associated.

What is known already:

Sperm count has been declining in the Western countries over the past few decades, and determining the risk factors for this is crucial. Coinciding with the decline in sperm count has been a decline in diet quality. A number of studies have examined the role of diet on semen quality but data on how adherence to different diet patterns influences testicular function is scarce among men in northern Europe. In addition, the population recruited in these studies have been relatively small and selective.

Study design, size, duration:

We assessed diet, semen quality, reproductive hormones, and lifestyle factors among 2,935 healthy young Danish men presented to a compulsory medical examination to determine their fitness for military service between 2008 and 2017.

Participants/materials, setting, methods:

Dietary patterns were identified by principal component analysis (PCA). We measured testicular function as conventional semen quality parameters (semen volume, sperm concentration, total count, percentages of motile and morphologically normal spermatozoa), and serum concentrations of reproductive hormones (LH, FSH, testosterone, Estradiol, SHBG, inhibin B). We used multivariable quantile regression to estimate the association between diet patterns and testicular function.

Main results and the role of chance:

We identified four dietary patterns: the "Western" pattern characterized by intake of pizza, chips, processed and red meats, snacks, refined grains, high-energy drinks, and sweets; the "Prudent" pattern characterized by intake of fish, chicken, vegetables, fruit, and water; the "Smørrebrød" pattern characterized by intake of cold processed meats, whole grains, mayonnaise, cold fish, condiments, and dairy; and the "Vegetarian" pattern characterized by intake of vegetables, soymilk, and eggs. After adjusting for confounders, the median sperm count for men in the highest quintile of adherence to the "Western" pattern was 25.6 (95% CI: -42.3,-8.86) million lower than that of men in the lowest quintile (P-trend=0.005) . Conversely, the median sperm count of men in the highest quintile of adherence to the "Prudent" pattern was 42.8 (22.9, 62.7) million higher than that of men in the lowest quintile (P-trend<0.0001). Adherence to the "Prudent" pattern was associated with the highest sperm count followed by adherence to "Vegetarian" and "Smørrebrød" patterns; adherence to the "Western" pattern was associated with the lowest sperm count. The "Western" pattern was associated with lower inhibin-B (P-trend=0.006) and higher free testosterone concentrations (P-trend<0.0001), while the "Prudent" pattern was associated with lower estradiol (P-trend=0.001) and higher SHBG concentrations (P-trend=0.04).

Limitations, reasons for caution:

This was a cross-sectional study, which limits our ability to determine causality.

Wider implications of the findings:

Our findings support the growing evidence that adhering to generally healthy diet patterns, including local variations, is associated to higher sperm counts and more favorable markers of sperm function.

Trial registration number:

Not applicable

O-179 Prenatal exposure to paternal smoking and semen quality in the adult offspring

S. Tøttenborg[1], K.K. Hærvig[1], B.B. Høyer[2], A. Giwercman[3], K.S. Hougaard[4], C. Ramlau-Hansen[2], I.O. Specht[5], G. Toft[6], J.P.E. Bonde[1]

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[6]Aarhus University, Department of Clinical Medicine- Department of Clinical Epidemiology, Aarhus, Denmark

Study question:
Is prenatal exposure to paternal smoking related to adult offspring semen parameters independently of prenatal exposure to maternal smoking?

Summary answer:
Paternal smoking is associated with lower sperm concentration and total sperm count and more non-progressive sperm independent of maternal smoking and other confounding factors.

What is known already:
The negative impact of maternal smoking during pregnancy on offspring semen quality is well established. Evidence is emerging that also preconception paternal smoking can induce epigenetic alterations in the sperm genome which can be transmitted to the cells of the offspring. Two recent studies investigating offspring semen quality in relation to prenatal exposure to parental smoking found that paternal smoking was associated with lower sperm counts independently of maternal smoking. However, these studies lacked information on key confounders, relied on retrospectively collected smoking information, and had small populations. Larger follow-up studies with extensive confounder information are needed to corroborate findings.

Study design, size, duration:
Population-based follow-up study of 536 young men aged 19 years from the Fetal Programming of Semen Quality (FEPOS) cohort, a sub-cohort of sons to mothers enrolled in the Danish National Birth Cohort (DNBC) during 1996-2002. Semen parameters were assessed at the Department of Occupational and Environmental Medicine at Bispebjerg Hospital in Copenhagen and the Department of Occupational Medicine at Aarhus University Hospital in Aarhus from April 2017 through May 2018.

Participants/materials, setting, methods:
Parental smoking information was based on maternal report around gestational week 16. Semen and testicle size were analyzed according to WHO-recommendations. Incidence rate ratios of semen volume, sperm concentration, count, and motility were calculated using negative binomial regression, difference in morphology with linear regression, and risk of testicle size< 12 ml with logistic regression, adjusting for maternal smoking and age, pre-pregnancy BMI, alcohol and caffeine consumption, paternal age, household occupational status, and son's abstinence time.

Main results and the role of chance:
In adjusted analyses, sons of fathers who smoked daily had a 15% lower sperm concentration (IRR 0.85, 95%CI 0.69;1.04), and 21% lower total sperm count (IRR 0.79, 95%CI 0.62;1.02), but same semen volume as compared to sons of paternal never smokers. While the differences in percentages of progressive and immotile sperm were not statistically significant, a 21% increase in non-progressively motile sperm was observed for sons of father's who smoked compared to those who never smoked (IRR 1.21, 95%CI 1.01;1.46). Although not statistically significant, analysis suggested that paternal daily smoking was associated with a 16% higher risk of having small testicle size as compared to paternal never smoking.

Limitations, reasons for caution:
Paternal and maternal smoking relied on mother's self-report and was not cotinine verified. Further, paternal smoking was assessed around gestational week 16 and may be less frequent than pre-conceptional smoking, though studies have shown a high correlation between pre-and postconceptional smoking.

Wider implications of the findings:
This study corroborates previous findings that paternal smoking affect offspring sperm concentration and total sperm count independently of maternal smoking. In our study, the association was also independent of other prenatal risk factors for adult semen quality including parental age, alcohol and caffeine consumption, pre-pregnancy BMI, and household occupational status.

Trial registration number:
not applicable

O-180 Only high-risk human papillomavirus in semen is associated with high sperm DNA fragmentation index in primary infertile men

P. Capogrosso[1], L. Boeri[2], E. Ventimiglia[3], W. Cazzaniga[3], F. Chierigo[3], E. Pozzi[3], F. Montorsi[3], A. Salonia[3]

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Study question:
We aimed to assess the impact of the presence of HPV in semen over the seminal parameters and SDF values in primary infertile men

Summary answer:
HPV seminal infection, in particular infections involving exclusively HR genotypes, was associated with impaired sperm progressive motility and SDF values

What is known already:
Human papillomavirus (HPV) is commonly present in semen sample. However, whether the presence of HPV in semen is actually associated with impaired semen parameters and sperm DNA fragmentation (SDF) values has yet to be elucidated

Study design, size, duration:
Cross sectional study of 729 infertile men

Participants/materials, setting, methods:
Comorbidities were scored with the Charlson Comorbidity index (CCI). Serum hormones and sperm DNA fragmentation index (SDF; SDF $\geq$30% was defined as pathologic) were measured in every patient. Semen analysis was based on 2010 WHO reference criteria. Amplification by nested polymerase chain reaction (PCR) was used to detect HPV-DNA sequences in semen samples. Descriptive statistics and linear regression models tested the association between HPV presence and clinical and seminal characteristics in the whole cohort

Main results and the role of chance:
The overall HPV positivity was 15.5% (113/729). Of all, 78/729 (10.7%) and 35/729 (4.8%) patients had high-risk (HR) HPV+ and low-risk (LR) HPV+, respectively. HPV16 was the most prevalent type (22.1%), followed by HPV43 (10.6%), HPV56 and HPV42 (both 8.8%). No differences were found in terms of clinical and hormonal characteristics between patients with or without seminal HPV. Sperm progressive motility was lower (16% vs. 22%; p=0.01) while SDF values were higher (35.9% vs. 28.3%; p=0.005) in HPV+ than in – HPV patients. HR HPV+ men had lower sperm progressive motility (12.5% vs. 22%; p=0.007) and higher SDF values (40.3% vs. 28.3%; p=0.003) than -HPV. Univariable linear regression analysis showed that HR HPV+ was associated with impaired sperm progressive motility (p=0.002) and SDF values (p=0.003). At multivariable analysis, age (beta -0.36), FSH levels (beta -0.51) and testicular volume (beta 0.66) were associated with impaired sperm progressive motility (all p$\leq$0.04). Conversely BMI, CCI, smoking habits and HPV status were not. Only age (beta 0.4; p=0.02) and FSH (beta 1.38; p=0.01) were associated with SDF, after accounting for BMI, CCI, testicular volume, smoking habits and HPV status.

Limitations, reasons for caution:
Retrospecrive design

Wider implications of the findings:
These findings outline the role of an accurate investigation of seminal HPV presence over the diagnostic work-up of primary infertile men

Trial registration number:
NA

O-181 Human sperm morphology as a marker of its nuclear quality and epigenetic status: implications for oocyte injection.

M. Bendayan[1], L. Caceres-Sanchez[1], J. Selva[1], L. Alter[1], J. Firmin[1], K. Fathallah[1], S. Bouba[1], V. Duranthon[2], A. Bonnet-Garnier[2], F. Boitrelle[1]

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Study question:

Is there any morphological characteristic of one alive spermatozoon that could predict its nuclear quality and its epigenetic status.

Summary answer:

Observed under high-magnification, spermatozoa with vacuoles present retained histones H3 and the mark H3K4me3. Spermatozoa with no vacuole present higher degree of protamination and H3K27me3.

What is known already:

In human sperm, histones are replaced by protamines during spermiogenesis to allow chromatin condensation. During this replacement, multiple epigenetic events can occur (such as post-translational histone modifications PTMs). Observed under high-magnification, alive spermatozoa with vacuoles were associated to low chromatin condensation levels and vacuoles were defined as DNA – free structures. These results have suggested a difference in terms of sperm nuclear quality between vacuolated ones and those with no vacuole. Nothing was known about epigenetic marks of these spermatozoa.

Study design, size, duration:

Between January and September 2018, we studied sperm samples of 20 infertile patients with differing sperm profiles. Epigenetic marks, known to be present on human spermatozoa and known as potentially implied in embryo development of animals were selected. Firstly, we performed immunostaining of epigenetic marks at one-cell (sperm) scale. Then, we assessed epigenetic profiles of a total of 4000 alive spermatozoa in function of the presence of one vacuole or not.

Participants/materials, setting, methods:

For each sperm sample, 200 alive normal spermatozoa were selected under high-magnification (100 without vacuole and 100 with a vacuole occupying >15% of head-area). For each sample, we have analysed by specific immunofluorescence two nucleoproteins: Protamine and Histone H3 and eight epigenetic marks (PTMs): H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me3 and H4k20me2. A total of 4000 spermatozoa were assessed. Three-dimensional deconvolution microscopy enabled us to reconstruct images of each sperm nucleus and epigenetic patterns.

Main results and the role of chance:

The degree of histone-replacement differs from one spermatozoa to another. Spermatozoa with vacuoles were significantly more prone to retain histone H3 and H3K4me3 than spermatozoa with no vacuole. Morphologically normal spermatozoa without vacuole presented a significantly higher degree of protamination (P2) and retained significantly more H3K27me3 than vacuolated spermatozoa. These epigenetic profiles suggest that vacuolated spermatozoa have an immature chromatin closest to spermatids which could have, according to literature data, negative impacts on embryo development. 3D visualization of vacuolated spermatozoa showed that protamins were located at the basement of vacuoles while H3, H3K4me3 and H3K9me1 (all related to DNA decondensation) filled these area, described until now as DNA – free spaces. These results argue in favour of a nuclear nature of sperm vacuoles, more precisely as an epigenetic nature of sperm vacuoles that should be considered as good individual markers of chromatin non-condensation in one human spermatozoon. Thus, this is the first time here that a morphological characteristic of alive spermatozoa could be linked to epigenetic marks.

Limitations, reasons for caution:

The impact of these marks on embryo development until blastocyst-stage (as suggested by literature) should be proved in further studies.

Wider implications of the findings:

This study was the first to identify the epigenetic pattern of one alive spermatozoon according to its morphology. Selection of an alive spermatozoon, knowing its epigenetic status before oocyte injection, becomes possible. To avoid spermatozoa with vacuole allows to deselect spermatozoa with bad nuclear quality.

Trial registration number:

not applicable

O-182 Reference ranges of male genital tract ultrasound parameters and correlations with clinical, biochemical, seminal characteristics in healthy-fertile men: the European Academy of Andrology (EAA) project.

F. Lotti[1], G. Balercia[2], H. Behre[3], A. Calogero[4], F. Francavilla[5], A. Isidori[6], S. Kliesch[7], S. La Vignera[4], A. Lenzi[6], A. Pilatz[8], M. Punab[9], O. Rajmil[10], O. Shaeer[11], W. Weidner[8], M. Maggi[1]

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[11]*Andrology, Cairo University, Cairo, Egypt*

Study question:

Which are the reference ranges and the clinical, hormonal and seminal correlations of the ultrasound parameters of the male genital tract in healthy-fertile men?

Summary answer:

The reference ranges and clinical, hormonal, seminal correlations of the male genital tract ultrasound parameters in healthy-fertile men have been assessed by the EAA project.

What is known already:

Infertility affects up to 12% of all men. Despite much progress, mainly in genetics, its etiology remains obscure in about half of cases. To fill this gap, imaging of the male genital tract has progressively expanded, providing useful information in the assessment of male genital tract abnormalities. However, sonographic imaging of the male genital tract still suffers from a lack of standardization, and often tends to produce subjective and vague diagnoses. This is the main reason why the European Academy of Andrology has promoted a multicenter study (see http://www.andrologyacademy.net/studies) aimed at defining the anatomic and functional characteristics of healthy-fertile men.

Study design, size, duration:

Cohort, multicentric, international (11 EAA Centers), observational study. Sample size estimated according to the Clinical and Laboratory Standard Institute Guidelines (2000) as 200 subjects. Duration of the project: 36 months. Study design: evaluation of at least 200 healthy-fertile men according to Standard Operation Procedures (http://www.andrologyacademy.net/studies) performed within the same day: 1)personal and medical history; 2)physical examination; 3)hormonal and glyco-metabolic evaluation; 4)scrotal and transrectal ultrasound before and after ejaculation; 5)semen analysis (according to the WHO, 2010).

Participants/materials, setting, methods:

Evaluation using clinical and ultrasound Standard Operation Procedures of at least 200 healthy-fertile men in Outpatient Clinic of 11 EAA Centers. The inclusion criteria were: 1.mens aged >18 years; 2.partners of a pregnant woman in the second or third trimester of pregnancy, or men who fathered a child during the last year, under natural conception; 3.capacity to give consent for study participation. The exclusion criteria were: previous or current systemic diseases and/or medications affecting fertility.

Main results and the role of chance:

We enrolled 248 healthy-fertile men (35.3±5.9 years). Data on 248 scrotal and 188 transrectal ultrasound evaluations are available. The main results are the following: 1)Production, for the first time, of normative values and reference ranges of the organs of the entire male genital tract assessed by color-Dopper ultrasound. 2)Evaluation of the main fertiliy, life-style, medical history and physical examination characteristics of healthy-fertile men, showing median time to pregnancy as 3 months; current smokers and alcohol consumption in 25% and 35%, respectively; physical activity in 50%; absent history of cryptorchidism; mumps in 30% but without orchitis; history of genito-urinary infections in 15%. 3)Positive associations between mean testis volume and sperm concentration and progressive motility (but not normal morphology), confirmed after adjusting for confounders (age, waistline, calculated free testosterone, #EAA centers) (adj.r=0.229, p<0.0001 and adj.r=0.154, p=0.02, respectively). 4)Negative associations between mean testis volume and LH and FSH levels (but not testosterone levels), confirmed in adjusted models (adj.r=-0.363, p<0.0001 and adj.r=-0.197, p<0.005, respectively). 5)Varicocele prevalence 38%. 6)Positive associations between prostate volume, age and body mass index (both p<0.005), but not with testosterone levels. 7)Positive association between seminal vesicles emptying volume documented by ultrasound and semen volume, confirmed in adjusted model (adj.r=0.493, p<0.0001).

Limitations, reasons for caution:

The population studied is made of healthy-fertile men, but fertile men may be not healthy by definition. In addition, investigator meetings have been organized to standardize the clinical and ultrasound method to be followed by the EAA Centers: other sonographers should follow Standard Operation Procedures (http://www.andrologyacademy.net/studies) to standardize their results.

Wider implications of the findings:

This study will: 1)allow a better understanding of male infertility, by comparison of cohorts of infertile men with a true control group of healthy-fertile men; 2)allow the categorization of risk factors (e.g. smoking, alcohol, sedentary, criptorchidism); 3)allow a different management of male genital tract abnormalities (e.g. varicocele).

Trial registration number:

Florence Ethical Committee (6 June 2013; Prot.2013/0024124) and Azienda Ospedaliero-Universitaria Careggi (11 November 2013; Prot.37896/2013, Rubrica n.60/13) approval.

SELECTED ORAL COMMUNICATIONS

SESSION 52: ART FROM THE POINT OF VIEW OF SAFETY

Tuesday 25 June 2019 Haydn 2 15:15 - 16:30

O-183 Long-term risk of ovarian tumours after assisted reproductive technology

M. Spaan[1], A.W. Van den Belt-Dusebout[1], C.B. Lambalk[2], H.H. Van Boven[3], C.W. Burger[4], F.E. Van Leeuwen[1]

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Study question:

Does assisted reproductive technology (ART) increase the risk of invasive and borderline ovarian tumours?

Summary answer:

After a median follow-up of 22 years, the risk of invasive ovarian cancer is not increased.

What is known already:

Despite the large number of women treated with ART every year, evidence about the long-term risk of ovarian tumours after ART is lacking. Previous studies, with a median follow-up of 4 to 17 years, showed inconsistent results with respect to invasive ovarian cancer, while for borderline ovarian tumours an increased risk was found in several reports.

Study design, size, duration:

Long-term risk of ovarian tumours was assessed in the OMEGA study, a nationwide historical cohort with prospective follow-up. The cohort includes 30,636 women who received ovarian stimulation for ART (ART group) between 1983 and 2001 in one of the 12 Dutch IVF centers and 9,969 women who underwent subfertility treatments other than ART (non-ART group) between 1980 and 2001. Median follow-up amounted to 22 years.

Participants/materials, setting, methods:

Detailed information on subfertility cause and treatment was collected from the medical records. Data on reproductive and lifestyle factors were obtained from the women through a questionnaire. Cancer incidence was ascertained through linkage with the Netherlands Cancer Registry (1989-July 2018). Ovarian cancer risk in the cohort was compared between the ART group and non-ART group using multivariable time-dependent Cox regression analyses.

Main results and the role of chance:

After a median follow-up of 22 years 143 invasive ovarian cancers were observed in the entire cohort, 108 in the ART group (36/10,000) and 35 in the non-ART group (35/10,000). The risk of ovarian cancer in ART-treated women was not increased (hazard ratio (HR): 1.03, 95% confidence interval (CI) 0.70-1.52) compared with the non-ART group. In addition, no trend emerged with a higher number of ART cycles (1-2 cycles HR:1.23, 95% CI 0.79-1.91, 3-4 cycles HR:0.82, 95% CI 0.51-1.32, 5-6 cycles HR:0.77, 95% CI 0.40-1.50, >7 cycles HR: 1.50, 95% CI 0.80-2.80). The risk did also not increase after long follow-up (>25 years: HR: 1.03, 95% CI 0.37-2.88).

As expected, parous women had a reduced risk of ovarian cancer (HR: 0.57, 95% CI 0.41-0.80) compared with nulliparous women. Although there was no significant interaction between parity and ART, ovarian cancer risk tended to be slightly increased in parous women treated with ART compared to parous women not treated with ART (HR: 1.40, 95% CI 0.50-1.51), while among nulliparous women ART-treatment did not increase ovarian cancer risk (HR:0.87, 95% CI 0.80-2.42).

Limitations, reasons for caution:

Risk of borderline tumours could not yet be analysed because data collection through the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) is currently being completed. The final results, including the risk of borderline ovarian tumours, will be available for presentation at the ESHRE conference.

Wider implications of the findings:

This large study, with an internal comparison group of subfertile women not treated with ART, importantly contributes to knowledge of the long-term risks of ART. The reassuring results with respect to invasive ovarian cancer can be used to inform couples who are contemplating to start or continue with ART.

Trial registration number:

not applicable

O-184 No decreased chance of a live born child in women with epilepsy after assisted reproduction treatment: a nationwide cohort study

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Study question:

We assessed the chance of a live birth following assisted reproductive technology (ART) treatments in women with epilepsy compared to women without epilepsy receiving ART.

Summary answer:

Women with epilepsy receiving ART treatment did not have a decreased chance of a live birth per embryo transfer compared to other women.

What is known already:

In the general population up to 10–15% of all women have problems of infertility. Studies within this area have indicated that ART treatments are less effective in women with chronic inflammatory bowel disease and rheumatoid arthritis, than in infertile women in the general population. Some evidence

has suggested a role of inflammation in the pathophysiology of epilepsy, but in general epilepsy is not regarded as an autoimmune disease. The efficacy of ART in women with epilepsy has never been examined, and therefore, we want to study the chance of a live born child after ART in women with epilepsy.

Study design, size, duration:

This nationwide cohort study comprised all embryo transfers in Denmark from 1995 to 2017 including follow-up on pregnancies until November 2018. Women undergoing ART treatment included 2,693 embryo transfers in 916 women with epilepsy (the exposed cohort), and 200,259 embryo transfers in women without epilepsy (the unexposed cohort).

Participants/materials, setting, methods:

We used multilevel logistic regression analyses to compute crude and adjusted relative risk estimates (prevalence OR with 95% CI) for live births following ART treatments in women with epilepsy relative to women without epilepsy, and the model accounted for multiple embryo transfers in the same woman. We adjusted for Charlson Comorbidity Index, women's age at the time of embryo transfer, calendar year of infertility treatment, type of infertility treatment, and cause of infertility.

Main results and the role of chance:

There were 631 live births in the epilepsy cohort (by 535 women), and 48,336 live births in the non-epilepsy cohort (by 40,290 women). The cause of infertility in the epilepsy cohort referred to as a female factor in 22.7% of the cases and a mixture of factors in 44.2% of the cases. Among embryo transfers in patients with epilepsy, the median duration of epilepsy at the time of embryo transfer was 15 years (25%–75% percentiles: 8–22 years). The odds ratio for a live born child in women with epilepsy, relative to women without epilepsy undergoing ART, was 0.94 (95% CI 0.85-1.05) and adjusted 0.92 (95% CI 0.83-1.02).

Limitations, reasons for caution:

In this large register-based nationwide study, we cannot obtain information on clinical details on disease severity or types of seizures. These preliminary results are not adjusted for medication.

Wider implications of the findings:

This is the first population-based study to examine the impact of epilepsy on the efficacy of ART. The chance of a live birth per embryo transfer in women with epilepsy was similar to women without epilepsy. This finding is novel, and it is reassuring for women with epilepsy.

Trial registration number:

The study was approved by the Danish Data Protection agency (j. nr. 2012-58-0018, 17/37434).

O-185 Time trends in pregnancy complications over three decades of assisted reproduction technology (ART) – a registry-based study from the Committee of Nordic ART and Safety

S. Petersen[1], M. Gissler[2], C. Bergh[3], B.O. Åsvold[4], L.B. Romundstad[5], A. Tiiteten[6], S.C. Rasmussen[7], M. Petzold[8], A.B. Pinborg[9], U.B. Wennerholm[3], A.K. Henningsen[9], S. Opdahl[1]

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[9]Rigshospitalet Copenhagen University Hospital, The Fertility Clinic, Copenhagen, Denmark

Study question:

Do trends in occurrence of pregnancy complications differ for assisted reproductive technology (ART) and spontaneously conceived pregnancies during three decades of ART treatment?

Summary answer:

ART pregnancies follow the same trends for pregnancy complications as the background population, except for placenta previa, where risk has increased over time.

What is known already:

ART conception comprises an increasing proportion of birth cohorts in the Nordic countries. Perinatal health in ART pregnancies has improved over time, approaching health in the background population. Risk of pregnancy complications is higher after ART compared to spontaneously conceived (SC) pregnancies. Whether this excess risk has remained stable over time, is unknown.

Study design, size, duration:

Registry-based cohort study including nation-wide data from four Nordic countries. In total, 6,830,578 pregnancies resulting in delivery between 1988 and 2015 were included. Among these, 146,998 (2.2%) were ART pregnancies.

Participants/materials, setting, methods:

Data were collected from several national health registries in Denmark, Finland, Norway and Sweden, including ART, medical birth, and patient registries, and linked using the unique identification number for residents. We used logistic regression with post-estimation to estimate absolute risks and risk differences (RDs) over time for hypertensive disorders in pregnancy (HDP), placental abruption and placenta previa, adjusting for parity, maternal age and country. We repeated analyses for singleton and twin pregnancies, separately.

Main results and the role of chance:

The risk of each pregnancy complication was consistently higher in ART compared to SC pregnancies across the study period, with the exception of HDP in twin pregnancies, where risks were similar. In a sub-sample with information on maternal body mass index and smoking in pregnancy, adjustment for these factors did not influence the results.

Risk of HDP increased over time in twin pregnancies for both conception methods (RD 1.73 percentage points [pp] per 5 years, 95% confidence interval [CI] 1.35 to 2.11 for ART and 0.75 pp, 95% CI 0.61 to 0.89 for SC). No clear trend was found for HDP in singletons pregnancies. Risk of placental abruption decreased over time in all groups (RD -0.16 pp, 95% CI -0.19 to -0.12 and -0.06 pp, 95% CI -0.06 to -0.05 for ART and SC, respectively, for singletons and multiple pregnancies combined). The risk of placenta previa increased over time among both ART singletons (RD 0.21 pp, 95% CI 0.14 to 0.27) and twins (RD 0.30 pp, 95% CI 0.16 to 0.43), but remained stable in SC pregnancies. Trends for placenta previa persisted when restricting analyses to primiparous women and to placenta previa with caesarean section.

Limitations, reasons for caution:

Results may be influenced by changes in registration practice over time for placental complications, although registration practice is similar for ART and SC pregnancies. Residual confounding cannot be excluded.

Wider implications of the findings:

Risks of pregnancy complications are consistently higher in ART. Trends in HDP and placental abruption follow those in the background population. The increasing risk of placenta previa over time in ART pregnancies diverges from a stable risk in the background population and warrants further investigation.

Trial registration number:

ISRCTN11780826

O-186 Reduced multiple birth after 85,559 eSET over 233,548 non-eSET cycles, but twinning rate in eSET cycles is above expected levels

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Study question:
What is the prevalence of multiple births after elective single embryo transfer (eSET)?

Summary answer:
There was significant reduction of twins with eSET (1.4%) compared to non-eSET (20.7%) after 85,559 eSET and 233,548 non-eSET cycles. Triplets occurred in both groups.

What is known already:
Prevalence of multiple pregnancies with zygotic splitting after SET was 1.36% after 937 848 single embryo transfer cycles in Japan. UK data is from HFEA database.

Several risk factors for zygotic splitting include manipulations of the zona pellucida for example assisted hatching, ICSI, embryo biopsy leading to herniation of blastomeres through the zona and embryo splitting during blastocyst expansion. Extended blastocyst culture can weaken intracellular bonding in the inner cell mass, leading to zygote splitting. Genetic factors, ovarian stimulation, embryo quality and maternal age are potential risk factors. Some dizygotic pregnancies or triplets from sexual intercourse cannot be ruled out.

Study design, size, duration:
A retrospective observational study was performed, based on 85,559 eSET and 233,548 non- eSET treatment cycles registered on the UK HFEA database from 2012-2016. Additional data on associated risks was gained under the Freedom of Information act (FOI). Data submission to the HFEA is a mandatory requirement.

Participants/materials, setting, methods:
Numbers of eSET and non- eSET treatment cycles registered on the UK HFEA database from 2012-2016 were obtained from the large UK regulated HFEA database coupled to FOI request for information on risk aligned to IVF practices. The level of twins and triplets from each was compared and comparative z-statistical analyses were performed. Incidences of < 5 triplets or quadruplets were not further analysed as it potentially breaches HFEA laws on patient identification.

Main results and the role of chance:
For the 233,548 non eSET cycles there were 54,460 births (23.3%/cycle) and 11,498 multiple births (21.1%). In contrast, from 85,559 eSET cycles the birth rate was 31,585 (36.9%/cycle) and multiple births numbers of 448 (1.4%). The twin births from non-eSET were 20.7% whereas in eSET this was 1.4%. The triplet rate for non-eSET was 0.4% whereas for eSET cycles this was described numerally as < 5 so as not to potentially identify patients. Likewise quadruplets occurred minimally in the non-eSET cycles. From the stakeholders and patients perspective 76.7% of non-eSET treatment cycles failed whereas in the eSET group this was 63.1% failure.

The rate of multiple pregnancy as a proportion of all births was significantly lower following eSET than following IVF without eSET [eSET: 1.42% (1.29-1.55); non-eSET: 21.11% (20.77-21.46). Absolute difference: 19.69% (19.33-20.06). Relative risk: 14.88 (13.56-16.34) p<0.001].

The proportion eSET increased significantly between 2012 and 2016: [2012: 19.85% (19.53-20.17; 2016: 34.65% (34.29-35.01). Absolute difference: 14.80% (14.32-15.28). Relative risk: 1.75 (1.71-1.78) p<0.001]

In eSET cycles there were no quadruplets but there were insignificant levels of triplets.

The practice of non-eSET vs eSET was 2.7 fold higher respectively, with e SET twins higher than background monozygotic twinning rate.

Limitations, reasons for caution:
Although, as expected, eSET is significantly better towards reducing twins compared to non-eSET cycles, it is still higher than the 0.40–0.45% universal monozygotic twinning prevalence. IUI as first line treatment option offers an alternative strategy for lessening the multiple births, several risk factors and numerous associated costs with IVF.

Wider implications of the findings:
Although, as expected, eSET is significantly better towards reducing twins compared to non-eSET cycles, it is still higher than the 0.40–0.45% universal monozygotic twinning prevalence. IUI as first line treatment option offers an alternative strategy for lessening the multiple births, several risk factors and numerous associated costs with IVF.

Trial registration number:
not applicable

O-187 Association between oocyte number retrieved with neonatal outcome after freeze-all strategy

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Study question:
Is there a relation between the number of oocytes retrieved and neonatal outcomes following IVF/ICSI treatment for patients using a freeze-all strategy?

Summary answer:
There is an increased risk of very preterm birth (VPTB, <32 weeks gestation) in women with a low number (1-3) of oocytes retrieved.

What is known already:
Recent studies have found that there is an increased risk of PTB (<37 weeks gestation) and low birthweight (LBW, <2500g) following IVF in women with a high number(>20) of oocytes retrieved in fresh ET cycles. Still others found that there is an association of oocytes retrieved with placenta praevia. However, the association between the number of oocytes retrieved and neonatal outcomes in freeze-all strategy cycles is unknown.

Study design, size, duration:
This retrospective cohort study included 14170 women with singleton deliveries achieved after freeze-all strategy conducted between November 2006 and December 2017, in China. Only the first delivery from one episode of ovarian stimulation were calculated.

Participants/materials, setting, methods:
Patients were categorized into five groups according to the number of oocytes retrieved:1-3, 4-9, 10-15, 16-20 or >20 oocytes. A number of ten to fifteen oocytes was used as a reference. The number of oocytes retrieved was analyzed as a continuous variable as well as categorized variable. Logistic regression analysis of the association between ovarian response (quantified as numberof oocytes retrieved) and outcomes of preterm birth (PTB), VPTB and low birthweight(LBW) was performed.

Main results and the role of chance:
The gestational age, birthweight z-scores, risk of PTB, LBW and congenital malformations did not differ significantly between the groups. There was a significantly higher risk of VPBT among women with a poor ovarian response (≤3 oocytes) compared with women with a normal response (10–15 oocytes) (1.5% vs 0.8%), crude odds ratio (OR) 2.001, 95% confidence interval (CI) 1.159–3.465, P=0.03;adjust OR 1.799, 95%CI 1.027–3.153, P=0.040.

Limitations, reasons for caution:
As with any retrospective study, unknown confounders may affect outcomes.

Wider implications of the findings:
In the absence of ovarian stimulation, a higher number of oocytes retrieved was not associated with adverse neonatal outcome. However, women with low ovarian response associated with a higher risk of VPTB. Factors that may underlie the unfavourable outcomes warrant further research.

Trial registration number:
Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 53: NEW FINDINGS IN REPRODUCTION GENETICS

Tuesday 25 June 2019 Haydn 4 15:15 - 16:30

O-188 Mitochondrial DNA mosaicism in early human development

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Study question:

Do day-3 human preimplantation embryos show mitochondrial DNA mosaicism?

Summary answer:

90% of embryos carry variants unique to one blastomere, showing that mitochondrial DNA mosaicism is already present at day three of human development.

What is known already:

In the past years it has become evident that all the cells in one human being are not as genetically homogenous as previously assumed. Historically, the mitochondrial genome was known to show heterogeneity by different family members carrying the same variants in different frequencies and with diversity across tissues. By now, this diversity has been further refined to the level of the single cell, and often individuals consist of different cell populations that differ substantially in their variants and their mutational burden. Conversely, the timing of the appearance of these cell lineages has not yet been identified.

Study design, size, duration:

We hypothesized that mitochondrial DNA mosaicism begins during preimplantation development. We studied 19 top-quality day-3 embryos, donated by 8 couples, resulting in all but two embryos having at least one sibling in the study cohort. The embryos were surplus to ART treatment and donated after informed consent. The embryos were disaggregated and 119 single blastomeres were deep sequenced for a large part of the mitochondrial DNA.

Participants/materials, setting, methods:

The zona pellucida was removed using pronase, and the blastomeres were isolated by briefly incubating them in Ca^{2+}- and Mg^{2+}- free medium. The single blastomeres were collected in alkaline lysis buffer and long-range PCR was used to generate amplicons of 13 Kbp. Deep massive parallel sequencing was carried out on an Illumina NovaSeq platform and the reads were aligned with reference genome (NC_012920.1) using BWA-MEM. Variants >2% were further analyzed with mtDNA server and MuTect.

Main results and the role of chance:

Fifteen of the 19 embryos carried the same heteroplasmic variant in all of their blastomeres. These had a load ranging between 2-49% and showed a mean difference in frequency from blastomere to blastomere of 3%. Of these variants, 61% were in the hypervariable region and 39% in protein coding regions, of which 60% non-synonymous. Only one pair of sibling embryos showed the same variant but at different loads. All other variants were specific to each embryo. In 17 embryos, we found variants that were unique to one of their blastomeres (33-85% of the blastomeres of one embryo). Of these 147 unique variants, 48% were found in the RNA coding region, 17% in the hypervariable and 35% in protein coding sequences (65% of which non-synonymous). Most of these variants had a low load (±5%), except for 16 variants that had a load over 10% and up to 49%. These variants may be the result of asymmetric distribution of mutant mitochondria and show that the first events of mitochondrial somatic bottleneck are indeed occurring as early as day three of human development.

Limitations, reasons for caution:

These findings apply to non-disease related variants and should be taken with caution if trying to extrapolate to, for instance, preimplantation genetic diagnosis for mitochondrial disease. For pathogenic variants at high loads, different forms of segregation may apply.

Wider implications of the findings:

This is the first study of this kind in the human and shows that it is very likely that the cell lineages found in the adult start originating in the early developmental stages.

Trial registration number:

not applicable

O-189 Human granulosa cells are of very young DNA methylation age following a different epigenetic drift than other somatic cell types

K.W. Olsen[1], A. Cardona[2], A. Chi-Ho Chan[3], R. Borup Helweg-Larsen[3], J.R.B. Perry[2], E.R. Hoffmann[3], N. La Cour Freiesleben[4], A. Zedeler[5], M.L. Groendahl[1]

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Study question:

Does the DNA methylation age (DNAm age) in mural granulosa cells from human ovarian follicles differ from the chronological age of the women?

Summary answer:

The DNAm age of granulosa cells from preovulatory follicles is substantially younger compared to the chronological age of the women.

What is known already:

A multi tissue age predictor based on of 353 CpG sites was developed by Steve Horvath in 2013 suggesting that age-associated DNA methylation changes are shared between cell-types and independent of mitotic activity.

Several studies have shown age-associated hypermethylation in specific genes. Furthermore, higher DNAm age compared to chronological age is associated with a higher risk for all-cause mortality.

In reproduction, the DNAm age of cumulus cells have shown to be younger than the chronological age in infertile women with normal and poor response to ovarian stimulation (Scott et al. 2018).

Study design, size, duration:

This study is a multicenter cohort study based on retrospective analysis of prospectively collected data and material derived from healthy women undergoing IVF or ICSI treatment following controlled ovarian stimulation with antagonist protocol. The granulosa cells were collected from 64 women between September 2016 and January 2018 in in the public fertility clinics at the Copenhagen University Hospitals in Herlev and Hvidovre, and in the private fertility clinic, Stork IVF.

Participants/materials, setting, methods:

Granulosa cells were obtained from women with varying ovarian reserve status (defined from age dependent anti-Müllerian hormone levels) by isolation from pooled follicles immediately after oocyte retrieval. DNA from the granulosa cell aggregates were extracted and analysed with the Illumina EPIC array, at the Human Genotyping Facility Genetic Laboratory, Dept. of Internal Medicine Erasmus MC Rotterdam. Subsequently the DNAm age was calculated using Steve Horvath's age predictor.

Main results and the role of chance:

The DNAm age in the granulosa cells are considerable younger (4-12 years of age, mean: 6.6 years) than the chronological age (26-43 years of age, mean: 33.0 years). There was no correlation between DNAm age and the chronological age of the women (p-value = 0.44), suggesting that the somatic cells of the follicle follow a different epigenetic drift compared to other somatic cell types. Stratification by ovarian reserve status (diminished, normal and high) had no effect on the results.

Limitations, reasons for caution:

The granulosa cells are collected during ovarian stimulation which may influence DNA methylation. As DNA was extracted from granulosa cells from pooled 3-5 follicles we were not able to address if variation in DNAm age is present between follicles in the same women.

Wider implications of the findings:

An accelerated ageing of the somatic cells of the follicle seems not to be involved in the age dependent decline in oocyte competence. This young DNAm age of granulosa cells may reflect that DNAm aging is 'on hold' in the years as pre-granulosa cells before primordial follicle activation.

Trial registration number:

Not applicable.

O-190 Asn680Ser single nucleotide polymorphism in the follicle stimulating hormone receptor (FSHR) gene does not affect semen quality

C.G. Petersen[1,2], A.L. Mauri[1,2], L.D. Vagnini[2], A. Renzi[2], B. Petersen[1,2], M. Canas[2], M. Mattila[1], C. Zamara[1], F. Dieamant[1,2], J.B.A. Oliveira[1,2], R.L.R. Baruffi[1,2], J.G. Franco Jr[1,2]

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Study question:

Are there correlations between the follicle stimulating hormone receptor (FSHR) gene Asparagine/Asn 680 Serine/Ser (2039A>G; rs6166) polymorphism and semen quality or DNA damage in sperm?

Summary answer:

There seems to be no association between FSHR Asn680Ser single nucleotide polymorphism (SNP) and semen quality or sperm DNA damage.

What is known already:

Given the significant role of FSH in fertility, genetic mutation of the FSHR gene might lead to impaired fertility. FSHR gene Asn680Ser SNP exchanges Asn for Ser in the intracellular domain of the receptor and affects the sensitivity of FSHR to FSH in women. However, in men, the impact of this SNP is unclear. Some studies have assessed the association between FSHR Asn680Ser polymorphisms and male infertility. However, the results remain inconsistent.

Study design, size, duration:

A prospective cohort study enrolled 347 men seeking fertility care. DNA was extracted from peripheral blood, and the FSHR Asn680Ser SNP was genotyped using real-time PCR with the Taqman Universal PCR Master Mix and Taqman SNP genotyping assays. For purposes of comparisons, the individuals were divided into three groups according to their genotypes: Asn/Asn; Asn/Ser; and Ser/Ser. Semen analyses were compared between genotype groups

Participants/materials, setting, methods:

A portion of each semen samples was used for analysis according to the WHO guidelines/morphological analysis by motile sperm organelle morphology examination(MSOME). The remainder of the semen samples were tested for sperm DNA fragmentation using TUNEL assays; sperm apoptosis was analysed using the annexin V assay; sperm chromatin packing/protamination was assessed using chromomycin A3(CMA3) staining; and sperm mitochondrial membrane potentia (MMP) was analysed using MitoTracker Green. At least 200 spermatozoa were examined in each evaluation.

Main results and the role of chance:

Hardy–Weinberg genotype distributions indicated concordance between the observed/expected frequencies. No correlations between FSHR Asn680Ser genotypes and potential confounders (age, abstinence time, smoking, and drinking alcohol) were observed. There was no association between FSHR Asn680Ser genotypes and general semen parameters or DNA damage in sperm. Table 1 shows the data.

Limitations, reasons for caution:

Sperm was obtained from men seeking fertility care. Generalisations of the results to the general population should be performed with caution. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings:

Although an association between the FSHR Asn680Ser SNP and human fertility has been reported, there appears to be no link between the genotype and semen quality or sperm DNA damage. The genotype of this polymorphism is probably not useful in the evaluation of male infertility.

Trial registration number:

Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

O-191 Rare variants in genes involved in Integrin-linked-kinase pathway identified in patients developing severe ovarian hyperstimulation syndrome (OHSS) with a predicted low-risk of OHSS.

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Study question:

Do genetic variants in patients developing severe OHSS but with predicted low risk of OHSS, provide clues to the mechanisms involved in the syndrome?

Summary answer:

Genes with variants in OHSS patients with low-risk of OHSS enrich the Integrin-linked-kinase (ILK) and Focal-adhesion-kinase (FAK) signaling pathways, both linked to VEGF signaling.

What is known already:

Severe OHSS is a systemic disorder including high vascular permeability correlated to an excessive ovarian response following ovarian stimulation (OS) and ovulation trigger with hCG. However, severe OHSS is also, though rarely, seen in women with low ovarian response to OS and in spontaneous pregnancies. VEGF is involved in the high vascular permeability. OHSS-predisposing genes have primary been confined to known and frequent of polymorphisms (i.e. *FSHR, LHR, CYP11A, AMH, VEGFR2*). Recently, a case report (severe OHSS after GnRHa trigger) focusing on rare genetic variants, suggested *VEGFR3* (official name *FLT4*) to have a role in OHSS (Stouffs et al., 2018).

Study design, size, duration:

Among participants in a prospective randomized study including 1050 women in 1st IVF cycle (2009-2013) (Toftager et al., 2016), six women with no risk factors of OHSS developing severe OHSS (grade 4 and 5, Golan classification) were selected. In the same cohort, six matched (age, diagnosis, protocol, ovarian response, estradiol level) controls (grade 0, no OHSS) were selected. In connection to oocyte retrieval, cumulus cells were isolated and snap frozen until analysis.

Participants/materials, setting, methods:

DNA was extracted from cumulus cells from six patients with OHSS (grade 4-5) and six controls (grade 0). Whole exome sequencing (WES) was performed by SureSelect library preparation and sequenced on the Illumina High-Seq2500. DNA variants call was performed in GATK 4.0 pipeline, variants and pathways filtering was done using the Ingenuity Variant Analysis tool (Qiagen). Variants were manually validated in Integrative Genomics Viewer (IGV 2.4.9), only class 3, 4 and 5 variants were included.

Main results and the role of chance:

Variants called (number: 30911) from the six patients and six controls were submitted to pathway filtering. Among the most significant enriched pathways in the OHSS group as compared to control was the ILK signaling pathway (23 genes, 24 variants, $p<0.00001$) recently connected to play a central role in VEGFR3 signaling (Urner et al., 2019). Furthermore, FAK signaling, converging signaling point between VEGFR2 and integrins and focal adhesions in endothelial cell was enriched (17 genes, 18 variants, $p<0.00001$). Genes with variants in ILK signaling were genes coding for cell junctional proteins (*ITBG2, ITBG5, ITBG7, PXN*) and protein phosphatases and kinases (*PTPA, PIK3R5, PIK3R6, PIK3C2G, PIK3R4, PIK3CD*). Each OHSS patient had two-six variant genes in the pathway, while variants were absent in the controls.

Limitations, reasons for caution:

A potential role of ILK and FAK signaling in development of OHSS needs to be validated by WES on additional OHSS in women with high risk cases and followed by functional studies.

Wider implications of the findings:

Clarification of the mechanism as well as potentially defining genetic predisposition of the high vascular permeability is important for future treatment and prevention of OHSS.

Trial registration number:

Not applicable.

O-192 Endometrial microbiome analysis using long-read nanopore sequencing technology

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Study question:

In this pilot study we aim to investigate if the human endometrial microbiome can be analyzed by using a handheld third-generation nanopore DNA sequencing device.

Summary answer:

Our data suggest the feasibility of 16S rDNA amplification and sequencing the microbiome from endometrium biopsies by using long-read nanopore sequencing technology.

What is known already:

Metagenomics sequencing enlightened the fact that the healthy endometrium hosts a normal, non-infectious microbiome and is not sterile as historically thought. Recent studies suggest that the microbiome constellation contributes to fertility and pregnancy outcome. Especially the amount of *Lactobacillus* species in the endometrial microbiome influences pregnancy outcome, with higher live birth rates in *Lactobacillus*-dominated (>90%) microbiota.

Novel third-generation sequencing technologies, like the portable MinION sequencing device from Oxford Nanopore Technologies (ONT) allow real-time sequencing analysis and significantly reduce initial hardware investment costs, library preparation time and cost per sequence. Additionally, the long-reads allow full-length sequence analysis of 16S rRNA genes.

Study design, size, duration:

Here we investigate the feasibility of targeted 16S microbiome sequencing of ten endometrial biopsy samples on the Oxford Nanopore MinION device. The results were compared with RT-PCR microbiome panel analysis from DNA Technology (FEMIFLOR® Screen).

Participants/materials, setting, methods:

Endometrium biopsy was taken from patients undergoing assisted reproductive technology (ART) in our institution due to repeated implantation failure for further analysis. The biopsy was performed using Probet endometrial suction curette (Gynétics) and left-over material was used for this pilot study after patient's informed consent.

After DNA extraction, 16S rDNA was amplified using ONT's SQK-RAB204 kit. The sequencing was performed on the handheld MinION sequencing device and data were analyzed using EPI2ME 16S workflow.

Main results and the role of chance:

In a first step, we analyzed the feasibility of unbiased 16S rDNA sequencing with the MinION using ZymoBIOMICS™ Microbial Community Standard. By comparing different DNA extraction methods and PCR conditions, we minimized the extraction and PCR bias for equal sequencing of different gram-positive and gram-negative bacterial species. Microbiota were analyzed on genus-level.

With these optimized conditions, we were able to amplify 16S rDNA from six out of ten endometrial biopsy samples. In four of six samples *Lactobacillus* spp. was the dominant genus, with three of these showing >90% *Lactobacillus* reads. One sample was *Streptococcus*-dominated, and one was dominated with *Staphylococcus* reads. Microbiome RT-PCR analysis from DNA Technology did not lead to any reliable results due to low bacterial load in all six samples, which were reliably amplified for nanopore sequencing. This highlights the importance of optimized 16S amplification protocol and the necessity of highly sensitive detection methods like high-throughput sequencing for the analysis of low bacterial load tissues.

These results open new avenues for endometrial microbiome analysis for assessing IVF-outcome chances. Our study proofs that it is possible to reliable detect and analyze bacterial specimen even in tissues with low bacteria infiltration by using 16S amplification and long-read nanopore sequencing.

Limitations, reasons for caution:

Nanopore sequencing technology is fast, cost-effective and much progress has been made recently. Nevertheless, sequencing still produces high error rates what currently enables assessment of microbiota only down to genus level.

Additionally, not all endometrium samples could be amplified. This is possibly due to low bacterial load in these samples.

Wider implications of the findings:

This study is a proof of concept for the feasibility of rapid, cost-effective and easy endometrial microbiome screening for human reproduction.

Clinical impact of endometrium microbiome sequencing for pregnancy outcome, especially for ART treatment is still under debate and needs to be further investigated in prospective clinical trials.

Trial registration number:

not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 54: FERTILITY PRESERVATION 2

Tuesday 25 June 2019 Strauss 1+2 15:15 - 16:30

O-193 Encapsulation of mice immature testicular tissue in alginate supplemented with nanoparticles delivering a necrosis inhibitor improved spermatogonial survival and seminiferous tubule integrity in autografts

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Study question:

Can we improve the outcome of immature testicular tissue (ITT) autotransplantation using tissue engineering techniques that involve localized and sustained delivery of a necrosis inhibitor?

Summary answer:

Seminiferous tubule integrity and spermatogonial survival were improved in 21 day grafts of mice ITT encapsulated in alginate supplemented with nanoparticles delivering necrosis inhibitor NecroX-5™.

What is known already:

Anticancer therapy often induces gonadal damage leading care providers to develop fertility preservation measures. For prepubertal boys, cryostorage of ITT containing spermatogonial stem cells is so far the only option to give them a hope for future fatherhood. Fertility restoration using frozen-thawed human ITT has not been achieved yet but autotransplantation appears to be a promising strategy based on non-human primate data. Unfortunately, spermatogonial loss is a constant observation in ITT transplantation experiments, although ITT encapsulation in 1% alginate containing nanoparticles delivering VEGF (vascular-endothelial growth factor) improved tissue vascularization and doubled spermatogonial survival compared to non-encapsulated tissue.

Study design, size, duration:

ITT pieces recovered form 15 mice were encapsulated within a matrix of 1% alginate supplemented with PLGA/PEG (Poly(D,L-lactide-co-glycolide) poly(ethylene glycol)) nanoparticles (Nps) delivering NecroX-5™, a necrosis inhibitor. One fragment per mice was auto-transplanted orthotopically for 21 days and grafts were compared to non-encapsulated and alginate-encapsulated ITT auto-grafts.

Participants/materials, setting, methods:

1mm^3 testicular fragments were obtained from 15 NMRI (naval medical research institute) prepubertal mice after bilateral orchidectomy. Orthotopic auto-transplantation of non-encapsulated, alginate-encapsulated and alginate/Necrox-5™ Nps- encapsulated ITT was carried out for 21 days. Grafts were evaluated on hematoxylin-eosin histological sections using a scoring system for seminiferous tubule integrity and after immunohistochemical staining using promielocytic leukemia zinc-finger (PLZF) , active caspase-3 and malondialdehyde as markers of undifferentiated spermatogonia, apoptotic cells and lipid peroxidation, respectively.

Main results and the role of chance:

Histological evaluation of grafts showed that when an alginate tissue embedding matrix supplemented with Nps delivering NecroX-5™ was used, the number of seminiferous tubules scored as intact (as cells adhesion to the basement membrane, cell cohesion and absence of sclerosis were observed) was increased compared to both non-encapsulated (p=0,0068) and alginate-encapsulated grafts (p=0,0036). The number of undifferentiated spermatogonia was also increased in grafts supplemented with NecroX-5™ Nps compared to alginate- encapsulated (p=0.0009) and non-encapsulated tissue (p<0.0001), as well as in alginate- encapsulated compared to non-encapsulated grafts

(p<0.0001). No statistical significant difference was found when the results obtained in the three groups were compared for both active-caspase 3 positive cells and malondialdehyde positive cells.

Limitations, reasons for caution:

Several questions need to be answered before a translation to clinical practice could be considered. Experiments involving animal tissue should be replicated using human tissue. Moreover, no information was obtained concerning the reproductive potential of surviving spermatogonia, nor on genetic and epigenetic consequences on tissue engineered grafts.

Wider implications of the findings:

Supplementation of an alginate matrix with Nps containing a necrosis inhibitor allowed improved mice ITT auto-transplantation outcome. Application of tissue engineering involving tissue embedding and controlled delivery of growth factors and other molecules using nanoparticles represents a powerful tool for future perspectives to restore fertility with cryostored ITT transplantation.

Trial registration number:

not applicable

O-194 Pubertal development after testicular tissue biopsy for fertility preservation in pre- and peripubertal boys having received gonadotoxic treatment

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Study question:

Does a testicular tissue biopsy procedure at pre- or peripubertal age affect the pubertal development of young boys on the long-term?

Summary answer:

FSH and testosterone serum levels and bone age seem affected by the biopsy procedure; whereas testicular volume, LH, INHB, AMH and bone density are not.

What is known already:

Male infertility due to gonadotoxic treatment and the search for an adequate fertility preservation strategy becomes clinically more relevant because of the improved survival rate of children with cancer or haematological disorders. The only fertility preservation option for pre- and peripubertal boys who do not produce mature spermatozoa yet, is to undergo a testicular tissue biopsy (TTB) followed by banking before spermatogonial stem cell loss. Although recent evidence demonstrates that immediate adverse effects of the biopsy procedure are rare (1%), nothing is known on the effects on the long-term.

Study design, size, duration:

Testicular volume, LH, testosterone, FSH, INHB and AMH serum levels, bone age and bone density were retrospectively collected from patients' medical records between 2002 and 2017 at Universitair Ziekenhuis Brussel. Because Tanner stages were not available, data were coded according to patients' age at measurement and compared between boys who underwent TTB (biopsy group) and those who did not (control group) to identify possible association between TTB at pre- or peripubertal age and pubertal development.

Participants/materials, setting, methods:

109 pre- and peripubertal boys needing gonadotoxic treatment with >80% risk of later fertility problems were offered TTB and banking. Exclusion criteria were testicular cancer diagnosis, substitution treatments for testosterone and mature spermatozoa present in the semen sample. Seventy-two boys were diagnosed with cancer and 37 with haematological disorders. Prader orchidometer, the Greulich & Pyle method and z-scores were used for the measurement of testicular volume, bone age and bone density respectively.

Main results and the role of chance:

The testes of 63 boys were biopsied, while 46 boys had chosen not to cryopreserve their testicular tissue and served as control. TTB was performed in 52 prepubertal boys (< 12 years) and 11 peripubertal boys (12-16 years). Up to five years and seven months after the biopsy procedure, no statistically significant difference in testicular volume was found between the biopsied and the contralateral non-biopsied testis in both pre- and peripubertal boys. The number of boys showing normal FSH serum levels was significantly decreased in the biopsy group compared to the control group (68% vs. 94%; p=0.006). For testosterone, significantly more boys showed low serum levels after the TTB procedure (52% vs. 6%; p<0.001). No statistically significant differences were observed for the serum levels of LH, INHB and AMH. Less boys had a delayed bone age after TTB (17% vs. 50%; p=0.038). The TTB procedure had no impact on the bone density.

Limitations, reasons for caution:

Because this retrospective study revealed plenty missing data in clinical files, no firm conclusions could be made. A prospective study could obtain a more representative picture of long-term effects of TTB. This study proves the need for an improved and standardized follow-up protocol for boys undergoing TTB for fertility preservation.

Wider implications of the findings:

This was the first study investigating long-term effects of TTB at pre- or peripubertal age on pubertal development of boys with cancer or haematological disorders. A more standardized follow-up after TTB will allow better counseling of patients interested in fertility preservation and optimize selection of patients eligible for fertility preservation.

Trial registration number:

Not applicable.

O-195 Fertility of women with Rheumatoid Arthritis (RA) : The inflammatory activity of the pathology is negatively correlated with AMH serum level.

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Study question:

Is there an impact of Rheumatoid Arthritis and its treatment on the ovarian reserve to evaluate if fertility preservation would be necessary for these patients?

Summary answer:

Rheumatoid Arthritis (RA) activity is negatively correlated with AMH serum level.

What is known already:

Patients with RA have a higher prevalence of infertility compared with general population. Inflammation related to the pathology and the treatments of RA seem to be the main hypothesis to explain the infertility of these patients. Nevertheless, the tangible link of these different factors with the decline of women's fertility has never been clearly demonstrated.

Study design, size, duration:

Patient data and serum analysis were derived from the French national prospective cohort ESPOIR (Etude et Suivi des POlyarthrites Indifférenciées Récentes). Enrolled patients (n= 106, 18 to 37 years old) with the ACR/EULAR2010 criteria for RA didn't receive any medication at the time of enrolment (T0). Patients were examined and AMH serum level was measured at T0, 6(T6), 12(T12), 24(T24) and 36(T36) months after the diagnosis.

Participants/materials, setting, methods:

The measurement of serum AMH level was performed with electrochemiluminescence method (analyseur Cobas® e411). The impact of RA inflammatory activity (evaluated by DAS28 score and level of CRP) and treatments (methotrexate only/or with other medications) was evaluated at each time of our study.

Main results and the role of chance:

We observed a gradual decrease of AMH serum level of patients over time similar with the decrease curve described for the healthy women in the literature (Kelsey *et al*.,2011). AMH serum level of the RA patients compared with the values considered as normal graduated by age did not show any significant difference at T6, T12, T24 and T36 ($p > 0.05$). We did not observe an impact of the treatments on AMH serum level whatever the type of medication performed (methotrexate only/or with other medications). We showed an inverse correlation between AMH variation and inflammation parameters (DAS28: $r = -0.27$, $p = 0.003$; CRP: $r = -0.16$, $p = 0.06$).

Limitations, reasons for caution:

The evaluation of the ovarian reserve should be completed by an ultrasound at the 5th day of the cycle as well as an additional hormonal assessment.

Wider implications of the findings:

This study is the first to study AMH serum level follow up of a large cohort of young RA patients during 36 months. The quick limitation of inflammation seems necessary to limit alterations of ovarian reserve. Oocytes and/or ovarian tissue don't seem necessary if inflammation is quickly controlled.

Trial registration number:

Clinical trial registration number is in progress.

O-196 Fertility preservation by ovarian tissue freezing in patients with genetic and chromosomal disorders

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Study question:

Should ovarian tissue cryopreservation (OTC) be considered in girls and young women with genetic and chromosomal disorders that may cause premature ovarian insufficiency (POI)?

Summary answer:

OTC may be considered in patients with galactosemia, thalassemia and in a highly selected group of patients with mosaic Turner syndrome (TS).

What is known already:

Genetic and chromosomal disorders such as TS, galactosemia, and thalassemia are often associated with poor ovarian function. The main reproductive effect of these disorders is POI, and the prevalence of spontaneous pregnancy is low. Further, these diagnoses are often associated with increased risk during pregnancy. A few studies have detected follicles in ovarian biopsies from adolescent girls with TS and in young girls with galactosemia, and some centers offer OTC for these patients. However, the speed at which the follicle pool is depleted and the subsequent fertility potential of these follicles has not been clarified.

Study design, size, duration:

This retrospective study included 15 girls and young women with TS (aged 5 to 22 years), 6 patients with galactosemia (aged 0.3 to 11 years), 11 patients with thalassemia (aged 3 to 17 years) and 42 control patients (aged 2 to 26 years), who had one ovary removed for fertility preservation for other reasons prior to gonadotoxic treatment. All patients underwent OTC between the years 2002 and 2017.

Participants/materials, setting, methods:

A total of 74 girls and young women aged 0.3 to 26 years (mean age 13.6 years), who underwent OTC for fertility preservation were included in this study. Follicle density (follicles/mm^3), morphology and health were assessed in ovarian cortex biopsies from patients with genetic/chromosomal disorders and compared to controls.

Main results and the role of chance:

Follicles were found in 60% (9/15) of the biopsies from TS ovaries; 8 girls with a mosaic karyotype and one 45,X. In 78% (7/9) of the ovaries with follicles, the mean follicle density was -0.7 standard deviations below age-related predicted density of the control group. There was a high rate of abnormal follicle morphology in the TS ovaries. In girls with galactosemia, morphological normal follicles were found in 83% (5/6) of the biopsies and the mean density was -0.2 standard deviations below age-related predicted density of the control group. No follicles were detected in the ovary from an 11.7-year-old girl with galactosemia. In girls with thalassemia follicles were detected and the densities were within the 95% CI of the control group. 5 follicle specific proteins were expressed similarly in ovaries with genetic and chromosomal disorders and in control ovaries.

One women with thalassemia had her tissue grafted and conceived following IVF treatment and delivered a healthy baby where the oocyte derived from the transplanted tissue.

These findings suggest that OTC may be considered for highly selected patients with genetic/chromosomal disorders. It is important to note that frozen/thawed ovarian tissue has not yet been grafted to women with TS or galactosemia.

Limitations, reasons for caution:

The pathophysiology of the genetic/chromosomal disorders leading to an accelerated follicle loss is unknown and it is currently unknown to what extent transplanted ovarian tissue can sustain fertility.

Wider implications of the findings:

The benefits of OTC may be limited to a highly selected group of patients with genetic/chromosomal disorders, in whom a sizeable pool of normal follicles is present at OTC and where endocrine assessment does not indicate POI, and if other health issues do not preclude pregnancy.

Trial registration number:

Approved by the ethics committee of Copenhagen and Frederiksberg (H-2-2011-044)

O-197 Secondary follicle rescue feasibility and in vitro follicle culture after initiation of treatment with cyclophosphamide in prepubertal mice – Implications for fertility preservation

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Study question:

How a recent treatment with cyclophosphamide (CPA) affects the feasibility of isolation of secondary follicles for culture aiming fertility preservation vs untreated controls?

Summary answer:

Follicle isolation, culture and oocyte maturation were feasible after CPA treatment, although significantly smaller follicle size and reduced maturity rate were obtained vs controls.

What is known already:

Clinical guidelines recommend the application of fertility preservation methods before cancer treatment, however, in some cases, this is not possible. Up to

date, there is a lack of knowledge about the growth dynamics of ovarian follicles in vitro after cancer therapy initiation.

Study design, size, duration:

Experimental controlled study using mice. Prepubertal B6CBA/F1 females 12 days old were randomly distributed to CPA treatment (n=18) or control (n=8). CPA 50, 75 or 100mg/kg was injected intraperitoneally. The mice were sacrificed by cervical dislocation after 3 days and ovaries collected. Secondary follicles were isolated and cultured for 12 days up to oocyte maturation according to the method described by Cortvrindt [1].

Participants/materials, setting, methods:

Main outcome variables included the number of isolated follicles obtained per ovary, follicle growth estimated by mean diameter throughout the culture, survival at day 12 of culture, expulsion of the cumulus-oocyte complex (COC) and metaphase II (MII) oocytes ratios. Comparison between groups was performed using Chi-square test with Yate's continuity correction and Welch's ANOVA test. The results were considered significantly different for $p<0.05$.

Main results and the role of chance:

The size of secondary follicles isolated and put in culture was similar among the groups. However, on day 12 the size of follicles obtained from CPA-treated ovaries was significantly smaller (575.2mm ±253,0; 560,4mm ±225,0 and 600,1mm ±205,4 in the 50, 75 or 100mg/kg, respectively) than in the controls (666,5mm ±191,0) ($p<0,01$ for all). In all groups follicle survival ratio was high (>80%). There was a trend towards decreasing COC expulsion rates in association with increasing CPA dose received ($p=0.07$). The MII oocyte ratio was reduced in all CPA-treated groups vs control ($p<0,01$).

Limitations, reasons for caution:

The data presented herein are based on morphological follicle characteristics observed during culture. Further investigation of molecular markers would improve the knowledge on the mechanisms of cytotoxic drugs affecting follicle development and oocyte viability.

Wider implications of the findings:

Secondary follicles could be retrieved from pubertal mice ovaries after CPA-treatment. Comparable numbers of follicles could be isolated, survival and COC expulsion were not affected by CPA treatment. However, the final size of the follicles and the MII oocyte ratio were significantly reduced in follicles obtained from CPA-treated ovaries.

Trial registration number:

not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 55: NON-INVASIVE APPROACHES FOR PREDICTING EMBRYO PLOIDY

Tuesday 25 June 2019 Haydn 1 17:00 - 18:00

O-198 The blastocyst score, integrating morphologic grade and day of blastocyst development, is a predictor of blastocyst ploidy

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Study question:

Can the blastocyst score (BS), which comprises numeric values for blastocyst expansion, inner cell mass (ICM), trophectoderm (TE), and day of blastocyst development, predict ploidy?

Summary answer:

A numeric blastocyst score, integrating blastocyst (BL) morphologic grades and speed of development (D5 or D6), can be used as a predictor of ploidy status.

What is known already:

Preimplantation genetic testing for aneuploidy (PGT-A) has been shown to enhance the implantation rate per transfer and decrease the miscarriage rate, particularly in advanced maternal age patients. However, the technique is invasive, sometimes inconclusive, and expensive. Non-invasive blastocyst morphology (grade) and morphokinetics have been shown to be associated with implantation, suggesting a possible correlation with ploidy, as reported in several studies. Moreover, blastocysts developed on D5 exhibit higher implantation and euploidy rates compared to D6 blastocysts. The use of blastocyst quality and its developmental speed as a way to determine ploidy should be validated.

Study design, size, duration:

This is a retrospective cohort study conducted in an academic institution from November 2011 to June 2017. A total of 11,364 biopsied blastocysts from 2,309 IVF cycles (female age, 21-48; avg. 36.8±4.7) were included. Embryos were biopsied on days 5 or 6, contingent on reaching the expanded blastocyst stage, and tested using aCGH/SNP or NGS.

Participants/materials, setting, methods:

Blastocyst scores were based on a modified Gardner grading. Numerical blastocyst grades of 1-4 were given individually for expansion, ICM, and TE (1 being the best) based on clinical implantation rates. The blastocyst score was calculated as the sum of the blastocyst grades and development day (D5 or D6; a value of 2 was added for D6 blastocysts). Logistic regression analysis was used to estimate the odds ratio between scores with a 95% CI.

Main results and the role of chance:

To predict blastocyst euploidy, the following factors were significant (ranked in descending order of significance): age of female patient (oocytes), blastocyst score, and blastocyst developmental day (D5 or D6). The oocyte source (donor vs. autologous) and the method of fertilization (ICSI vs. IVF) were not significant. Blastocyst euploidy decreased by 0.86 times (95% CI, 0.84-0.87) per year of advancing age. Blastocysts from 21-year-old oocytes were 66.7 times more likely to be euploid than those from 48-year-old oocytes (95% CI, 50.1-84.4). The blastocyst score showed a linear correlation with euploidy rate in all female age groups. For each numerical rise in blastocyst score, embryos were 0.83 times less likely to be euploid (95% CI, 0.82-0.85). The embryo with the best blastocyst score (score 3) had euploid probability 12.9 times higher than the worst-scored one (score 17; 95% CI, 10.6-15.9). Euploid prediction for patients <35 years old with the best blastocyst score was 80%. In comparison, euploid prediction in 40-year-old patients with the same blastocyst score was 40%.

Limitations, reasons for caution:

This study is limited by its retrospective nature. PGT-A was performed on different platforms.

Wider implications of the findings:

This study underscores the importance and the role of evaluating blastocyst morphology and developmental speed. The numeric blastocyst score can be used as a non-invasive predictor of euploidy and a tool for patients considering undergoing PGT-A.

Trial registration number:

N/A

O-199 Morphokinetics may provide a useful adjunct for selecting Euploid embryos with a high chance of implantation

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Study question:

Can a universal morphokinetic model based on Known Implantation Data (KID) increase the chance of selecting euploid embryos with a higher implantation potential, than just morphology.

Summary answer:

Selecting euploid embryos with higher morphokinetic scores by a universal time-lapse algorithm gives a higher probability of implantation than selection by morphology.

What is known already:

PGT-A is increasingly used to select chromosomally normal blastocysts, however despite advances in genetic testing techniques, pregnancy rates of euploid embryos are still around 60%. When multiple euploid embryos are available, further selection must be applied. Morphokinetic models have been developed by correlating embryo development pace and morphology, with Known Implantation Data (KID) of transferred blastocysts. Several studies have shown that morphokinetic scores can be used to select untested blastocysts with higher implantation potential. Few studies have examined the adjunct use of

morphokinetics to select higher implantation potential euploid embryos, instead of morphology alone.

Study design, size, duration:

This retrospective analysis embryos were cultured in the EmbryoScope time-lapse system (Vitrolife A/S) and evaluated for morphokinetics, morphology and ploidy. KIDScore D5 v2.0 scores were generated in the EmbryoViewer Software. Blastocysts were categorized into three KIDScore groups: Poor, Fair, Good according to score range (2-5.9, 6.0-7.9 and 8.0-9.9, respectively). Morphological scoring was performed using Gardner score. Following trophectoderm biopsy on day 5 or day 6, PGT-A was performed using NGS platform (VeriSeq protocol, Illumina).

Participants/materials, setting, methods:

Between October 2017 and December 2018 we analysed a total of 1498 embryos from patients between ages 20 to 50 (mean age 30.564). Euploid blastocysts for frozen embryo transfer were selected by morphology only. Mosaics and aneuploid blastocysts were not selected for transfer. For this subset of 200 transferred embryos the different scoring options were compared to implantation rates. Statistical significance of implantation data was calculated using chi square test.

Main results and the role of chance:

The analysis of the ploidy status for all embryos showed that increasing Day 5 KIDScore categories (poor / fair / good) were correlated with an increasing rate of euploid embryos (48.1% / 51.7% / 63.3%; $P<0.05$) and a decreasing rate of aneuploid embryos (25.6% / 35.7% / 41.6%), whereas the rate of mosaic embryos ranged from 10.2% to 12.5% and was not different. A similar result was found for morphology alone versus ploidy.

Implantation rates for euploid embryos of all morphology types in the KIDScore categories good / fair / poor were 73.9%, 59.5% and 47.7%, respectively. Within the different KIDScore subgroups the embryos with good morphology alone gave implantation rates of 58.7% (KIDScore good), 43.2% (KIDScore fair) and 27.3% (KIDScore poor), respectively. Comparing morphology only versus KIDScore only showed implantation rates of 63.5% versus 73.9% for good morphology score versus good KIDScore, 59.6% versus 59.5% (fair/fair) and 27.3% versus 47.7% (poor / poor), thus showing that in the direct comparison subselecting euploid embryos by KIDScore gave better or equal results compared to subselection by morphology only scoring.

Limitations, reasons for caution:

These data are from a single center. Larger numbers and verification in another center are needed.

Wider implications of the findings:

In cases where multiple Euploid embryos are available for transfer, morphokinetic analysis may identify other viability factors, which are not related to chromosomal status and morphology alone and therefore be a valuable adjunct to PGT-A for selecting embryos with the highest implantation potential.

Trial registration number:

None

O-200 Slow embryo development predicts higher aneuploidy rates among high grade blastocysts but does not influence pregnancy outcomes in euploid blastocyst transfer cycles

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Study question:

Does blastocyst formation rate predict rates of embryo aneuploidy, independent of morphologic grading, and subsequent pregnancy outcomes in euploid frozen blastocyst transfer cycles?

Summary answer:

Early blastocyst development only predicts higher euploidy rates among high-grade embryos in women <40-years-old and does not correlate with pregnancy outcomes in euploid transfer cycles.

What is known already:

Current strategies for embryo assessment primarily rely on either non-invasive morphologic parameters that have limited accuracy for determining embryo viability or invasive embryo biopsy techniques for pre-implantation genetic testing (PGT-A). Although previous studies found an association between blastocyst morphology and euploidy rate, there is conflicting evidence regarding the value of embryo development rate as a non-invasive marker for predicting embryo aneuploidy and implantation potential of euploid embryos. Understanding the role of embryo development rate and blastocyst morphology in PGT-A cycles may help improve to embryo selection and optimize cycle management and clinical outcomes.

Study design, size, duration:

A retrospective cohort study was conducted at an academic fertility center and included 535 patients undergoing in-vitro fertilization (IVF) and preimplantation genetic testing for aneuploidy (PGT-A) between 2014-2018. 2305 embryos were biopsied on either day 5 (n=481) or day 6 (n=1824) depending on rate of embryonic growth and blastocyst expansion. A standard infertility evaluation was performed within 12 months of treatment and the decision to undergo PGT-A was a joint discussion between patient and physician.

Participants/materials, setting, methods:

Euploid rate was the primary outcome of interest and was calculated according to the day of biopsy and stratified by morphologic grading (good: 5/6AA-BB, average: 5/6BC-CC, poor: 3/4BB-CB) and patient age. Secondary outcomes included implantation (IR) and ongoing pregnancy rate (OPR) in subsequent euploid transfer cycles. Implantation rates were calculated based on a positive pregnancy test after embryo transfer while OPR was defined by the presence of a viable fetal heart rate >7 weeks' gestation.

Main results and the role of chance:

A higher euploidy rate was observed among day 5 compared to day 6 blastocysts overall (53.01 vs. 39.75%, $p<0.0001$). As expected, aneuploidy rates increased with maternal age and no difference in euploid rates was observed among day 5 vs. 6 blastocysts in women ^{3}41-years-old (33.33 vs. 23.17%, p=0.22). A significantly higher euploid rate was also observed among high grade embryos compared to low grade embryos (47.43% vs. 28.23%, $p<0.0001$); however, day 5 biopsied blastocysts demonstrated a significantly higher euploid rate compared to day 6 biopsied blastocysts among high grade embryos (55.15% vs. 44.87%, p=0.0002), while no difference in euploid rates was observed among lower grade embryos (23.68 vs. 28.64%, p=0.64).

Overall, no significant difference in IR or OPR between day 5 and day 6 blastocysts was observed (72.41 vs. 70.91% and 71.42 vs. 65.63%, respectively). High grade embryos demonstrated improved IR compared to lower grade embryos (73.20 vs. 62.50%, p=0.047) but OPR were not significantly different (69.35 vs. 55.00%, p=0.10). Using regression logistic analysis to stratify embryos by morphologic grade and day of biopsy, high grade embryos biopsied on day 5 yielded similar IR and OPR compared to day 6 (72.29 vs. 73.47%, p=0.87, and 71.67 vs. 68.06%, p=0.57, respectively).

Limitations, reasons for caution:

The retrospective nature of this study is its major limitation and a prospective randomized design would be required to validate these results. Live birth outcomes were also not reported in this dataset.

Wider implications of the findings:

Contrary to previous studies, our results demonstrate that early blastocyst formation is only associated with higher euploidy rates among high-grade embryos in women <40-years-old. Early embryo development does not predict improved pregnancy outcomes in PGT-A cycles, but may be a useful embryo selection adjunct to morphologic grading in non-biopsied cycles.

Trial registration number:

Not applicable

O-201 Assessment of the impact of blastocyst morphology on the accuracy of non-invasive preimplantation genetic testing (NIPGT-A) utilizing culture-conditioned embryo culture medium combined with blastocoel fluid

S. Madjunkova[1], V. Kuznyetsov[1], R. Antes[1], G. Motamedi[2], R. Abramov[1], Z. Ibarrientos[2], A. Zaman[2], A. Novoselska[1], I. Kuznyetsova[2], C. Librach[3,4,5,6,7]

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Study question:
Does blastocyst morphology influence the concentration of cell-free DNA isolated from embryo culture-conditioned media(ECCM) and blastocoel fluid(BF), as well as the accuracy of non-invasive preimplantation genetic aneuploidy-testing(NIPGT-A) using this DNA?

Summary answer:
Cell-free DNA concentration in embryo culture-conditioned media (ECCM) and blastocoel fluid (BF) and the accuracy of NIPGT-A are not affected by blastocyst morphology grade.

What is known already:
Recently cell-free embryonic-DNA(cfeDNA) has been found in both BF and ECCM. These studies showed that the BF, ECCM or combined ECCM and BF could be potentially used for non-invasive aneuploidy and monogenic disease detection. However, this approach needs to be optimized before it could be routinely used clinically. This includes a better understanding of the nature of the cfeDNA releasing from human embryonic cells into BF and ECCM. Previous studies have reported that the concentration of cfeDNA correlates with apoptotic events. Therefore, lower quality blastocysts may have a higher rate of apoptosis and, as result, a higher quantity of cfeDNA.

Study design, size, duration:
This is a prospective study where we analysed the amount of amplified DNA and results from next generation sequencing (NGS) from 71 combined ECCM and BF samples and their corresponding 71 trophectoderm (TE) biopsy samples derived from fresh blastocysts (n= 71). The embryos were obtained from 21 patients undergoing PGT-A cycles between October 2018 and December 2018 with age ranging from 29-42 years (37.0 ± 3.3).

Participants/materials, setting, methods:
Embryos were zona-breached on d4 and transferred into fresh 15µl-droplets of Global-HP HSA medium. Morphology of Day5/6 full/expanded blastocysts was assessed based on the simplified SART scoring system: good (≥BB:-AA,AB,BA,BB) (n=35), and moderate/low quality (<BB:–AC,CA,BC,CB,CC) (n=36). Both ECCM and BF were collected as one NIPGT sample after pulse-laser collapse of the blast. The corresponding TE-samples were used as controls. Whole genome amplification (WGA) products were assessed with the Qubit3.0-Fluorometer, BioAnalyzer™ and VeriSeq™ PGS-NGS kit.

Main results and the role of chance:
WGA-DNA was detected in all NIPGT-A (n=71) and TE (n=71) samples. The mean amount of NIPGT-A sample WGA-DNA was lower for good quality blastocysts compared with moderate/low quality blastocysts 13.9 ± 0.9 ng/µl (5.1-30.0 ng/µl) vs. 15.7 ± 1.0 ng/µl (6.3-36.0 ng/µl), respectively, however the difference was not statistically significant. Average size of WGA-DNA fragments of NIPGT samples from good quality blasts was 746.9±42.6bp, and 736.9±26.9bp from moderate/low quality blasts. Informative NIPGT-A results were obtained from 98.6% TE biopsies (70/71) and from 88.7% NIPGT-A samples (63/71). Concordant rate for ploidy status (euploid/aneuploid) between NIPGT and TE biopsy samples for good (31/31, 100%) and moderate/low (32/32, 100%) quality blastocysts was not statistically different. NIPGT-A correctly determined the gender of embryos and aneuploidy in all NIPGT samples. Considering the amount of NIPGT-A WGA DNA from different blastocysts and the size of NIPGT-A WGA DNA fragments (not close to nucleosomal size), it is likely that cell apoptosis is not the only mechanism for DNA to be released into BF and ECCM from the ICM and TE. Therefore, other mechanisms for release of embryonic DNA, which are either passive or active or both, are likely involved.

Limitations, reasons for caution:
This study was limited to the number of samples. The amount of possible maternal contamination of non-invasive samples due to residual cumulus-corona radiata cells could be further decreased, and there is a room for additional optimization of the techniques for isolation, amplification and analysis of embryonic DNA from NIPGT-A samples.

Wider implications of the findings:
Our findings provide evidence that ECCM combined with BF has great potential for use with NIPGT-A. Also, NIPGT-A samples can be used as a backup source of embryonic DNA in case of inconclusive NGS results from the TE biopsy, potentially avoiding re-biopsy.

Trial registration number:
not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 56: FACTORS AFFECTING THE OVARY

Tuesday 25 June 2019 Haydn 3 17:00 - 18:00

O-202 Climacteric status at the age of 46: impact on metabolic outcomes in population-based study

S. Savukoski[1,2], H. Mäkelä[1,2], J. Auvinen[2,3], J. Jokelainen[4], K. Puukka[2,5], T. Ebeling[2,6], E. Suvanto[1,2], M. Niinimäki[1,2]

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Study question:
Does earlier climacterium impair cardiovascular risk-associated metabolic outcomes in women?

Summary answer:
Being climacteric at the age of 46 was associated with unfavorable changes in metabolic features, as compared to preclimacteric controls.

What is known already:
Several adverse changes in cardiovascular disease (CVD) risk factors during menopausal transition have been described in earlier studies: increased waist circumference, higher total cholesterol and low-density lipoprotein, altered concentrations of inflammatory markers, increased fat mass, central fat accumulation, and higher blood pressure. Many studies have suggested that EM (early menopause) and POI (premature ovarian insufficiency) increase the risk of cardiovascular morbidity.

Study design, size, duration:
A prospective cohort study including 2685 women of the Northern Finland Birth Cohort 1966 (NFBC1966). Cohort members have been followed since antenatal period with questionnaires and clinical examinations. This study was based on clinical examinations at the ages of 14, 31 and 46, on laboratory samples taken at the ages of 31 and 46 and on a follow-up questionnaire at the age of 46.

Participants/materials, setting, methods:
Study subjects were divided into two groups: climacteric cases and preclimacteric controls, by their menstrual anamnesis and FSH values at the age of 46. Between these groups, cardiovascular risk-associated anthropometric, hormonal and metabolic parameters were compared. The analyses were adjusted for age at menarche, parity, smoking, level of education, and physical activity. The possible effect of HRT (hormone replacement therapy) on outcomes was taken into account by including and excluding HRT users in respective sub-analysis.

Main results and the role of chance:
There were 381 climacteric and 2304 preclimacteric women in the study population. Women who were climacteric at the age of 46 had lower BMIs (body mass index) (P = 0.029), testosterone levels (P = 0.018) and FAIs (P = 0.009) at the age of 31. At the age of 46, they had less skeletal muscle (P < 0.001), a higher fat percentage (P = 0.016), higher cholesterol levels (total cholesterol [P < 0.001], low-density lipoprotein [LDL-C] [P < 0.001], high-density lipoprotein [HDL-C] [P = 0.022], and triglycerides [P = 0.008]) and higher alanine aminotransferase (ALT) (P=0.023) and gamma-glutamyltransferase (GGT) (P < 0.001) levels compared to preclimacteric women. Waist circumference, WHR, BP and hs-CRP levels did not differ between the groups. 111/381 of the climacteric women were using hormone replacement therapy (HRT). In sub-analysis that excluded the HRT-users, triglycerides, HDL-C and body fat percentage did not differ between the groups.

Limitations, reasons for caution:

Data concerning menstrual anamnesis and hormonal medications were based on self-reporting. In the sub-analysis excluding HRT users, the difference in body fat percentage and HDL-C became insignificant, even though the means of those parameters did not change, probably due to the smaller number of study subjects included in the analyses.

Wider implications of the findings:

Medical professionals should be aware of the impairing effect of climacteric transition on metabolic features, and that women who reach climacterium earlier may be more at risk for adverse long-term health consequences.

Trial registration number:

Not applicable

O-203 Does one size fit all? Female age impacts the number of oocytes retrieved to maximise fresh live birth rates: an analysis of 256,643 cycles

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Study question:

What is the number of oocytes retrieved to maximise live birth rates (LBR) after fresh assisted reproductive technology (ART) cycles for women of different ages?

Summary answer:

The maximal LBR was observed at 6-11, 11-16, 9-17 and 15-17 oocytes for women less than 30, 30-34, 35-39 and 40-44 years old, respectively.

What is known already:

Previous studies have shown that the number of oocytes retrieved is an important prognostic factor in fresh ART cycles. While some have advocated that large oocyte yields lead to increased LBR, others have suggested an excessive response results in impaired endometrial receptivity and decreased oocyte quality, leading to decreased LBR. High yields have also been associated with an increased risk of developing ovarian hyperstimulation syndrome (OHSS). Although previous studies have attempted to determine an optimal number of oocytes, most have not investigated the potential effect-modifying role of female age and whether this number changes for women of different ages.

Study design, size, duration:

This is a retrospective population-based registry study, with full ascertainment of cycles, using data extracted from the Australian and New Zealand Assisted Reproduction Database (ANZARD). The study included 129,023 women undergoing 256,643 fresh autologous cycles with at least one oocyte retrieved, performed from January 2009 to December 2015.

Participants/materials, setting, methods:

Live birth (LB) was defined as ≥1 liveborn baby at ≥ 20 weeks gestation. The secondary outcome was the incidence of ovarian hyperstimulation syndrome (OHSS) requiring hospitalisation. Generalised estimating equation (GEE) regression models were fitted to model LB with the number of oocytes as the primary independent variable and adjusted for confounders. An interaction term between the number of oocytes retrieved and female age was included to evaluate the potential effect-modifying role of age.

Main results and the role of chance:

Overall, the median age of patients was 36 years and the median number of oocytes retrieved was 8.

The number of oocytes remained a statistically significant predictor (P<0.001) of LB after adjusting for female age, parity and fresh cycle count. Compared with the reference category of 10-14 oocytes, the retrieval of 1-4 oocytes (adjusted odds ratio-AOR 0.46, 95% CI: 0.44-0.48) and 5-9 oocytes (AOR 0.85, 95% CI: 0.83-0.88) had lower odds of LB. At greater oocyte yields, the adjusted odds of LB reached a plateau followed by a decline when >20 oocytes were retrieved (15-19 oocytes: 0.96 (95% CI: 0.92-0.99), 20-24 oocytes: 0.73 (95% CI: 0.70-0.77) and ≥ 25 oocytes: 0.43 (95% CI: 0.40-0.47)).

Following stratification by female age, the predicted probability of LB was found to peak at different oocyte yields. Overall, the maximal LBR was observed at 6-11, 11-16, 9-17 and 15-17 oocytes for women less than 30, 30-34, 35-39 and 40-44 years old, respectively.

The risk of OHSS requiring hospitalisation increased significantly with the number of oocytes retrieved, being 1.1% with the retrieval of 15 oocytes and reaching 6.4% with the retrieval of ≥ 25 oocytes.

Limitations, reasons for caution:

The present study is subject to the potential presence of confounding bias due to unmeasured variables, including ovarian stimulation protocols. Due to the observational nature of the study, a cause-effect relationship between the number of oocytes retrieved and LBR should not be assumed.

Wider implications of the findings:

The results from this study highlight that ovarian stimulation protocols should be informed by female age. Younger women require less oocytes retrieved to maximise LBR in fresh cycles which in turn reduce OHSS occurrences.

Trial registration number:

not applicable

O-204 Ovarian Reserve and Exposure to Environmental Pollutants (ORExPo study)

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Study question:

to assess the association existing between environmental pollutants and AMH. The hypothesis is that exposure to pollutants may be associated to an age-independent decrease in AMH, suggesting decreased ovarian reserve.

Summary answer:

AMH serum levels are significantly affected by high levels of air pollution.

What is known already:

Many chemicals present within the environment, as well as natural and artificial components of our diet, have the potential to interfere with the physiological role of hormones, interfering with hormone biosynthesis, signalling or metabolism. In the last years AMH, a protein secreted by granulosa cells, has emerged as a reliable marker of ovarian reserve. The influence of age and smoking on AMH serum levels is largely accepted, although a clear effect of environment was not demonstrated so far.

Study design, size, duration:

A longitudinal, observational, retrospective, real-world big data trial was performed. All laboratory AMH measurements of women living in the area surrounding the city of Modena performed from January 2007 to October 2017 at the Central Laboratory of Modena Hospital were extracted and collected in a large database.

Participants/materials, setting, methods:

The study design did not foresee any inclusion/exclusion criteria, since the database was formed by the entirety of data produced by the laboratory. AMH was measured with commercial assay. A computing data warehouse was created, in which AMH data were connected to patients' age and residential address. The database was completed, including environmental data and considering the place where each patient lived for geo-localization. The environmental exposure considered daily particulate matter (PM) and NO2 values.

Main results and the role of chance:

1,463 AMH measurements were collected for 1,318 women. Serum AMH levels (mean 1.94 ng/mL and median of 0.90 ng/mL) were inversely related to patients' age (Rho=-0.437, p<0.001), although not related to age in patients younger than 25 years (Adjusted R- squared 0.068 p=0.055). On the contrary, AMH was inversely related to age after 25 years of age (Adjusted R-squared 0.120, p<;0.001).

AMH was inversely related to environmental pollutants, such as PM10 (Rho=-0.088, p=0.001), PM2.5 (Rho=-0.062, p=0.021) and NO2 (Rho=-0.111, p<0.001). This association was age–independent. No relationships were found between AMH and environmental temperatures.

Limitations, reasons for caution:

The study was based on a retrospective analysis. The assay for the AMH measurement has been revised during the study period and this may introduce variability in absolute values for the hormone

Wider implications of the findings:

It is well known that there is a large genetic component in the ovarian reserve, but other factors may influence the extent of the follicular pool such as environmental factors. Results of this study strongly suggest that environmental factors may modify the downward dynamics of AMH and ovarian reserve.

Trial registration number:

na

O-205 Chronic elevation of serum Progesterone levels and ovarian macrocysts in infertile and/or amenorrheic women: think of Cytochrome P450 oxidoreductase deficiency!

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Study question:

Can Cytochrome P450 oxidoreductase deficiency (PORD) be revealed lately in adult women through infertility and/or cycle disorder?

Summary answer:

PORD was biologically and genetically confirmed in 4 women with chronically elevated serum progesterone (P) levels who were referred for infertility and/or cycle disorder.

What is known already:

PORD is a rare autosomal recessive genetic disease most often revealed by ambiguous genitalia and/or skeletal abnormalities in infants and children. It is responsible for a decrease in the activity of P450 enzymes including *CYP21A2*, *CYP17A1* and *CYP19A1*.

Very few undiagnosed cases were described in adults where PORD was often misdiagnosed as Polycystic Ovary Syndrome (PCOS) or non-classical 21-Hydroxylase deficiency (NC-CAH). Little is known about fertility and pregnancy outcomes in such cases with late-onset PORD.

Study design, size, duration:

In this series we report 4 patients recruited in 3 different institutions between January 2017 and September 2018.

Participants/materials, setting, methods:

4 patients (P1, P2, P3 and P4) aged 19, 30, 36 and 38, respectively, were included in this study. They all underwent full clinical examination, basal hormonal assessment, ACTH stimulation test, pelvic ultrasound and sequencing of the *CYP21A2* and *POR* genes. When possible, a family study was performed.

In Vitro Fertilization (IVF) with conventional procedures followed by frozen embryo transfer was performed in P3 and P4, under glucocorticoid therapy

Main results and the role of chance:

All patients had irregular cycles or amenorrhea. None had hyperandrogenism. P1 had a supsicion of Shprintzen syndrome (craniosynostosis). Basal estradiol and total testosterone levels were low (<0.15 and <0.5 nmol/L, respectively). Serum FSH and LH levels were normal to moderately elevated. P and 17 hydroxyprogesterone (17OHP) levels were increased (3.11 to 35.23 and 5.7 to 11.6 nmol/L, respectively). The P, 17OHP, 21-deoxycortisol and corticosterone rises 60 min after ACTH stimulation were largely excessive (4.8 to 31.5-fold, 3.6 to 11.5-fold, 5 to 70-fold and 2.6 to 14.1-fold, respectively). Conversely, basal serum cortisol levels were low-normal, with weak rises under ACTH (1.2 to 2.6-fold). In all patients, pelvic ultrasound showed ovarian bilateral macrocysts. All 4 patients were homozygous or compound heterozygotes for *POR* gene (NM_000941.3) mutations. P1: c.859G>C, p.(Ala287Pro) homozygous ; P2: c.1648C>T, p.(Arg550Trp) and c.1826_1849del, p.(Leu612_Leu619del) ; P3: c.1324C>T, p.(Pro442Ser) and c.1249-1G>C ; P4: c.1825C>T, p.(Gln609*) and c.1859G>C, p.(Trp620Ser).Despite low E2 levels at ovulation triggering, pregnancy was obtained in P2 and P3 after IVF and frozen embryo transfer under hydrocortisone that was continued throughout pregnancy. P3 presented hypertension, preeclampsia and partial adrenal insufficiency. P2 had a normal twin pregnancy. The male (P4) and the twin female (P3) newborns were healthy.

Limitations, reasons for caution:

Hormonal assessment and ultrasound were done in 3 different institutions while genetic testings were done in 2 units.

Wider implications of the findings:

The diagnosis of PORD must be considered in infertile women with chronic anovulation, elevated P levels and ovarian macrocysts, after exclusion of NC-CAH. Genetic counseling must be provided to the patients, as beside reproductive issues, PORD may induce adrenal deficiency, disordered sex development, and skeletal malformations in the offspring.

Trial registration number:

0

SELECTED ORAL COMMUNICATIONS

SESSION 57: THE INFLUENCE OF ENDOMETRIOSIS ON PREGNANCY RATES AND METHODS FOR IMPROVEMENT

Tuesday 25 June 2019 Haydn 2 17:00 - 18:00

O-206 Implantation after embryo transfer (ET) of equal quality embryos is impaired in endometriosis compared to male subfertility patients: a matched case-control study

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Study question:

Investigating the uterine receptivity in IVF (In vitro fertilisation)/ICSI (Intracytoplasmic sperm injection) cycles following equal quality ET, compared between endometriosis patients and male factor subfertility.

Summary answer:

Implantation and subsequent IVF/ICSI outcomes in endometriosis showed to be significantly lower compared to outcomes after equal quality ET in a male subfertility population.

What is known already:

Endometriosis is a benign, gynaecological disease affecting approximately 10% of the female population, with prevalences peaking during reproductive life. Endometriosis is often accompanied by different degrees of adenomyosis, and can be visible in up to 79% of these women. Both pathologies are frequently associated with subfertility. Most studies focused on ovarian dysfunction and impaired oocyte and embryo characteristics, while no major impact on uterine receptivity is considered.

Study design, size, duration:

First fresh IVF/ICSI cycles resulting in a single embryo transfer on day 5, performed in Ghent University hospital, were included. In this subfertile population of 1053 subjects, 121 women with endometriosis and 361 patients undergoing IVF/ICSI because of male subfertility were eligible. 118 endometriosis cases were matched 1:1 to 118 male subfertile controls stratified by female age (±1 year), parity (±1 delivery) and embryo quality (identical ALPHA grading categories).

Participants/materials, setting, methods:

A retrospective matched case-control study was executed between 01/07/2015 and 31/08/2017 at the Department for Reproductive Medicine in Ghent University Hospital. Endometriosis was diagnosed by laparoscopy, transvaginal ultrasound or magnetic resonance imaging and was classified according the revised American Society for Reproductive Medicine (rASRM) score into grade I/II (34.7%) vs. grade III/IV (65.3%). Male subfertility was defined by the World Health Organization (WHO) criteria (5th edition).

Main results and the role of chance:

A multiple logistic regression (MLR) model was generated using the Enter method, including the matching variables and variables showing a significant

difference during univariate tests, including stimulation protocol, IVF/ICSI application and dysmenorrhea, to correct for possible confounding. A significant difference between the endometriosis and male subfertility population was found for positive HCG test on day 16 (p=0.047; OR=2.077;CI=[1.009;4.276]), clinical pregnancy rate (p=0.038;OR=2.265;CI=[1.048; 4.893]), ongoing clinical pregnancy rate (p=0.046;OR=2.292;CI=[1.016;5.173])) and live birth rate (p=0.043; OR=2.502;CI=[1.029;6.087]). However, undiagnosed co-existent adenomyosis can have an important attribution to the impaired IVF/ICSI outcomes observed in this study. Other factors contributing to the role of chance were minimized by matching and by controlling for confounding by applying MLR. Besides, case and control populations were highly comparable on characteristics of reproductive capability (AMH, age male, age female and number of previous IVF/ICSI cycles).

Limitations, reasons for caution:

Due to the tertiary hospital setting, a higher frequency of co-existent subfertility diagnoses in women with endometriosis can play an important role in the reported impaired IVF/ICSI outcomes. It needs further research to what extent a combination of adenomyosis with endometriosis is associated with the impaired fertility chances.

Wider implications of the findings:

These results are only valid for IVF/ICSI cycles due to possible positive/negative influences of the IVF/ICSI process, whereby results can not be extrapolated to the general population. This study confirmed that an altered uterine receptivity is present in women with endometriosis, complementary to the existing knowledge on ovarian disease.

Trial registration number:

not applicable

O-207 Short-term down-regulation of Gonadotropin-releasing hormone agonists does not improve pregnancy rate and live birth rate in frozen embryo transfer cycles in patients with endometriosis

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Study question:

Does Gonadotropin-releasing hormone agonists (GnRH-agonist) down-regulation increase pregnancy rate (PR) and live birth rate (LBR) in frozen embryo transfer (FET) cycles in women with endometriosis

Summary answer:

Down-regulating GnRH-agonist for less than three months does not increase the PR and the LBR in FET cycles in women with endometriosis including adenomyosis

What is known already:

According to the 2014 ESHRE guidelines for management of women with endometriosis, the prolonged down-regulation of GnRH-agonists prior the controlled ovarian hyperstimulation and the in-vitro fertilization treatment increases the chance of pregnancy in women with endometriosis.That is because of the suppression of hostile peritoneal microenvironments and improve the endometrium receptivity in women with endometriosis. However, whether the pre-treatment with GnRH-agonist prior the FET would increase the PR and LBR in women with endometriosis including adenomyosis is still unknown

Study design, size, duration:

This retrospective cohort study has been performed between January 2016 to January 2018. The selected female patients with endometriosis or adenomyosis have undergone frozen embryo-transfer (FET) at the Reproductive Medicine Center of The First Affiliated Hospital of Sun Yat-sen University

Participants/materials, setting, methods:

This study has included 911 patients with endometriosis or adenomyosis undergone 1286 FET cycles,376 cases have down-regulated GnRH-agonist ,while 909 cases were natural cycles (NC) or hormone replacement treatment (HRT) (NC/HRT group). In GnRH-agonist down-regulation group, patients were injected 3.75mg triptorelin14 days to 3 months before the use of estrogen. If the down-regulation duration is more than 1 month, patients have 3.75mg triptorelin every 28 days. SPSS 20 was used to analyze statistics

Main results and the role of chance:

Analysis of data includes the base line of age, basic FSH, BMI, embryo transfer number and the endometrial thickness did not found any significant differences.

Both In <38-year-old patients group and in ≥38-year-old patients group with endometriosis, PR and LBR in the GnRH-agonist down-regulation group and in NC/HRT group were found no statistically significance(52.44% and 42.38% VS 46.80% and 40.10%;P=0.147;0.612;23.5% and 20.0% VS 25.41% and 16.47%; P=0.589;0.349)

Both In <38-year-old patients group and in ≥38-year-old patients group with adenomyosis,PR and LBR in the GnRH-agonist down-regulation group and in NC/HRT group were found no statistically significance(43.61% and 27.53%VS 48.89% and 18.42%; P=0.589;0.349;12.5% and 5%VS31.80% and 22.22%;P=0.159;0.170).

We further analyzed data of the GnRH-agonist down-regulation groupusing median down-regulation duration (DRD). In patients with endometriosis, when DRD < 1 month, PR and LBR were 50.00% and 40.65%, respectively, when DRD ≥ 1month, PR and LBR were 45.78% and 37,30%, respectively. No significant difference was found as compared to NC/HRT group (PR=43.71%, LBR=37.07%). In patients with adenomyosis, when DRD < 1.5 month, PR and LBR were 40.30% and 22.41%, respectively, when DRD ≥1.5m, PR and LBR were 34.00%,15.56%. No significant difference was found as compared to NC/HRT group (PR=43.28%,LBR=19.64%)

Limitations, reasons for caution:

This is a retrospective cohort study, the selection bias is inevitable and the duration of GnRH agonist down-regulation was not uniform in each group. The total number of adenomyosisis 185 cycles which is relatively few and may cause false negative answer.

Wider implications of the findings:

Our research indicated that a short-term GnRH agonistspretreatment of less than three months prior FET does not increasethe PR and the LBR of women with endometriosis or adenomyosis. Whether long-term down-regulation of GnRH agonistswill benefit the outcome of FET is beyond studied

Trial registration number:

The study has no external funding. There are no competing interests.

O-208 Long-term gonadotrophin-releasing hormone agonist (GnRHa) therapy before in vitro fertilisation (IVF) for improving fertility outcomes in women with endometriosis: a Cochrane systematic review and meta-analysis

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Study question:

Does long-term GnRHa therapy versus no GnRHa pre-treatment before standard IVF in women with endometriosis improve reproductive outcomes?

Summary answer:

There is evidence of an increase in the clinical pregnancy rate with GnRHa therapy before IVF and no evidence of an increased complication rate.

What is known already:

Endometriosis has a negative impact on fertility through mechanisms that are currently not well established. Women with endometriosis often require

IVF to increase their chances of pregnancy. Studies have suggested that surgical treatment of endometriosis may improve IVF outcomes whereas surgical treatment to ovarian endometriomata could negatively impact ovarian reserve. Long-term GnRHa treatment, which is known to improve endometriosis pain via pituitary suppression, has been proposed as an alternative to surgical intervention. Studies have suggested that GnRHa use prior to IVF may also improve reproductive outcomes compared to standard IVF protocols although efficacy and safety have not been well established.

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 and CINAHL were conducted to January 2019 to identify relevant randomised controlled trials (RCTs). In addition, the following trial registers were searched for RCTs: www.clinicaltrials.gov and www.who.int/trialsearch/Default.aspx.

Participants/materials, setting, methods:

Participants: Infertile women diagnosed with endometriosis via laparoscopy-/laparotomy undergoing IVF or intracytoplasmic sperm injection (ICSI).

Intervention: GnRHa therapy for minimum 3 months before standard IVF/ICSI.

Comparison: standard IVF/ICSI only.

Two independent authors screened studies and extracted data. Risk ratios (RR) were calculated for dichotomous data and mean differences (MD) for continuous data, with 95% confidence intervals (CI). Heterogeneity was examined via the I^2 statistic. Primary analysis was conducted on data per woman randomised.

Main results and the role of chance:

Seven studies were included, incorporating 496 women. Eight studies are awaiting classification and two are ongoing studies.

On comparing long-term GnRHa therapy before IVF/ICSI to standard IVF/ICSI, there was no evidence of a difference in the primary outcomes of live birth rate (RR 7.31, 95% CI 0.97 to 55.29, one RCT, n = 67, I^2 not applicable) or complication rate (RR 0.64, 95% CI 0.16 to 2.57, one RCT, n = 116, I^2 not applicable).

There was evidence of an increase in the clinical pregnancy rate (RR 1.31, 95% CI 1.05 to 1.64, seven RCTs, n = 488, I^2 = 16%) and mean number of oocytes (MD 1.09, 95% CI 0.32 to 1.86, five RCTs, n = 333, I^2 = 73%) with GnRHa therapy. There was no evidence of a difference in the multiple pregnancy rate (RR 0.22, 95% CI 0.01 to 4.40, one RCT, n= 61, I^2 not applicable), miscarriage rate (RR 0.36, 95% CI 0.02 to 8.39, one RCT, n = 60, I^2 not applicable) or mean number of embryos (MD -0.20, 95% CI -1.68 to 1.28, one RCT, n = 119, I^2 not applicable). No studies were found that looked at ectopic pregnancy or fetal abnormality rates.

Limitations, reasons for caution:

The quality of some of the included studies as well as the significant degree of heterogeneity are the major limitations of this systematic review. In particular, live birth rate data are currently based on the results from one small single-centre RCT with wide confidence intervals.

Wider implications of the findings:

The clinical pregnancy rate appears to be higher with GnRHa therapy before IVF/ICSI in women with endometriosis. Further high-quality RCTs are required to determine whether this impacts on live birth rate and to more closely examine potential complications.

Trial registration number:

Not applicable.

O-209 Uterine administration of CXCL12 (SDF-1) increases pregnancy rates in mice with induced endometriosis

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[4]*Yale University - School of Medicine, Gynecology and Obstetrics- School of Medicine of Ribeirão Preto, New Haven, U.S.A.*

Study question:

Does CXCL12 (SDF-1) injected in the uterus, increase Bone Marrow Derived Stem Cells migration to the endometrium and improve fertility in mice with induced endometriosis?

Summary answer:

CXCL12 injection improves pregnancy rates in mice with endometriosis (30%increase), with no association between fertility improvement and a higher migration of BMDSCs to the uterus.

What is known already:

Stem cells migration plays a critical role in the pathogenesis of endometriosis.Using a mouse model of bone marrow transplantation (BMT) with marked cells (donor GFP+), we have previously demonstrated that the presence of endometriosis reduced the migration of BMDSCs to the uterus, with high concentration of these cells engrafting the lesion instead. Impaired BMDSCs cell migration to the uterus could explain the lower pregnancy rates seen in women with endometriosis. The administration of stem cell chemotaxic molecules to the uterus could potentially improve fertility.

Study design, size, duration:

Cross sectional study with 40 C57BL/6 female wild type mice that underwent BMT with BM from C57BL/6 GFP+ female donors. After 4 weeks the animals underwent surgery to induce experimental endometriosis or a sham control surgery (N=40). Each group was then subdivided to receive a single injection of CXCL12 (SDF-1) or placebo in both uterine horns, composing four study groups. Three weeks after surgery the animals were caged with proven males (C57BL/6) to assess fertility.

Participants/materials, setting, methods:

Endometriosis was induced by suturing endometrial tissue on the peritoneal cavity. Injection of CXCL12 or placebo in the uterine horns was done during a second laparotomy. For breeding, one male was placed with four females for four weeks. The reproductive outcomes were evaluated. Animals were euthanized in diestrus after delivery and embryo implantation markers were evaluated by immunohistochemistry (Hoxa11, $\alpha V\beta 3$ integrin and PR). Quantification of GFP+ BMDSCs in the uterus was also evaluated by immunofluorescence.

Main results and the role of chance:

Four study groups were composed: Endometriosis+CXCL12 (Edt+CX), Endometriosis+Placebo (Edt+Pl), Sham+CXCL12 (Sh+CX) e Sham+Placebo (Sh+Pl). The placebo treated endometriosis groups had lower pregnancy rates than the Sham groups (50% vs 100%; $p<0.0001$). There was a 28% increase in pregnancy rates in the endometriotic mice treated with CXCL12 (Edt-CX) (78%) in relation to the Edt-Pl group (50%) ($p<0,0001$). This increase was not associated to a higher engraftment of GFP+ BMDSCs in the uterus that persisted in the postpartum period ($p>0.05$); a transient recruitment prior to or during pregnancy was not evaluated. However, there was a significant increase in the abundance of PR and $\alpha V\beta 3$ integrin in the Edt+CX group when compared to Edt+Pl group ($p<0.0001$ and $p=0.005$, respectively).

Limitations, reasons for caution:

These experiments were performed in a murine model.

Wider implications of the findings:

CXCL12 (SDF-1) improves pregnancy rates of mice with endometriosis. The mechanism of action includes induction of molecules involved in endometrial receptivity. CXCL12 has potential as a therapeutic agent in women with endometrioisis and infertility.

Trial registration number:

Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 58: SQART IN TOXICITY. TARGETED GENE-EDITING AND MONOZYGOTIC TWINNING

Tuesday 25 June 2019 Haydn 4 17:00 - 18:00

O-210 Reprotoxicity tests performed by manufacturers of IVF disposable devices lack transparency and sensitivity

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Study question:
Can the reprotoxicity tests performed by manufacturers of disposable devices for IVF laboratories be trusted?

Summary answer:
Reprotoxicity tests used for IVF disposable devices lack standardization and sensitivity for detecting toxic substances. Hence, users should be cautious when choosing IVF devices

What is known already:
Many components used in IVF laboratories, such as culture media and disposable consumables may impair human embryonic development. Consequently, it is necessary to screen all reagents and materials used that will be in contact with gametes and embryos. Numerous reprotoxicity tests have been developed as objective quality control tools independent of clinical factors, e.g. Mouse Embryo Assay (MEA), Human Sperm Survival Assay (HSSA), Limulus Amoebocyte Lysate (LAL) test, biocompatibility and sterility tests. However, presently this field is not regulated, and there is no consensus defining the methodology used or the thresholds applied in quality control tests.

Study design, size, duration:
Through a questionnaire manufacturers of IVF disposable devices were contacted during the period November to December 2018 to explore and compare the methodology of the MEA, HSSA and LAL tests performed to ensure the overall quality of raw materials and final products used in IVF laboratories; we focused in specific on dishes, catheters, tubes, needles, pipettes, straws, syringes, and cones.

Participants/materials, setting, methods:
We approached 9 major IVF companies (Cook Medical, CooperSurgical, Biocare, Merck Serono, Vitrolife, CCD, BD, ThermoScientific, CryoBioSystem) and asked for details about methodology of the MEA, HSSA and LAL tests performed to validate the absence of toxicity in the IVF disposable devices. All specific parameters like mouse strains, number of embryos used, culture conditions (media, temperature, atmosphere, pH), subcontracting, and thresholds were registered and compared between the companies for the MEA, HSSA and LAL tests.

Main results and the role of chance:
There were significant differences in the methodology and thresholds of the reprotoxicity bioassays used by different manufacturers to test their IVF disposable devices. The MEA was performed either with frozen or non-frozen, 1- or 2-cell mouse embryos from different strains. Fifteen to 30 embryos were cultured either in micro-drops or in wells with or without protein supplementation under 5% or 20% O_2. The objectives varied from 70 to 80% of blastocysts or fully expanded blastocysts within 96h sometimes a quantifying the inner cell mass and trophectoderm cells. The HSSA was performed with donor sperm, using gradient centrifugation or swim-up at a final concentration from 4 to 60 M/mL. The sperm incubation with the tested device was performed during 24h at room temperature, 27°C or 32°C. The total motility or the progressive motility were assessed with a threshold of 70%. For LAL test, the extraction mode and the measurement method varied (kinetic turbidimetric or chromogenic tests). Importantly, being aware of the lack of sensitivity of some reprotoxicity tests, some manufacturers developed more sensitive tests using new technologies such as time-lapse or Computed Automated Sperm Analysis (CASA). More detailed results of this study will be presented at the conference.

Limitations, reasons for caution:
Until now 9 IVF industrial companies were approached in this study, however, our results call for the inclusion of a larger number of benchmarked manufacturers.

Wider implications of the findings:
Our study confirms the heterogeneity of the reprotoxicity tests performed by manufacturers when validating their IVF disposable devices. Professionals should call for and request standardization and a future higher degree of transparency as regards reprotoxicity testing from supplying companies.

Trial registration number:
Not applicable

O-211 Low frequency of exogenous DNA template-mediated repair of double strand breaks in human embryos

N. Marti Gutierrez[1], D. Liang[1], S.W. Park[2], A. Koski[1], H. Ma[1], H. Darby[1], Y. Lee[1], R. Ahmed[1], T. Hayama[1], C. Van Dyken[1], Y. Li[1], E. Kang[1], P. Amato[3], J.S. Kim[2], S. Mitalipov[1]

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[2]Institute for Basic Science, Center for Genome Engineering, Daejeon, Korea- South
[3]Oregon Health & Science University, Division of Reproductive Endocrinology, Portland, U.S.A.

Study question:
Repair frequency of DNA double strand breaks (DSBs) by non-homologous end joining (NHEJ) vs. homology directed repair (HDR) with synthetic DNA template in human embryos.

Summary answer:
Repair by NHEJ is the dominant pathway ranging from 95-99%, while the incidence of HDR is much lower (1-5%) when targeting homozygous loci.

What is known already:
CRISPR-Cas9 can be designed to target a specific genomic region in human embryos and induce a DSB. Embryos quickly respond to such DNA damage and repair DSBs by either NHEJ or HDR using the provided exogenous single-stranded oligodeoxynucleotide (ssODN) as a template. NHEJ is detrimental for most therapeutic purposes since it generates small indel mutations often leading to gene inactivation. In contrast, HDR provides means for repair of heritable mutations and thus is critical for development of germline gene therapy.

Study design, size, duration:
Pre-tested CRISPR-Cas9 was injected into human zygotes to target heterozygous MYH7 (g.15819 C>T; NG_007884.1) and homozygous wild-type (WT) MYBPC3 (g.9836, NC_000011.10) loci. A total of 88 injected zygotes were cultured for 3 days and individual blastomeres of cleaving embryos were isolated and target regions were analyzed by sequencing.

Participants/materials, setting, methods:
One sperm and seven oocyte donor volunteers were recruited. Selected sgRNA for both loci, Cas9 protein and ssODNs were co-injected into zygotes 18 hours after fertilization. Each blastomere from injected and control embryos was disaggregated, whole genome amplified and target regions genotyped by Sanger sequencing.

Main results and the role of chance:
DSBs at the heterozygous *MYH7* locus were repaired by NHEJ in 55% (50/91) of embryonic cells resulting in additional indel mutations, while the remaining 45% (41/91) of DSBs were repaired by gene conversion using the intact maternal MYH7 allele as a template. No samples with ssODN-mediated HDR were detected. Targeting of homozygous *MYBPC3* locus resulted in repair by NHEJ in 93% (110/118) of cases leading to indel mutations either in one or both alleles. HDR using the exogenous ssODN was seen in 7% (8/118) of cells with both alleles repaired in only 4 blastomeres.

These results demonstrate that gene conversion is one of the major repair pathways in human heterozygous embryos for the target region. However, HDR with ssODN is low or absent. Repair by NHEJ dominates when targeting homozygous loci.

Limitations, reasons for caution:
Human embryos demonstrate limited HDR via conventional ssODN and exogenous templates of different sizes and design should be tested.

Wider implications of the findings:
Our study provides prospects for development and application of future germline gene therapy

Trial registration number:
not applicable

O-212 Risk factors for Monozygotic Twinning (MZT) in Frozen Embryo Transfer (FET) Cycles

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[3]*Boston IVF, Embryology, Boston, U.S.A.*

Study question:

Is there a difference in the incidence of monozygotic twinning between individual infertility centers in FET cycles and, if so, can it be explained?

Summary answer:

Individual IVF centers are independent risk factors for MZT in FET cycles. There are likely multiple intrinsic factors contributing to this difference.

What is known already:

The incidence of MZT is greatly increased among infertility patients undergoing fresh IVF cycles compared to the general population (0.7-13% versus 0.45%, respectively), but the reason for this remains unclear. Several risk factors have been proposed and, of those, prolonged embryo culture (blastocyst transfer) appears to be the most significant independent risk factor for MZT. The use of FET cycles is increasing annually, coinciding with an increased utilization of preimplantation genetic testing for aneuploidy (PGT-A) and a hypothesis of a more physiologic uterine environment. There is limited data in the literature on risk factors for MZT in FET cycles.

Study design, size, duration:

This was a retrospective, multicenter, cohort study. Using an electronic medical record system (eIVF, PracticeHwy), clinical pregnancy data (confirmation of a gestational sac(s) and presence of a fetal pole with a heartbeat(s) on ultrasound) was obtained from single embryo transfer (SET) FET cycles from June 1st, 2004 to December 31st, 2016. 122,531 IVF cycles were identified in the database. Our analytic sample comprised 4,991 SET thaw cycles. Of these, 151 (3%) resulted in MZT.

Participants/materials, setting, methods:

Four large infertility centers in the U.S (both academic and private) were included in the study. Only cycles using homologous oocytes were included in the final analysis. Monozygotic pregnancies were identified when the number of fetal heart beats (FHB) seen on ultrasound exceeded the number of embryos transferred (≥2 FHB). A logistic regression analysis was performed to ascertain independent risk factors for MZT within our cohort.

Main results and the role of chance:

The mean (±SD) patient age in the cohort was 37±5 years. 216/4991 patients (4%) had a single cleavage stage embryo transfer and 4775/4991 (96%) had a single blastocyst transfer.

In the logistic regression we controlled for patient age at cycle start, day of embryo transfer, PGT-A performed, media type, freezing technique and clinic number.

Firstly, we examined the media type in which the embryos were cultured. There was not a significant independent difference in the incidence of MZT in those cultures in single step (2961/4991, 59%) or sequential media (2030/4991, 41%), (p=0.35).

Cryopreservation technique performed- slow freeze (5%) or vitrification (95%), also did not have an independent association with MZT rates (p=0.63).

However, when we examined incidence of MZT amongst different clinics in FET cycles we noted a significant difference (range 1.7-4.6%). This difference was significant and independent of all factors examined and listed above in 2 of the 3 clinics (p<0.01), with the largest clinic serving as a reference.

Of note, patient age at cycle start, day of embryo transfer and embryo biopsy for PGT-A were not found to be significant, independent risk factors for MZT in our cohort.

Limitations, reasons for caution:

A limitation of the study is its retrospective nature. We examined data from four infertility centers over a 12-year period. Undoubtedly there have been changes in practice over this period. Without access to genetic information confirming zygosity of pregnancies, it is impossible to be certain of the incidence of MZT.

Wider implications of the findings:

With increasing utilization of FET cycles, it behooves us to counsel patients on the risk of MZT. This risk varies between clinics and each individual clinic should continuously monitor their respective MZT rates over time and investigate potential causative factors in time periods where an increased incidence is seen.

Trial registration number:

Not applicable

O-213 Monozygotic splitting and multiple births following embryo biopsy: analysis of 149 182 single embryo transfers

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[3]*Christian Medical College, Department of Reproductive Medicine, Vellore, India*

Study question:

Does embryo biopsy increase the risk of monozygotic splitting following ART?

Summary answer:

The present study demonstrated an increased risk of monozygotic splitting with embryo biopsy and warrants validation by further research.

What is known already:

Multiple pregnancies resulting from transfer of multiple embryos is an iatrogenic adverse outcome following ART. Single embryo transfer (SET) has been promoted to avoid the risk of multiple pregnancies. Extended embryo culture has facilitated SET by allowing for better embryo selection. There has been evidence for increased risk of monozygotic twinning following blastocyst transfers. There have also been speculations that certain other ART procedures are associated with a risk of monozygotic twinning. With the expanding use of preimplantation genetic testing (PGT) it is of interest, whether interventions such as embryo biopsy affect the risk of zygotic splitting and monozygotic twinning.

Study design, size, duration:

Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of ART in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Recent data from 2001 to 2016 comprising a total of 149 182 SET cycles (4 544 following PGT and 144 638 following autologous IVF) were analysed.

Participants/materials, setting, methods:

Data on women undergoing SET either with embryo biopsy for PGT or autologous IVF between 2001-2016 were analysed to compare the risk of zygotic splitting and monozygotic twins. Zygotic splitting was established when two or more gestational sacs were identified on ultrasound following SET. Twins following SET were considered as being monozygotic. Logistic regression analysis was performed adjusting for potential confounders; female age, IVF or ICSI, day of embryo transfer and fresh or frozen transfers.

Main results and the role of chance:

The live birth rate following SET was 31.9% (95% CI 30.5% - 33.2%) for PGT cycles and 28.9% (95% CI 28.6% - 29.1%) following IVF. The incidence of zygotic splitting following PGT involving embryo biopsy was 2.4% (95% CI 1.7% - 3.3%) versus 1.4% (95% CI 1.3% - 1.5%) following IVF (without embryo biopsy). The incidence of monozygotic twins/ multiple births was 2.2% (95% CI 1.5% - 3.1%) with PGT versus 1.4% (95% CI 1.2% - 1.5%) with IVF. There was a significantly higher risk of monozygotic twinning/ multiples with embryo biopsy cycles versus IVF, unadjusted odds ratio (OR) 1.72, 95% CI 1.24 - 2.38. After adjusting for potential confounders such as female age category, IVF or ICSI, day of embryo transfer and fresh or frozen transfers, there was a trend towards an increased risk, adjusted odds OR 1.55, 95% CI 0.98 - 2.46.

Limitations, reasons for caution:

Limitations with observational data would apply to this study including residual confounding. This is the first study to address this study question, the results need validation by further studies, particularly given the low event rates with monozygotic twinning.

Wider implications of the findings:

The risk of zygotic splitting associated with embryo biopsy during PGT is useful information for clinicians to counsel patients.

Trial registration number:

Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 59: DECISION-MAKING AND ADJUSTMENT TO TREATMENT: BEFORE, DURING AND AFTER

Tuesday 25 June 2019 Strauss 1+2 17:00 - 18:00

O-214 Why are frozen oocytes still frozen? A five year follow up of women after social egg freezing

A. Tsafrir[1], T. Miron-Shatz[2], T. Eldar-Geva[1], M. Gal[1], A. Weintruab[1], J. Hyman[1], D. Goldberg[3], H. Levi[1], N. Dekel[1], O. Schonberger[1], N. Srebnik[1], K. Rotshenker-Olshinka[1], E. Zivi[1], R. Nabulsi[1], H. Holzer[1]

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[2] *Ono Academic College, Center for Medical Decision Making, Kiryat Ono- Israel, Israel*

[3] *Clalit Health Services, fertility clinic- Central district, Modi'in Illit, Israel*

Study question:

While social oocyte freezing is gaining popularity, it remains unclear why usage rates of frozen oocyte are low.

Summary answer:

Five years after freezing, most women either attempted conception naturally or with fresh oocytes, or chose to defer pregnancy due to lack of partner.

What is known already:

According to studies with short term follow-up periods, less than 10% of women who froze oocytes tried to conceive using those oocytes.

Study design, size, duration:

An anonymous internet based survey for all women who underwent social oocyte freezing in one unit in the preceding four to seven years. The participants completed the survey independently, responding to both open and closed - ended questions.

Participants/materials, setting, methods:

Patient records and laboratory data were used to create a comprehensive database of all cycles of social oocyte freezing performed in our unit since this procedure was first approved in Israel in 2011. Women treated during the years 2011-4 were contacted by phone between August 2018 and January 2019. Links for an online survey using a secure web application were emailed to women who agreed to participate in the study.

Main results and the role of chance:

87 women who underwent social oocyte freezing during 2011-4 were invited to participate, and 61 women responded (70%). The main reason for oocyte freezing was lack of interest in pregnany without a partner (85%). Age at freezing was 37.1± 2.3 (range 30-41) and 42.5±2.6 (range 35-48) at time of participation in the study. Time from first cycle of oocyte freezing to participation was 5.4±1.2 years (range 4-7).

During the follow–up period, 39 women (64%) tried to conceive, with husband or partner (66.6%), or with donor sperm (33.3%). Of these, 22 women achieved a live birth (36% of all respondents, 56% of those who tried to conceive). 13 respondents reported using their frozen oocytes (21%), and 4 had a livebirth or ongoing pregnancy using those oocytes (30% of women who used the frozen oocytes). The reasons for not using frozen oocytes included: becoming pregnant spontaneously or with fresh oocytes (37.5%), lack of interest in pregnany without a partner (33.3%), still trying without the frozen oocytes at time of the study (18.8%), or lack of interest in pregnancy for other reasons (10.4%).

Limitations, reasons for caution:

Potential bias regarding women who chose to respond, who possibly had better reproductive outcomes than those who did not. Follow-up period and age of participants do not reflect full potential of natural fertility and of frozen oocytes since most participants did not use their oocytes at time of study.

Wider implications of the findings:

The current study could help fertility professionals provide improved counselling and advice to women considering fertility preservation options.

Trial registration number:

not applicable.

O-215 Nudging to promote elective single embryo transfer (eSET): A randomized controlled experiment

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[2] *Chulalongkorn University, Population Studies, Bangkok, Thailand*

Study question:

Our study evaluates the effect of a novel approach based on a behavioral economic theory—nudging— to promote elective single embryo transfer (eSET).

Summary answer:

Nudging technique can significantly increase preference for eSET regardless of twin preference, gender and age. Therefore, nudging can be an effective intervention to promote eSET.

What is known already:

Multiple pregnancies are associated with both maternal and fetal complications. Elective single embryo transfer (eSET) is an effective strategy to reduce multiple pregnancies; however, uptake is low. Traditional interventions (e.g., education or counseling) have not been effective.

Study design, size, duration:

A randomized controlled experiment. There were 56 participants in the control group and 52 participants in the intervention (nudging) group. We conducted the study from September to November 2018.

Participants/materials, setting, methods:

Infertile patients attending an information session at a university, teaching hospital in Bangkok, Thailand were randomized into two groups. In the control group, participants simply reported their preference for either eSET or double embryo transfer (DET). In the intervention group, eSET was presented as a default choice and participants were given a chance to opt-out.

Main results and the role of chance:

108 out of 110 participants completed the experiment (response rate = 98.1%). There were no differences between the control group and intervention group in regards to twin preference, gender, age, education and income, $p > .05$ for all characteristics.

Overall, preference for eSET was significantly higher in the intervention group compared to the control group (59.6% vs 19.6%), $p < .001$.

Subgroup analyses revealed that preference for eSET was significantly higher in the intervention group across all subgroup-comparisons. First, 46.7% vs. 10.5% in those preferring twins, $p = .001$ and 76.2% vs. 43.8% in those preferring singletons, $p = .047$. In regards to participants' gender, 70.8% vs. 24.0% in male participants, $p = .001$ and 50.0% vs. 16.1% in female participants, $p = .006$. Lastly, 57.1% vs. 17.9% in participants 36 years old and younger, $p = .005$ and 61.3% vs. 21.4% in participants 37 years old and above, $p = .002$.

Limitations, reasons for caution:

The nudging effect might be influenced by various factors and should be further tested in other contexts and settings.

Wider implications of the findings:

Nudging can be used effectively in reproductive medicine and healthcare settings. It can be an effective strategy to promote more efficient and safer care.

Trial registration number:

not applicable

O-216 Dealing with endometriosis and infertility: Partners matter as interrelation influences stress and sexual satisfaction

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Study question:

Investigate the interrelations in couples with endometriosis and/or infertility in matters of stress and sexuality.

Summary answer:

How couples deal with the impact of endometriosis pain and infertility interrelates with the partners stress and sexual satisfaction.

What is known already:

Endometriosis is a common gynecological disorder, often associated with severe dysmenorrhea, pelvic pain and dyspareunia. It is the major cause for female infertility and has a high impact on daily life as well as sexuality. Partnership quality positively influences the course of various diseases. Furthermore, a positive relationship between partnership and perceived stress can be found in reproductive treatment, with resilient partnerships being able to buffer high levels of stress associated with treatment. However, studies focusing on the male part in couples with endometriosis and wish for a child are rare, and even less about the reciprocal influences in couples.

Study design, size, duration:

A quantitative study was devised, approved by the Ethics Committee of the Heidelberg Medical Faculty and Medical University Innsbruck (MUI). The study was conducted from September 2016 to August 2018 at the Department of Gynecological Endocrinology and Fertility Disorders, Heidelberg University Hospital and from June 2017 to August 2018 at the Department of Gynecological Endocrinology and Reproductive Medicine, MUI with n=322 participants, resulting in n=140 couples.

Participants/materials, setting, methods:

Participants completed a questionnaire with 88 items regarding endometriosis, wish for a child, partnership, sexuality, pain, stress, psychosocial well-being and sociodemographic items. All women undergoing laparoscopy during their treatment at Heidelberg University Hospital and MUI and their partners were invited to participate in the study. Data were analyzed using the Actor-Partner-Interdependence Modell, which stipulates that one person's behavior does not only affect him-/herself (actor effect) but also his/her partner (partner effect).

Main results and the role of chance:

In total, n=140 couples (women: mean age: 32.6 years; men: 36.2 years) participated in the study. Mean partnership duration was 8.5 years. Of all participants, 26.5% already had children, 79.4% had a present wish for a child, on average for 3.7 years. Of all subjects, 63.6% had high school or university graduation. EM was clinically confirmed in N=104 women (74.3%). All analyses have been controlled for participant's age, duration of endometriosis and wish for a child, sex frequency and existing children.

Women and men were asked about the impact of women's endometriosis pain (IEP) in their everyday life (e.g. leisure time). Significant partner effects were evident: High stress scores in women led to high IEP in men (p=.012) and reciprocally (p=.049). Regarding sexuality, less sexual satisfaction in women led to a higher IEP in men (p=.035).

Concerning wish for a child, participants reported about their cognitions in terms of hopelessness and acceptance. The more men indicated cognitions of acceptance, the less stress women reported (p=.024). Hopeless cognitions in women led to more stress in men (p=.002) and reciprocally (p=.034). Furthermore, hopeless cognitions in women were interrelated to less sexual satisfaction in men (p=.021).

Limitations, reasons for caution:

Generalization of findings is limited due to the retrospective study design. Furthermore, participants in this sample had an above-average high educational background.

Wider implications of the findings:

The male partner should be focused when counseling or treating women with endometriosis and/or infertility. Thus talking about and improving sexual satisfaction may hold large benefits for dealing with endometriosis and infertility. Furthermore, stress reducing techniques for both partners should be addressed and enhanced actively.

Trial registration number:

Not applicable

O-217 How do people adjust to not realizing their parenthood goals? A test of the Three Tasks Model of Adjustment to Unmet Parenthood Goals (3TM)

S. Gameiro[1]

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

Study question:

Can the Three Tasks Model (3TM) explain the mental-health and well-being of individuals who do not realize their parenthood goals?

Summary answer:

The 3TM shows good explanatory ability, reflected in good model-fit to the data indexes and high proportions of explained variance in mental-health and well-being.

What is known already:

More and more people end their reproductive life without realizing their parenthood goals (none/less children than desired). This is associated with worse mental-health and well-being, with some individuals reporting never fully recovering. The Three Tasks Model of Adjustment to Unmet Parenthood Goals (3TM, Gameiro & Finnigan, 2017) predicts that people will try to adjust to this loss by creating meaning of their situation (meaning-making), integrating the loss in their identity and life-style (acceptance), and pursuing other life-goals (pursuit of new life-goals), and that these efforts will lead to better mental-health and more positive feelings (hedonic well-being) and life-fulfillment (eudaimonic well-being).

Study design, size, duration:

Cross-sectional online survey. To reach this non clinical heterogeneous sample the survey was advertised via fertility charities (Fertility Network, Resolve, Fertility Matters Canada, NISIG Ireland and Fertility New Zealand), forums (Fertility Friends, The Not Mom, Net Mums, Mums Net, and Health Unlocked) and Facebook and Google adds. Participants were given the chance to win one of four £50 vouchers.

Participants/materials, setting, methods:

Participants were individuals who had not met their parenthood goals and were not undergoing fertility treatment. We used well-validated and sound questionnaires (except when otherwise specified) to measure relevant 3TM variables. Determinants were age (in years), having stopped trying to conceive (no, yes), importance of parenthood (1 item scale), and perceived social-support; mediators were meaning-making (general efforts, positive re-framing), acceptance, and pursuit of new goals; and outcomes were mental-health and hedonic and eudaimonic well-being.

Main results and the role of chance:

Final sample were 420 individuals with unmet parenthood goals. 860 individuals accessed the survey and 516 (60%) met the inclusion criteria, but 86 (18.6%) were excluded because they did not fill any of the questionnaires. Average age was 35. Only 2 participants (0.5%) were men, the majority were in a relationship, had university education and were employed.

A test of the 3TM using Structured Equation Modelling (path analysis with maximum likelihood estimation) revealed a good model fit to the data ($X^2(18) = 44.936$, $p < .001$, CFI = 0.979, RMSEA = .060 [.038, .082]). The model explained 42%, 42% and 35% of variance in mental-health, hedonic and eudaimonic well-being, respectively. Older people reported higher acceptance (b=.119). Those who had stopped trying to have children and perceived more social-support reported higher positive re-framing (b=.192, .337), acceptance (b=.289, .243) and pursuit of new goals (b=.271, .332). Importance of parenthood was associated with more meaning-making general efforts (b=.139), less acceptance (b=-.291) and pursuit of new goals (-.198). Positive re-framing was associated with better eudaimonic well-being (b=.157), acceptance with higher hedonic well-being (b=.281) and pursuit of new goals with better mental-health and hedonic and eudaimonic well-being (b=.273, .223, .410).

Limitations, reasons for caution:

It was not possible to calculate the survey response rate. Individuals more affected by their inability to realize their parenthood goals might be more willing to participate, which may result in an under estimation of this population ability to adjust to this life challenge.

Wider implications of the findings:

The 3TM is useful to explain adjustment to unmet parenthood goals. Individuals who construct positive meanings of their situation experience higher life-fulfillment, individuals who are better able to accept it experience more positive

feelings, and individuals who pursue new life-goals experience better mental-health, more positive feelings and life-fulfillment.

Trial registration number:

not applicable

INVITED SESSION

SESSION 60: COCHRANE SESSION

Wednesday 26 June 2019 Haydn 1 08:30 - 09:30

O-218 Outcome reporting in fertility trials: what a mess! Why we need a core outcome set

J. Wilkinson[1]

[1] *University of Manchester, Centre for Biostatistics, Manchester, United Kingdom*

Abstract text

Outcome reporting in fertility trials: what a mess! Why we need a core outcome set

Recent reviews of fertility research have revealed massive inconsistencies in how outcomes are measured and reported. In our 2016 study of outcome reporting in fertility RCTs, we identified 361 numerators (for example, number of oocytes, clinical pregnancy and live birth) and 87 denominators (for example, per cycle started, per transfer procedure, per egg collection), resulting in 815 distinct combinations. We also observed that a variety of definitions were in use for many of the numerators, compounding the problem.

This heterogeneity undermines the evidence base in a variety of ways, several of which remain generally underappreciated. Most obviously, variation in outcome measurement hampers the comparison and combination of trials in meta-analysis. Recently published work from our group highlights the consequences of this for the evaluation of reproductive medical interventions; we found that meta-analyses contained in systematic reviews published by Cochrane Gynaecology and Fertility generally contained too few participants to detect anything other than implausibly large treatment effects, with results generally being too imprecise to determine which intervention was better. This concern has practical implications, since uncertainty around the effectiveness of interventions leaves room for the exploitation of vulnerable patients. Core outcome sets represent an important part of the solution to underpowered treatment comparisons.

A lesser appreciated point is that the choice of outcome measure can undermine the validity of an individual study. Many of the outcome measures commonly used in fertility trials discard the benefits conferred by randomisation. This results in biased observational comparisons being held in higher regard than they might warrant. Examples include live birth rates calculated per embryo transfer, miscarriage rates calculated per pregnancy, oocyte yields calculated per egg collection, and perhaps neonatal outcomes such as birthweight, which are necessarily only calculable for those women who have live births. I will discuss ongoing work where we attempt to quantify the implications of using these measures for the conclusions we make, and will outline a number of imperfect solutions. Core outcome sets, accompanied by clear guidance regarding presentation and analysis, offer one way to reduce these ubiquitous errors.

O-219 COMMIT: Developing a core outcome set for infertility research

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Abstract text

Despite the escalation in research activity and an exponential rise in published papers, many of the fundamental questions in infertility remain. Potential treatments for infertility should be evaluated in randomised controlled trials. The Priority Setting Partnership for Infertility (www.phc.ox.ac.uk/infertility) has prioritised unanswered research questions relating to the evaluation of potential treatments for infertility. However, complex issues including a failure to consider the perspectives of different stakeholders when designing trials, variations in outcome measures, and outcome reporting bias, could undermine the translation of future infertility research into clinical practice. Such research waste represents a substantial barrier in improving the outcomes of people with infertility.

The challenges of poor outcome selection, measurement, and reporting can be addressed by developing, disseminating, and implementing a core set of outcomes and outcome measures which would be common to future infertility research. An international initiative, embed within the Cochrane Gynaecology and Fertility group, has developed a core outcome set for infertility using robust consensus science methods, engaging 265 health care professionals, 58 researchers, and 55 patients representing 55 different countries.

Embedding the infertility core outcome set within future randomised trials, systematic reviews, and guidelines could make a profound contribution to advancing the usefulness of research to inform clinical practice, enhance patient care, and improve outcomes.

INVITED SESSION

SESSION 61: THE UTERUS AND THE SURGEON

Wednesday 26 June 2019 Haydn 3 08:30 - 09:30

O-220 The persisting challenge of Asherman's syndrome

M.H. Emanuel[1]

[1] *University Medical Center, Gynaecology and Reproductive Medicine, UTRECHT, The Netherlands*

Abstract text

The persisting challenge of Asherman's syndrome

Dysmenorrhea or pain in Asherman's Syndrome (AS; intrauterine adhaesions [IUA] present after an intrauterine pregnancy reated procedure leading to menstrual disorders and/or subfertility) related to menstruation is often explained by the theory that myometrial contractions try to evacuate blood trapped in the uterine cavity. However in patients with AS a haematometra is uncommon. Although Asherman and others have suggested that the endometrium becomes unresponsive when outlet adhesions are present, there is remarkably little objective evidence to support this. This lack of haematometra with occlusion of the lower (isthmic) part of the uterine cavity is in contrast to other types of lower congenital genital tract occlusion such as an imperforate hymen, a partially non-communicating bicornuate or septate uterus or a partially non-communicating hemi-vagina. Even occlusion at the external cervical os after cervical surgery, often leads to haematometra, haematocolpos and haematosalpinx on the long run. Between 2003 and 2013 a first-trimester pregnancy procedure preceded AS in 371 women (58.2%) and caused adhesions of grades 1–2 (ESGE/ESHRE). In 243 (38.1%) women, a postpartum procedure caused IUA of grades 3–5. Hysteroscopic adhaesiolysis with fluoroscopy guidance was successful in normalizing uterine cavity anatomy with at least one open tubal ostium in 606 (95%) women, and restoration of menstrual blood flow occurred in 97.8%; IUA spontaneously recurred in 174 (27.3%) of these cases. High grades of adhesions were predictive of a higher chance of spontaneous recurrence of adhesions. Representive endometrial biopsies were taken immediately after restoring the normal uterine cavity anatomy during hysteroscopical adhaesiolysis. At the same time blood samples were taken to determine the serum levels of follicle stimulation hormone, luteinizing hormone, estradiol and progesterone. All endometrial biopsies, taken from women with amenorrhea as a result of AS had microscopic characteristics mainly seen during the secretorial phase. Looking at the endocrinology in these randomly in the cycle taken biopsies 1/3 of them were actually taken during the follicular phase and only 2/3 during different stages of the luteal phase. This phenomenon which we named: "endometrial secretory arrest" in AS seems to prevent haematometra and severe dysmenorrhea in most patients. Future research should focus further on the pathophysiology of this typical clinical situation (mediators, immunology, gene expression, microbioma...).

O-221 Where are the limits of surgical treatment of uterine anomalies?

INVITED SESSION

SESSION 62: SPERM DNA MATTERS

Wednesday 26 June 2019 Haydn 2 08:30 - 09:30

O-222 Sperm DNA a clinically useful prognostic?

O-223 Finding the best sperm

N. Garrido[1]

[1]*IVI Foundation- Instituto de Investigación Sanitaria La Fe- Avenida Fernando Abril Martorell- 106 - Biopolo- Torre A- Planta 1ª, Innovation, Valencia, Spain*

Abstract text

The basic sperm analysis has limited predictive value in the achievement of success in either natural or assisted conception, in part because several non-measured molecular factors within the sperm cells are crucial to succeed.

There are upper limits of assisted reproduction success. If we look carefully to the results exhibited by all ART programs, we may find two similar patterns among them: first, often a couple needs several attempts to get a child. Second, not every patient succeeds.

In some cycles, there may be no embryos to transfer or they are unable to implant. Another subsequent cycle ends up in a child, even when using the same gamete's combination. Same gamete providers-different cycles-different results.

This, together with the fact that in an ART procedure, gametes are provided by the patients and afterwards the laboratory intervenes in the preparation and selection, lead us to wonder how much this intervention affects the reproductive performance of a couple. Regarding spermatozoa, you may find from thousands to millions within an ejaculate, they are different, they behave differently, and their adequate selection may be key for reproductive success. Using one specific spermatozoon leads oocyte fertilization, develops to a good quality embryo who implants, and a child is born. Choosing another instead, may result in an embryo that is arrested or is unable to implant. . .. even within the same ejaculate. . . even within different ejaculates from the same man.

Within an ejaculate, you may find that every single sperm is theoretically unique from the genetic viewpoint. The number of genetic combinations possible exceed the number of available spermatozoa even in the best ejaculates. Their genetic, and subsequently their molecular traits are exclusive, as expected after combining maternal and paternal chromosomes during spermatogenesis, and the DNA crossover between homologous regions. This has been previously confirmed sequencing and comparing single sperm DNA, and this uniqueness makes people have different children depending on the sperm that fertilizes the oocyte. But most importantly, they can lead you to success or failure.

When sperm is selected by an operator, they may have in their hands the possibility to choose, and their selection must be made according to the best criteria and available evidence. Most importantly, several millions of spermatozoa will be ruled out (unlike oocytes, here is where the key of sperm selection potential resides).

A good sperm selection protocol may change the whole picture and improve the entire embryos' cohort quality. Good sperm-Good embryo. Bad sperm-Bad embryo.

So far, several sperm phenotypical and molecular markers of fertility have been described as being related with reproductive success: DNA integrity, membrane charge, apoptotic traits, hyaluronic acid receptors, platelet activating factor, and a long etc.., and some can also be used to select sperm individually for using them in assisted reproduction techniques. Nevertheless, robust evidence for significant improvement in reproductive performance after its application is scarce, or almost inexistent.

From ultrahigh magnification to magnetic activated cell sorting, through physiological ICSI, hypoosmotic swelling tests, electrophoresis, microfluidic devices, birefringence, etc., several papers and research projects have aimed to separate ejaculates into two sperm populations or more, which contain the good and the bad. Available information is still limited, coming mainly from the developers of each technique, without enough evidence and non-contrasted by other independent groups.

First it´s a matter of finding and defining the molecular traits that identify the best sperm to be selected (or the worse to be removed), those strongly linked with reproductive results, and then developing "sperm-friendly" and cost/effective selection techniques, to lead us to move from auto or subjective sperm selection towards smart or objective sperm selection ultimately improving reproductive results.

INVITED SESSION

SESSION 63: IFS-ISAR EXCHANGE SESSION - CONTROVERSIES TO CONSENSUS IN RECURRENT IMPLANTATION FAILURE

Wednesday 26 June 2019 Haydn 4 08:30 - 09:30

O-224 Probing deep into RIF

O-225 Maternal factors in RIF

O-226 Improving embryo quality

K. Jain, M.D.[1]

[1]*KJIVF & LAPAROSCOPY CENTRE, department of reproductive medicine, DELHI, India*

Abstract text

Recurrent Implantation Failure- Improving Embryo Quality

Implantation a rate limiting factor in ART cycles & is a cause of concern. There is no consensual definition of RIF . common accepted definition is failure to achieve conception in three cycles in spite of transferring good quality embryos. Successful implantation depends on synchronization of multiple factors like immune quality of embryos, optimal culture conditions, receptively of endometrium & maternal system. Selecting good quality embryo & putting them at appropriate time is the mainstay of treatments in RIF.

Factors incriminated in RIF are embryo quality are suboptimal conditions compromising development, chromosomal abnormalities, zona hardening & improper timing and technique of embryo transfer.

A large proportion of patients of RIF show a high no of chromosomal anuploidy and other structural defects[maternal or paternal origin]. Routine morphological evaluation cannot distinguish between euploid or aneuploid embryos. High DNA fragmentation of sperms causes decreased implantation and chromosomal abnormality and need identification.

strategies to improve and select good quality euploid embryos to improve implantation are

1. optimizing culture condition to get a viable blastocyst to help in selecting embryos with best implantation potential. However reaching up to blastocyst stage may be challenging in elderly and poor responders because of fewer embryos and increased risk of aneuploidy.
2. Various stimulation protocols have been suggested to improve the outcome in RIF however due to lack of high quality evidence, none of the protocol seems superior to other in improving outcome in RIF.
3. Assisted hatching- zona hardening due to prolonged culture resulting in to failed implantation may be another cause of RIF. Assisted hatching has been proposed as a measure to correct the problem and thus increasing pregnancy rates in RIF. However role of assisted hatching remains controversial due to lack of any conclusive evidence.
4. Co- culture system Co- culture system with granulose cell, Homologus endometrial cells have been proposed to improve pregnancy rates in RIF however there role remains controversial.

5. PGS/ PGT- A; High order auploidy and chromosomally abnormal embryo. Leading to failed implantation is important factor in RIF. Electing euploid embryos by PGS/ PGT- A is logical choice to improve pregnancy rates in RIF. Biopsy at blastocyst stages is suggested to select euploide embryos in comparison to D3 biopsy. However there are multiple reasons for in efficiency of PGS in improving the pregnancy rate significantly, Using NGS/ array CGH is preferable than FISH to select chromosomally normal embryo.
6. Cytoplasmic transfer; procedure is still experimental and requires validation for clinical practice.
7. Newer methods for embryo selection of good quality embryo by using time lapse technique has been suggested however no robust data exists to suggest is as beneficial in RIF. When compared to conventional system analysis of spent culture media for byruvate, glucose consumption and amino acid turnover. All of them correlated with development of blastocyst viability, can be used to select the good quality embryo. Also measurement of specter profile of spent culture edia reflection of oxidative stress shows a good correlation with pregnancy outcome.

Conclusion: RIF poses a great challenge for both clinician and embryologist there are multiple measures which have been suggested to improve and select a good quality embryo to improve pregnancy outcome. Some of them like improving culture condition and blastocyst culture are applicable to all cases while rest of measure can certainly be used in selected group of patients.

O-227 Immunological Issues- Any evidence?

G. Devi[1]

[1] *Senior consultant-Gouri Hospitals ltd- Director-Ridge ivf pvt ltd, Reproductive medicine, Delhi, India*

Abstract text

RIF is defined as the failure to achieve clinical pregnancy after the transfer of two good quality embryos , in at least three fresh or frozen IVF cycles/embryotransfers (6 embryos in total) or in at least two egg donations(i.e., 4 embryos in total)

Despite growing new evidence of the involvement of immunological alterations on pregnancy outcome. There are no existing evidence-based guidelines focusing on immunological factors of RIF

Causes of RIF can be Maternal or Embryonic.One of the important maternal factors is immunologic.

Pathophysiological role of immunological factors in RIF:

1. Antiphospholipid syndrome:Inclusion of RIF as clinical criteria for APS has been proposed since aPL are pathogenic antibodies and the implantation failure could have analogous pathophysiology to that of an early miscarriage
2. Expansion of peripheral natural killer cells
3. Deregulation of uterine natural killer cells has a controversial role
4. Allorecognnition: MHC molecules and NK cells receptors: Successful implantation depends on a fine balance of NK cell activation and inhibition which might be influenced by the KIR gene frequencies of the patients and their partners
5. Untreated hypothyroidism:women with hypothyroidism have decreased chances of achieving pregnancy and optimal level of TSH prior to and during controlled ovarian hyperstimulation for IVF should be determined.
6. Cytokine imbalance

The Food and Drug Administration (FDA) provides pregnancy safety categories for immunomodulator and immunosuppressant therapies. IVIG, corticosteroids, tacrolimus and intralipid therapy are category C medications. Tacrolimus has demonstrated safety for both the mother and foetus in a number of studies. The TNFα inhibitors such as etanercept and adalimumab are classified as FDA Category B reflecting limited evidence in a small number of pregnant women and women of childbearing age, where these drugs have not shown any increase in the frequency of mal- formation or other direct or indirect harmful effects on the human foetus. Nevertheless, immunomodulator and immunosuppressive therapies for RIF and RPL have already been introduced to clinical practice with promising results.

- To conclude, defining the evidence-based immunological studies is essential for the appropriate evaluation and management of couples with RIF
- Immunological as well as haematological or hormonal factors play a relevant role in successful gestation as has been highlighted for the case of women with RM
- There is an urgent need of further validating these data with clinical trials and of standardizing both the evaluation and interpretation of immunological tests
- Research targeted to come up with better answers to improve the outcome of pregnancy and the life-quality of couples undergoing IVF is needed
- This will also impact the IVF-related costs

SELECTED ORAL COMMUNICATIONS

SESSION 64: MICROMANIPULATION REVISITED

Wednesday 26 June 2019 Mozart 10:00 - 11:45

O-228 ICSI does not offer any benefit over conventional IVF across different ovarian response categories: a European multicenter analysis

P. Drakopoulos[1], J.A. Garcia-Velasco[2], E. Bosch[3], C. Blockeel[1], M. De Vos[1], H. Tournaye[1], N.P. Polyzos[4]

[1] *UZ Brussel, Center for Reproductive Medicine, Jette- Brussels, Belgium*
[2] *IVI- RMA Madrid, Reproductive medicine, Madrid, Spain*
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[4] *Dexeus University Hospital, Department of Obstetrics- Gynecology and Reproductive Medicine, Barcelona, Spain*

Study question:

Does ICSI improve reproductive outcomes compared with IVF in different ovarian response categories?

Summary answer:

There is no advantage of ICSI over IVF as insemination method for non-male factor infertility, irrespective of the ovarian response.

What is known already:

Conventional IVF and ICSI are two techniques used to inseminate oocytes in-vitro. Although ICSI was originally developed for male factor infertility there has been an increase in the use of ICSI for all causes of infertility. The rationale that ICSI is associated with a higher likelihood of fertilization and a potentially increased number of embryos is controversial. Furthermore, ICSI still remains the first choice in many centers in case of low oocyte yield. There are currently no patient-based data on the effectiveness of ICSI compared with IVF according to the number of oocytes retrieved in patients with non-male factor infertility.

Study design, size, duration:

This is a large multicenter analysis using individual patient data, conducted in 15 tertiary referral hospitals across Europe. The study included the first cycle of all patients undergoing ovarian stimulation for IVF or ICSI in a GnRH antagonist protocol from 2009 until 2014. Each patient contributed with one cycle.

Participants/materials, setting, methods:

Only patients having either IVF or ICSI for non-male factor infertility were included. Patients were divided into 4 groups based on their ovarian response: Group 1: poor responders (1-3 oocytes), Group 2: suboptimal responders (4-9 oocytes), Group 3: normal responders (10-15 oocytes), Group 4: high responders (>15 oocytes).

Main results and the role of chance:

In total, 4891 patients were analyzed, of whom 4227 underwent ICSI and 664 IVF. There was no significant difference for the insemination method (ICSI vs. IVF) used among the different ovarian response categories: 87% vs. 13%, 87% vs. 13%, 86% vs. 14%, 84% vs. 16%, for groups 1, 2, 3 and 4, respectively, p value= 0.35. Mean fertilization rate defined as the ratio of 2PN oocytes and number of oocyte-cumulus complexes was comparable between ICSI and IVF groups [61% ± 23% and 60% ± 27%, p-value = 0.9). These findings were replicated for each ovarian response group. Mean embryo utilization rate defined as the total number of transferred and cryopreserved embryos per number of fertilized oocytes was also similar in the two insemination procedure groups, irrespective of the ovarian response category. Univariate analysis demonstrated that the insemination procedure was not significantly associated with either live birth rate (LBR) or cumulative LBR (p-value = 0.38 and 0.38, respectively). Multivariate regression analysis taking into account the ovarian response category and other relevant confounders showed that the insemination method was not significantly associated with LBR or with cumulative LBR (OR=1.1 CI 0.9-1.3/ OR =1.06 CI: 0.9-1.2).

Limitations, reasons for caution:

This is a cohort analysis based on retrospective data collection. Despite our robust methodological approach, the presence of bias related to the retrospective design cannot be excluded.

Wider implications of the findings:

To our knowledge, this is the first population-based cohort study to investigate the cumulative LBR following IVF and ICSI across different ovarian response categories. The number of oocytes retrieved has no value for the selection of the insemination procedure in case of non-male infertility.

Trial registration number:

N/A

O-229 ICSI is associated with lower live birth outcomes compared to IVF in couples having their first treatment cycle: A longitudinal analysis of 285,038 treatment cycles

H. Kamali[1], I. Gamaleldin[2], P. Wilson[3], V. Akande[4]

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[3]*Bristol Centre for Reproductive Medicine, Embryology and Andrology, Bristol, United Kingdom*
[4]*Bristol Centre for Reproductive Medicine, Medical Director, Bristol, United Kingdom*

Study question:

Is ICSI associated with improved outcomes over conventional IVF in couples undergoing their first treatment cycle?

Summary answer:

ICSI is associated with lower implantation, clinical pregnancy and live birth rates in women undergoing their first treatment cycle compared to conventional IVF

What is known already:

Currently, ICSI is the treatment of choice for couples with male factor infertility or with previous low fertilisation rates. An 'ICSI for all' approach has been suggested as the treatment of choice in all cases requiring assisted conception. However, previous studies comparing IVF and ICSI have shown inconsistent results.

Study design, size, duration:

Anonymised data on first fresh ICSI and conventional IVF treatment cycles (n=353,881) performed in the UK from years 2000 – 2016 were retrospectively obtained from the Human Fertility and Embryology Authority (HFEA). Women above 42 years old and those having donor oocytes/sperm were excluded. Primary outcome was live birth rates. Secondary outcomes were fertilisation rates, implantation rates and clinical pregnancy rates. Outcomes were adjusted for age, number of oocytes collected, and number of embryos transferred.

Participants/materials, setting, methods:

Following exclusion, complete data was available for 285,038 cycles. Poisson regression with restricted cubic splines was used to model events and rates. Covariates included year of treatment, age groups, cause/type of male infertility and total embryos transferred. In the subgroup analysis, we incorporated second order interactions among covariates to estimate the rate ratio within; each age group, in those with and without male factor infertility and at different time periods (year 2000,2010 and 2016).

Main results and the role of chance:

A total of 146,991 women had IVF and 138,047 women had ICSI. The overall live birth rates were 30% for ICSI and 29% for IVF. The adjusted analysis showed higher fertilisation rates in ICSI compared to IVF (68% vs 64%, RR:1.08 95% CI 1.08-1.08, p<0.001). However, the likelihood of implantation (27% vs 28%, RR:0.96, 95% CI 0.95-0.98, p<0.001) and clinical pregnancy (49% vs 50%, RR:0.98, 95% CI 0.97-0.99, p<0.001) were lower in ICSI compared with IVF. Although the observed rate of live birth was higher in ICSI compared to IVF, following adjustment the likelihood of live birth was lower in the ICSI group (RR:0.98, 95% CI 0.97-0.99, p<0.001). Sub-group analysis comparing ICSI to IVF persistently showed lower live birth outcomes in cycles with non-male factor infertility (RR:0.96, 95% CI 0.94-0.98, p<0.001). These findings were consistent across different age groups and different time periods.

Limitations, reasons for caution:

As a retrospective study, our analysis depends on previously recorded data, therefore certain variables (such as clinic specific protocols and duration of infertility) could not be collected. The reported cause of infertility is based on the treating clinician's classification and may be inconsistent

Wider implications of the findings:

In couples having their first fresh treatment cycle, ICSI offers no advantage over IVF in terms of live birth outcomes. These findings are consistent across different age groups and different time periods. The routine application of ICSI for treating couples with non-male factor infertility offers no benefit over conventional IVF.

Trial registration number:

N/A

O-230 Effect of the use of Polyvinylpyrrolidone (PVP)-coated injection pipette during intracytoplasmic sperm injection on clinical outcomes

T. Okimura[1], K. Uchiyama[1], T. Kobayashi[2], K. Kato[2]

[1]*Kato Ladies Clinic, IVF laboratory, Tokyo, Japan*
[2]*Kato Ladies Clinic, Department of Gynecology, Tokyo, Japan*

Study question:

Does the use of Polyvinylpyrrolidone (PVP) for coating injection pipette during intracytoplasmic sperm injection (ICSI) procedure influence embryonic and pregnancy outcomes?

Summary answer:

The application of PVP-coated injection pipettes for ICSI procedure may influence negatively embryonic outcomes.

What is known already:

ICSI is a well-established and accepted method for infertility treatment. Most centres use Polyvinylpyrrolidone (PVP) routinely during the ICSI procedure to improve the safety of the procedure, such as easier oocyte manipulation and sperm immobilization. Although there is evidence that the exposure of spermatozoa to PVP had detrimental effect on sperm structure and function, there is an inadequate emphasis on the potential hazards of PVP on clinical outcomes. Additionally, the effects of coating the pipette with PVP prior to ICSI on embryonic and pregnancy outcomes have not been investigated.

Study design, size, duration:

A retrospective cohort study of 951 cycles with 1,385 oocytes was conducted between April 2015 and January 2016. It consisted of 417 cycles (598 oocytes, mean age: 40.2 ±4.0 years) in which ICSI using PVP-coated injection pipette was performed (PVP-ICSI) and 534 cycles (787 oocytes, mean age: 40.2 ±4.2 years) in which ICSI using non-PVP coated pipette was performed (non-PVP-ICSI). Embryonic and pregnancy outcomes following single vitrified-warmed blastocyst transfer (SVBT) were compared among the groups.

Participants/materials, setting, methods:

Maturation status of oocytes was defined by confirming spindles using a Polscope. A small droplet of PVP was added to an ICSI dish and the injection pipette was washed there for coating. PVP was not used for sperm suspension. ICSI were performed by senior embryologists, using a pneumatically-driven injector. The survival, fertilization and good-quality blastocyst formation rates, clinical pregnancy (gestational sac observation), live birth at >22 weeks of pregnancy, and miscarriage rates were analysed.

Main results and the role of chance:

The survival rates of oocytes after ICSI in PVP-ICSI and non-PVP-ICSI groups were 96.8% (579/598) and 96.7% (759/785), respectively, there was no significant difference between the groups. There were no significant differences in normal fertilization and multiple nucleation (3PN<) rates between PVP-ICSI and non-PVP-ICSI groups [normal fertilization rates: 79.9% (478/598) vs 80.8% (634/785) and multiple nucleation rates: 5.2% (31/598) vs 3.7% (29/785), respectively]. Nonetheless, good-quality blastocyst formation rates (Gardner criteria: grade >3) were significantly higher in non-PVP-ICSI than those in the PVP-ICSI group [non-PVP-ICSI: 36.7% (288/785), PVP-ICSI: 31.4% (188/598), P<0.05]. A total of 161 and 237 cycles of SVBT were performed in PVP-ICSI and non-PVP-ICSI groups. The clinical pregnancy and live birth rates in PVP-ICSI and non-PVP-ICSI were 39.1% (63/161), 42.6% (101/237), 31.1% (50/161), and 29.1% (69/237), respectively. No significant differences were observed between the groups. The miscarriage rate did not significantly differ either [PVP-ICSI: 44.4% (28/63) and non-PVP-ICSI: 41.6% (42/101)].

Limitations, reasons for caution:

The main limitation of the present study is related to its retrospective design and a single-centre study. In addition, no comparisons with sibling oocytes were made. A further prospective sibling-split trial is required to confirm the potential adverse impact of PVP on embryonic outcomes.

Wider implications of the findings:

Our results demonstrate that good-quality blastocyst formation rates were significantly lower when ICSI was performed using a PVP-coated injection pipette, suggesting the potential adverse impact of PVP application on embryonic development. Moreover, this adverse effect may be expected even when a small amount of PVP is used.

Trial registration number:

None

O-231 Does the difficulty of blastocyst biopsy increased embryo mosaicism? A prospective cohort study

L. Guzman Masias[1], M. Jazmin[1], R. Lopez[2], J. Portella[3], P. Villanueva[1], L. Noriega-Portella[1], L. Noreiga-Hoces[1], N.P. Polyzos[4]

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Study question:

Is the technical difficulty of blastocyst biopsy associated with increased the embryo mosaicism rate?

Summary answer:

Loss of cellular integrity (CI) of more than 50% of biopsied cells almost doubles blastocyst mosaicism rate.

What is known already:

Biological mechanism and prevalence of mosaicism at blastocyst-stage remains difficult to be established. The technical limitations to determine an embryo as a mosaic are diverse, the fluctuation of copy number profiles can be explained by preferential DNA amplification, resulting in a DNA profile similar to the presence of mosaicism.

Additional biological sources of errors should be considered when evaluating mosaicism from thophectoderm biopsies. Reciprocal aneuploidies and trisomies at the same proportion might produce an euploid profile, leading to a false negative diagnosis. Another source of error could be the presence of cells in S-phase may appear as a mosaic aneuploidy.

Study design, size, duration:

This prospective study was performed in 242 IVF/ICSI cycles. From January 2017 to June 2018, 949 blastocysts were biopsied on day 5 or 6. NGS analysis was performed using Illumina platform following manufacturer's instructions.

Participants/materials, setting, methods:

Embryo biopsy was performed with the aid of laser with a power of 100% and a potency ranging from 350 to 400 µs. Biopsied cells were analyzed under microscope and classified by the number of cells biopsy and CI. The CI classification was decided prior to study initiation as follows: Grade I= more than 90% of CI; Grade II= 90-70% of CI; Grade III= 69-50% of CI and Grade IV= less than 50% of CI.

Main results and the role of chance:

The mean maternal age was 39.1±6.6. The overall euploidy rate was 43% (409/941), while mosaicism rate was 25% (239/941) and aneuploidy rate was 31% (293/941). mosaicism rate remains similar among the three first groups (25% (52/210); 26% (144/547); 21% (33/160) respectively) but significantly increased in group IV (42% (10/24)(p=0.024). On the contrary, eupliod rates was similar in group I, II and III ((45% (95/210); 43% (235/547) and 46% (74/160) respectively), but decreased significantly in group IV (21% (5/24), p=0.020). Finally, aneuploidy rate was similar for all groups.

To avoid cofounder factors, the maternal age, ICM and trophectoderm quality was classified by CI and any differences were found.

Limitations, reasons for caution:

Further work is needed to find other biological and technical variables which can explained the origen of mosacism in blastocyst.

Wider implications of the findings:

Our data suggest that mosaicism incidence may be overestimated when the CI of TE cells after embryo biopsy is extensively compromised. Embryo biopsy is an invasive technique that might impact genetics results.

Trial registration number:

None

O-232 IVF vs ICSI in non-male factor infertility: time to change course?

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Study question:

What are the reproductive outcomes of Intracytoplasmic Sperm Injection (ICSI) without indication of male factor, compared to conventional In-vitro fertilization (IVF)?

Summary answer:

ICSI performed without indication of male factor is associated with lower cumulative live birth rates (CLBR) compared to conventional IVF.

What is known already:

The widespread use of ICSI in patients with normal semen parameters is driven by an increase of both fertilization rates and number of available embryos. Nevertheless, little good-quality evidence supports ICSI effectiveness in this population. Published randomized-controlled trials do not take into account the results of subsequent frozen embryo transfers from the cycles studied and, therefore, do not report CLBR. We perform a comprehensive comparison of reproductive results between ICSI and conventional IVF cycles in a non-male factor infertility, combined with sub-analyses in women of advanced maternal age (≥40 years) and poor responders (≤ 4 retrieved oocytes).

Study design, size, duration:

Retrospective cohort of 956 autologous IVF or ICSI cycles, performed in 2008-2016. Only first cycles of patients were included, with men of normal semen parameters (WHO 2010 guidelines). Patients with abnormal karyotype in either man or women; history of recurrent pregnancy loss, chronic infections; need for PGT, BMI over 30 kg/m2 in the woman, presence of endocrine or uterine abnormalities and evidence of chronic, autoimmune or metabolic diseases were excluded.

Participants/materials, setting, methods:

Laboratory and reproductive outcomes of cycles with at least one oocyte at retrieval, following insemination by standard IVF (n=479) or ICSI (n=477) were compared using Chi-square tests and multivariate logistic regression analyses, adjusted for maternal age, number of retrieved oocytes, number of available embryos and number of transferred embryos. Sub-analyses were performed for poor responders and women of 40 years of age or more. Differences were considered significant if $p<0.05$.

Main results and the role of chance:

Per transfer live birth rate was significantly higher in the IVF group, both in the univariate (24.7% vs 18.9%; p=0.038) and multivariate analysis (OR 1.55; 95% CI 1.09-2.19). We found no difference in biochemical pregnancy (40.6% vs 35.5%; p=0.12), clinical pregnancy (34.3% vs 28.5%; p=0.06) or pregnancy loss (39.2% vs 46.8%; p=0.16). Fertilisation failure and cycle cancellation (no embryo transfer) were expectedly lower in the ICSI group (4.2% vs 7.1%; p=0.06 and 6.7% vs 9.8%; p=0.12, respectively). More importantly, the cumulative LBR was significantly higher in the conventional IVF group (36.2% vs 23.4%; p<0.001), and this was further confirmed in the multivariate analysis (OR 2.92; 95% CI 2.07-4.12). In poor responders, performing IVF significantly improved cumulative LBR (23% vs 13.8%; p=0.02 and OR 3.08; 95% CI 1.61-5.88), while this improvement, although present, did not reach statistical significance in women ≥ 40 years (17.8% vs 12.5%; p=0.201 and OR 1.87; 95% CI 0.95-3.69).

Limitations, reasons for caution:

The main limitation of our study is its retrospective nature. Although we adjusted our statistical analysis for known and suspected confounders, we cannot exclude the possibility of residual confounding factors.

Wider implications of the findings:

ICSI has been advocated in non-male factor infertility to increase fertilization rates and to prevent fertilization failure. Our data shows that adopting a systematical ICSI policy yields significantly worse reproductive results, both after the first ET and cumulatively, and call for a critical review of current practices.

Trial registration number:

Not applicable

O-233 Time interval between ovulation triggering and oocyte injection: does it affect the clinical outcome?

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Study question:

Does the time interval between ovulation triggering and oocyte injection affect clinical pregnancy and live birth rates?

Summary answer:

The timing of oocyte injection after ovulation triggering had no impact on clinical pregnancy and live birth rates.

What is known already:

In assisted reproduction, ovarian stimulation (OS) is used to induce the simultaneous growth of multiple follicles, followed by final maturation and ovulation triggering with exogenous hCG. Generally, oocyte retrieval (OR) is performed 35-36h later. In addition, 1-3 hours in culture of the cumulus-oocyte complexes 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 heavy workloads, respecting these exact time intervals is frequently challenging.

Study design, size, duration:

A single-center retrospective cohort analysis was performed including 8889 ICSI cycles from 2010 until 2015. Regarding the time interval between ovulation triggering and oocyte injection, seven categories were considered: <36h, 36h, 37h, 38h, 39h, 40h and ≥41h. The interval of <36h and 36h occurred only if OR was carried out before the planned 36h trigger interval and followed by immediate injection. As primary outcome measures, clinical pregnancy (CPR) and live birth rates (LBR) were considered.

Participants/materials, setting, methods:

During the study period, OR was routinely performed 36h post-triggering except in the <36h and 36h groups. Only cycles with fresh autologous gametes were included. Exclusion criteria were: injection with testicular/epididymal sperm, managed natural cycles, conventional IVF, combined conventional IVF/ICSI, PGT and IVM cycles. Statistical analysis was performed by multivariable multilevel mixed modeling regression analysis. Female age, number of oocytes, day of transfer, number of embryos transferred and embryo quality were considered as potential confounders.

Main results and the role of chance:

No effect of the time interval was observed on clinical pregnancy rates (range 21.9%-36.5%; p=0.286) and live birth rates (range 15.6%-27.9%; p=0.263), after adjusting for potential confounders. The adjusted analysis showed that the chance of having a live birth tends to be lower (OR 0.522, 95%CI: 0.247-1.104; P=0.075) when oocyte injection was performed before 36h compared to 38h after ovulation triggering, however, no significant difference was reached. Injection ≥41h post-triggering did not affect live birth rate (OR 1.038, 95%CI: 0.864-1.247; P=0.691) compared to injection at 38h post-ovulation.

Limitations, reasons for caution:

As this is a large retrospective study, the influence of uncontrolled variables cannot be excluded. These results should not be extrapolated to other ART procedures such as IVM, conventional IVF or injection with testicular/epididymal sperm.

Wider implications of the findings:

Our results indicate that the optimal insemination time window is less stringent than previously thought. This is reassuring, as busy centers might not always be able to follow strict time intervals. We observed a tendency to lower pregnancy and live birth rates when ICSI is performed <36h after ovulation triggering.

Trial registration number:

None

O-234 Is ICSI ethically supported by evidence as the method of fertilisation in donor oocyte cycles, in the absence of a medical indication?

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Study question:

Does ICSI improve success in donor oocyte cycles in the absence of a medically indicated reason?

Summary answer:

IVF results in a higher clinical pregnancy and live birth rate whilst ICSI has a lower failed fertilisation rate.

What is known already:

Donor oocytes have expanded the scope of assisted reproductive technology (ART) for women unable to conceive with autologous oocytes, and resulting in higher live birth rates. However, the degree of anxiety surrounding donor cycle outcomes is increased, with some advocating ICSI for all donor oocyte cycles to reduce the likelihood of failed fertilisation. ICSI has a clear role in the management of male factor subfertility, but its use for other indications has yet to be proven. A recent RCT performed in autologous oocyte cycles demonstrated no additional benefit with ICSI in cases of non-male factor subfertility.

Study design, size, duration:

Retrospective cohort analysis of all cycles conducted between 2002-2016, from the Human Fertilisation and Embryology Authority database. 23,589 fresh donor cycles (12,989 IVF cycles; 12,400 ICSI cycles), excluding male factor subfertility were undertaken. Assuming that the live birth rate for IVF is 30% and 40% for ICSI cycles, a power calculation demonstrated that 712 cycles would need to be analysed for 80% power and a 5% significance level to detect 10% difference.

Participants/materials, setting, methods:

The database was analysed for singleton live birth rate (SLBR), stratified by recipient age, donor age, number of previous IVF treatments and type of cycle (fresh IVF versus fresh ICSI). Cycles complicated by male factor subfertility were excluded from the final analysis as this is known to influence the method of fertilisation. Statistical analysis was performed using Logistic Regression and Chi-square; $p<0.05$ was considered statistically significant.

Main results and the role of chance:

The overall IVF to ICSI ratio for donor oocyte treatment cycles in the absence of male factor subfertility favoured IVF (68:32) in 2002, with a yearly rise seen in ICSI cycles reaching 42:58 in 2016.

The clinical pregnancy rate is statistically higher with donor IVF (n=4721;40.5%) versus donor ICSI cycles (n=4280;38.3%) (odds ratio [OR] 1.10, 95% confidence intervals [CI] 1.04-1.16, p=0.0009).

The overall live birth rate per embryo transferred was higher in the IVF group (n=5,230;26.6%) compared with ICSI (n=4,593;24.1%). The OR was calculated using binary logistic regression and adjusted for recipient age, donor age, number of previous IVF cycles and cause of subfertility, giving IVF an OR 1.13 (95% CI 1.04-1.23, p=0.004).

The failed fertilisation rate was higher for IVF (n=270;2.08%) compared with ICSI (n=196;1.58%) (relative risk [RR] 1.32, 95% CI 1.09-1.58, $p<0.0001$). Furthermore, the data from this study suggests that the number needed to treat to prevent one failed fertilisation through IVF is 200 cycles of ICSI.

Limitations, reasons for caution:

The accuracy of the database is dependent on the information submitted to the HFEA. Until 2007, this data was manually captured adding the risk of data entry error. Furthermore, information on embryo quality is not available including the inability to account for cumulative pregnancy rates in women with previous attempts.

Wider implications of the findings:

The reduction in the failed fertilisation rate does not translate to an improvement in the live birth rate and therefore, ICSI does not confer any additional benefit compared to IVF. This provides patients with the ability to make an informed choice of IVF over ICSI, with an overall cost saving.

Trial registration number:

Not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 65: IMPROVING IVF OUTCOME

Wednesday 26 June 2019 Haydn 1 10:00 - 11:45

O-235 Single follicular degarelix, a new long acting GnRH-antagonist for LH surge suppression during ovarian stimulation in oocyte donors. A randomised controlled trial

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Study question:

Could the use of a novel long acting, single dose GnRH antagonist, Degarelix, cause efficient pituitary downregulation during ovarian stimulation in oocyte donors?

Summary answer:

Single administration of a novel long-acting GnRH antagonist can efficiently downregulate LH, produce mature oocytes and achieve comparable pregnancy outcome to the classical short-antagonist protocol.

What is known already:

GnRH antagonists have started to be widely used in the last decade. Their immediate action, successful LH surge suppression and complete eradication of OHSS, especially when combined with GnRH agonist triggering and a freeze all strategy create a favorable-luring profile. Their action, though immediate is however short lived, requiring multiple doses to be administered in the mid follicular phase leading to increased cost and patient discomfort**.** Women faced with infertility undergoing IVF treatment experience repeated drug injections as traumatic and bothersome and the special population of oocyte donors could benefit from an even more convenient and patient friendly protocol.

Study design, size, duration:

This RCT recruited healthy women, under 35yrs undergoing oocyte donation and studied the efficacy of a single Day6 follicular dose of degarelix (Firmagon, Ferring Pharmaceuticals) compared to the classical daily dose protocol from January 2017-December 2018. Two groups of patients were compared: Group-A (Classical antagonist group) consisted of 89 women, who followed a fixed Day6 GnRH-antagonist protocol with daily injections of 0,25mg ganirelix, whereas, Group-B (Degarelix Group) involved 76 women undergoing the new antagonist protocol.

Participants/materials, setting, methods:

Ovarian stimulation was initiated on cycle Day2 with gonadotropins 175-300IU, daily, in both groups. In Group-A 0,25mg of antagonist (ganirelix or cetrorelix) was administered daily from stimulation Day6 in a fixed manner. On the same day a single bolus injection of 0,1 ml Degarelix was administrated subcutaneously in Group-B. Agonist triggering (Triptorelin 0,3ml) was employed for all and OPU performed at 36h. Fresh /frozen blastocyst-only transfer was performed following recipient endometrial oestrogen and progesterone priming.

Main results and the role of chance:

The mean age (27.0 vs 28.1 years), AMH (4.15 vs. 3.65ng/ml) and total gonadotropin dose (2400 vs 2508 IU) of participants were not different among group-A and group-B respectively. No LH rise or any OHSS was noticed in any groups. Although, statistically significant more oocytes retrieved (18.8 vs.16.6, $p<0.05$) with degarelix short antagonist group, not statistically different mean number of blastocysts produced among groups (6.6 in group-A vs. 7.5 in group-B). All recipients underwent 2 blastocysts transfer. Pregnancy is expressed per donor. Initial positive HCG per donor was significantly higher ($p<0.05$) in the Degarelix Short Antagonist (Group B) 80.3% (61/76) as compared with 65.9% (n=60/91) in classic short antagonist (Group A). Similarly, Cumulative delivery rate was 60.5% (46/76) in the new single shot Degarelix short antagonist group as compared to 49.5% (n=45/91) in classic antagonist group, however not statistically significant ($p>0.05$)

Limitations, reasons for caution:

Limitation is that, this is a proof of concept study, and the trial is still ongoing. Additionally, the titration of the degarelix dose was made for the first time by our group and maybe other doses can be tested in future studies.

Wider implications of the findings:

This is a new concept, with a fixed bolus follicular administration of a new long acting GnRH antagonist, which offers convenience and simplifies the IVF protocol. This single dose of degarelix, makes the antagonist protocol even more patient friendly.

Trial registration number:

NCT03240159

O-236 Mild vs. Conventional stimulation for in vitro fertilization: a stratified meta-analysis according to the level of ovarian response

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Study question:

Is conventional stimulation (CS) superior to mild stimulation (MS) (i.e., oral compounds, lower doses or shorter treatments) in poor, normal or high responders undergoing IVF?

Summary answer:

CS results in higher live birth rates (LBR) compared to MS in normal responder women. No difference is observed in either poor or high-responders.

What is known already:

Previous randomized trials and meta-analyses have shown that mild ovarian stimulation may result in equivalent pregnancy rates compared with conventional stimulation, with a potentially higher safety profile, patient satisfaction, and lower costs, suggesting that the higher cycle cancellation rate and fewer oocytes retrieved following mild stimulation, does not affect the final reproductive outcome. Still, none of the previous meta-analyses evaluated whether this is the case in all ovarian response categories and different mild-stimulation protocols, given that the possible benefit of a higher oocyte yield might be more evident in specific ovarian response categories.

Study design, size, duration:

We conducted a systematic search of relevant randomized controlled trials (RCTs). We searched electronic databases including MEDLINE, EMBASE, LILACS-BIREME, CINAHL, The Cochrane Library, CENTRAL (Cochrane Register), Web of Science, Scopus, Trip Database, and Open Grey, to identify all relevant studies published up to January 2019. We searched trial registries for ongoing trials. No publication-year or language restrictions were adopted. We examined the reference list of all included studies, reviews, and, abstracts of major scientific meetings.

Participants/materials, setting, methods:

We included subfertile women undergoing IVF/ICSI characterized as poor, normal or high responders and compared primary and secondary outcomes between the different protocols of mild stimulation IVF (i.e., oral compounds, lower doses or shorter treatments) and conventional IVF. We used the PICO (Patients, Intervention, Comparison, and Outcomes) model to select our study population. The primary effectiveness outcome is LBR.

Main results and the role of chance:

Overall, 48 randomized controlled trials were included in the meta-analysis: 18 trials for poor, 27 trials for normal and three trials for high ovarian responders. In women characterized as poor ovarian responders, LBRs were comparable between mild vs. conventional stimulation RR(95%CI) 0.90 (0.66-1.24), heterogeneity: I2=0%,(n = 958 patients, 5 studies). No difference was observed either when using utilizing oral compounds (OC) (i.e., letrozole and clomiphene), three RCTs, lower doses (LD), or with shorter treatment duration (ST).

In women with normal ovarian response LBRs were significantly higher in the conventional stimulation (MS vs. CS) RR(95%CI) 0.78 (0.69-0.89), I2=0%, (n = 1673 patients, 5 studies). Conventional stimulation was superior to mild either when using OC, LD or ST.

Finally, in high ovarian responders, LBRs were comparable between MS vs. CS RR(95%CI) 1.09 (0.86-1.38), I2=78%, (n =1282 patients, 3 studies). No difference was observed either when using LD, ST, whereas no studies with OC have been published.

Limitations, reasons for caution:

This meta-analysis reports data for ongoing and live-birth rates. Given the higher oocyteyield in the majority of the patients' categories, no guidance can be given on whether MS is comparable to CS with regards to CLBR. Results for high-responders need to be interpreted with caution owing to high statistical heterogeneity.

Wider implications of the findings:

This is the first systematic review, and meta-analysis comparing the three general protocols of mild stimulation (i.e., oral compounds, lower doses or shorter treatments) to the conventional ovarian stimulation, and the first to evaluate them regarding the profile of the patient (i.e., high-, normal-, and poor-responders).

Trial registration number:

NA

O-237 Long-Antagonist protocol versus Classical-day-6 Antagonist. Single luteal use of long-acting GnRH-antagonist Degarelix can efficiently downregulate LH during ovarian stimulation for IVF achieving comparable pregnancy outcome

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Study question:

Is the new protocol with only a single luteal dose of long-acting GnRH-antagonist comparable to the short follicular antagonist protocol in terms of pregnancy achievement?

Summary answer:

A single dose of long-acting antagonist Degarelix, during luteal phase, down-regulates LH, produces mature oocytes, implantable embryos and results comparable to classical short antagonist protocol.

What is known already:

GnRH-antagonists in daily doses from stimulation day-5 or 6 gradually becomes the protocol of first choice, as it is more patient friendly and reduces dramatically the risk for Ovarian Hyperstimulation Syndrome risk (OHSS). Nevertheless, due to the limited flexibility in cycle programming long-agonist protocol is still the preferred treatment in the majority of IVF clinics. We hypothesized whether a single luteal dose of long-acting antagonist causes prolonged suppression of LH rise allowing thus flexibility in the starting day of gonadotropins later in the follicular phase.

Study design, size, duration:

This proof of concept randomized control trial studied the efficacy of a single dose of long-acting antagonist, Degarelix. The study was performed during January-December 2018. Two groups of patients were compared: Group A (Short-Antagonist group) involved 59 women, following a classic fixed day-6 GnRH-antagonist protocol whereas, Group B (study group-New Long-Antagonist Group) involved 54 women undergoing the new long-antagonist protocol. Both groups included infertile women <40 years, prepared for IVF treatment in Assisting Nature Centre.

Participants/materials, setting, methods:

In the new protocol, a single bolus injection of 0.5ml Degarelix was administrated subcutaneously in late luteal phase (day-24). Ovarian stimulation with gonadotropins 200-300IU started from cycle-day-2 to 10. In the classical short-antagonist-group gonadotropins 200-300IU started on cycle day-2 or 3, while 0.25mg of antagonist (ganirelix) from stimulation-day-6. RecHCG or agonist triggering was used when three 18mm follicles were present, followed by oocyte-pick-up 36h later. Fresh/frozen day-5 only embryotransfer was decided upon response/ progesterone rise.

Main results and the role of chance:

The mean age and AMH of participants were not different (33,8 vs. 33,2) and (2,42 vs. 2,58) among groups respectively. Stimulation ranged from 9-10 days in control group A, whether in the Long Antagonist group ranged from 10-11 days and patients, even triggered with HCG up to cycle-day-22. Similar number of oocytes retrieved (10.8 vs. 11.9) and not statistically different mean number of blastocysts were produced in both groups (4.8 vs. 5.8). No LH rise or any OHSS was noticed in any groups. All patients underwent blastocyst transfer. Fresh embryotransfer was performed in 26 out of 59 patients in group-A achieving a 23.1% ongoing/delivery rate (n=6/26) and the rest 33 patients underwent frozen embryotransfer in a Freeze-All strategy achieving a 60.6% ongoing/delivery rate (n=20/33). Similarly, fresh embryotransfer was performed in 12 out of 54 patients in group B achieving a 25% ongoing/delivery rate (n=3/12) and the rest 42 patients underwent frozen embryotransfer in a Freeze-All strategy achieving a 64.3% ongoing/delivery rate (n=27/42). Cumulative ongoing/delivery rate was 44,1% (n=26/59) in classic

antagonist (group A) and 55,5% (30/54) in the new Long Antagonist (group B), p<0.05.

Limitations, reasons for caution:

Limitation is the small size of the groups; however, this is a proof of concept still ongoing trial.

Wider implications of the findings:

This pure novel concept for IVF couples combines security, flexibility and eventually pregnancy efficacy. This new Long-Antagonist protocol addresses cycle programming that was missing with antagonist protocols in the top of eradicating OHSS. However, larger studies are required to confirm the success of this protocol.

Trial registration number:

NCT03684421

O-238 A systematic review and meta-analysis comparing Biosimilar versus Originator Recombinant Follitropin Alfa in women undergoing IVF/ICSI

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Study question:

What is the efficacy and ovarian hyperstimulation syndrome (OHSS) risk with biosimilar compared with originator recombinant follitropin alfa for ovarian stimulation in women undergoing IVF/ICSI?

Summary answer:

Biosimilar follitropin alfa preparations result in significantly lower live birth and clinical pregnancy rates compared with the originator preparation; there is insufficient evidence on OHSS.

What is known already:

IVF is one of the cornerstones of the modern management of infertility. Ovarian stimulation with gonadotropins is an important part of IVF treatment, but these are expensive. Biosimilars have been introduced as they are presumed to be equally effective and safe, but cheaper than the originator preparation.

Study design, size, duration:

We performed a systematic review and meta-analysis comparing efficacy outcomes and OHSS (moderate to severe) with biosimilars compared with originator follitropin alfa. We searched Medline, Embase, Cochrane and Web of Science from origin to January 2019. We also searched clinical trial registries including ClinicalTrials.gov and the WHO international clinical trial registry platform for additional studies.

Participants/materials, setting, methods:

We searched for randomised controlled trials (RCTs) comparing biosimilars and originator follitropin alfa. The primary endpoint was live birth. Secondary endpoints included clinical pregnancy and OHSS (moderate to severe). Fixed effect meta-analysis was used to pool the data, and relative risks (RRs) with 95% confidence intervals (CIs) were calculated for each outcome. The I-squared statistic was used to evaluate heterogeneity.

Main results and the role of chance:

Our search identified 370 references, of which 12 were potentially eligible. After screening the full texts, three RCTs (Retenbacher 2015, Strowitzki 2016, NCT01687712; 1761 women) were found to be eligible. The biosimilar preparations were Bemfola in two studies and Ovaleap in one study.

Compared with the originator preparation, the live birth rate was significantly lower after the use of biosimilars (RR 0.82, 95% CI: 0.70 to 0.97; 3 RCTs) and the clinical pregnancy rate was also significantly lower (RR 0.83, 95% CI: 0.71 to 0.96; 3 RCTs). The overall RR for OHSS (moderate to severe) was non-significantly higher (RR 1.30; 95% CI: 0.83 to 2.70; 3 RCTs) when comparing biosimilars with the originator preparation. The heterogeneity was negligible for all outcomes (I-squared=0%).

Limitations, reasons for caution:

This meta-analysis was limited to the first treatment cycle. Two studies reported the second cycle separately but it was not possible to identify the participants with the same event in both cycles in the aggregated data. In addition, women with history of severe OHSS were excluded from the trials.

Wider implications of the findings:

In published RCTs in women undergoing IVF, ovarian stimulation with biosimilar follitropin alfa reduced the chances of live birth by 18% compared with the originator preparation.

Trial registration number:

not applicable

O-239 Follicular steroidogenesis in GnRH antagonist ovarian stimulation cycles with r-FSH vs. hp-HMG. A randomized controlled trial

E. Bosch Aparicio[1], P. Alamá[1], J.L. Romero[1], M. Mari[1], E. Labarta[1]

[1]*IVI-RMA, Human Reproduction, Valencia, Spain*

Study question:

Does ovarian stimulation with hp-HMG protect from elevated Progesterone in the follicular phase compared to r-FSH cycles through a different follicular steroidogenesis?

Summary answer:

Hp-HMG enhanced the Δ_4 pathway from Pregnenolone to Androgens, while r-FSH promoted the conversion of Pregnenolone to Progesterone leading to higher follicular phase serum Progesterone

What is known already:

Elevated Progesterone in the follicular phase has been related to lower clinical outcome in fresh IVF cycles. Progesterone levels are positively correlated to ovarian response, and some studies have shown that when r-FSH alone is used for ovarian stimulation, serum P levels the day of triggering are higher than when hp-HMG is given. Whether this is due to a lower ovarian response in hp-HMG cycles or to a different follicular steroidogenesis between both ovarian stimulation regimens has not been well characterized

Study design, size, duration:

Randomized controlled trial including 110 oocyte donors undergoing ovarian stimulation with GnRH antagonists and 225 IU/day of rFSH (n=56) or hp-HMG (n=54) in a University affiliated private infertility clinic. Subjects were recruited between October 2016 and June 2018

Participants/materials, setting, methods:

Women aged 18 to 35, with a regular menstrual cycle (25-35 days) and normal ovarian reserve (AMH=10-30 pMol/L) undergoing ovarian stimulation for oocyte donation. Serum FSH, LH, Estradiol, Estrone, Progesterone, Pregnenolone, 17-OH-Progesterone, Androstenodione, Dehidroepiandrostenodione, and Testosterone, were determined on stimulation days 1, 4, 6, 8 and triggering and in follicular fluid. Samples were frozen at -20°C until determination. Total exposures across the follicular phase were compared by polynomic extrapolation

Main results and the role of chance:

Subjects of both groups were comparable for age, body mass index and AMH levels. Ovarian response was also similar: 17.5±7.9 vs. 16.5±7.5 oocytes with r-FSH and hp-HMG respectively (p=0.49)Serum P (ng/mL) the day of trigger was 0.46±0.27 in the hp-HMG group vs. 0.68±0.50 in the r-FSH group (p=0.010).Differences were also significant on stimulation days 6 and 8. The Pregnenolone:Progesterone ratio was significantly increased in the r-FSH group from stimulation day 8 to the day of trigger (p=0.019).

Serum Androstenodione (ng/mL) the day of trigger was 3.0±1.4 in the hp-HMG group vs. 2.4±1.1 in the r-FSH group (p=0.015). Differences were also significant on stimulation day 8. The Pregnenolone:Androstenodione ratio was significantly higher in the hp-HMG group (p=0.012). There were no other significant differences between both groups for any other hormones in any stimulation day. Follicular fluid E2, FSH, LH, Dehidroepioandrostenodione, Androstenodione and Testosterone were significantly higher in the hp-HMG

group.No differences were observed for Progesterone, Estrone, 17-OH Progesterone and Pregnenolone. The multivariable regression analysis showed that ovarian stimulation with r-FSH remained as a significant factor for elevated serum P on stimulation day 8 and triggering after adjusting for age, ovarian response and body mass index

Limitations, reasons for caution:

All women included in the study were young, non infertile, had a normal body mass index and a good ovarian reserve. Findings might be different in other patients' subpopulations

Wider implications of the findings:

The findings of the present study suggest that stimulation with hp-HMG may prevent for Progesterone elevation at the end of the follicular phase because of a different follicular steroidogenesis, regardless of ovarian response. This should be considered particularly in high responders if a fresh embryo transfer is planned

Trial registration number:

NCT02738580

O-240 The dydrogesterone and FSH protocol in patients with polycystic ovarian syndrome undergoing controlled ovarian hyperstimulation in IVF/ICSI cycle

A.S. Gurbuz[1], F. Gode[2]

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[2]*Bahcesehir University, Obstetrics and Gynecology, Istanbul, Turkey*

Study question:

Can dydrogesterone be used as an alternative suppressor of LH surge in controlled ovarian stimulation in PCO patients.

Summary answer:

Dydrogesterone can be used as an alternative suppressor of LH surge in controlled ovarian stimulation in PCO patients.

What is known already:

Progesterone-primed ovarian stimulation (PPOS) is a new ovarian stimulation regimen based on a freeze-all strategy that uses progestin as an alternative to a GnRH analog for suppressing a premature LH surge during the follicular phase. Dydrogesterone (DYD), medroxyprogesterone acetate (MPA) and natürel micronized progesterone (NMP) has been successfully used as adjuvant to gonadotrophin in the PPOS protocol in normoresponders and poor responders . However, the use of freeze-all strategy is suitable for hyperresponders. DYD has less side effect than the other progestin.

Study design, size, duration:

A retrospective trial including 488 patients was performed between April 2017 and November 2018. The patients results were collected to at a 1:1 ratio into two treatment groups: an rec FSH +DYG group (242 patients) or an rec FSH + GnRH antagonist (Cetrorelix) group (246 patients) followed by IVF or ICSI with the freeze-all strategy. The primary outcome of the trial was the number of oocytes retrieved.

Participants/materials, setting, methods:

Patients under 35 years of age with PCO patients who were undergoing IVF/ICSI procedure, based on the oral progestin or antagonist protocol used: rec FSH+DYG or GnRH Antagonist protocol: rec FSH+cetrorelix . The DYD was simultaneously administered at the beginning of menstrual cycle 3. The cetrorelix was administered on 6th day of controlled ovarian stimulation. Oocyte maturation was triggered by GnRH agonist (0,2mg triptorelin). All viable embryos from both protocols were cryopreserved for later transfer.

Main results and the role of chance:

Basic characteristics in both groups were similar. There was no significant difference in the number (mean±SD) of oocytes retrieved (14,2±5.3 for the rec FSH + DYG group versus 15.1±5.8 for the rec FSH+Cet group, (P=0.31) or the oocyte retrieval rate (76.2±15.3% for the rec FSH + DYG group versus 76.7 ±17.5% for the rec FSH + Cet group, P=0.71) between the groups. The viable embryo rate per oocyte retrieved did not differ between the two groups (odds ratio (OR): 1.06, 95% CI: 0.96–1.19, P=0.17): 36% (1314/3642) for the rec FSH + DYG group versus 35.8% (1331/3718) for the rec FSH +Cet group. During the whole process of ovarian stimulation, the mean LH level in the rec FSH + DYG group was always higher than that in the rec FSH+ Cet group ($P < 0.001$); however, no patient from either group experienced a premature LH surge. No patients experienced ovarian hyperstimulation syndrome . No significant difference was found in the clinical pregnancy rate of the FET cycle between the two groups (OR: 0.81, 95% CI: 0.55–1.24, P = 0.33): 56.7% for the rec FSH + DYG group (118/208) versus 52.0% for the rec FSH + Cet group (101/194).

Limitations, reasons for caution:

Main limitation of this study is that is retrospective study. This study has only clinical pregnancy rates because of live birth rates were not observed in the follow up period.

Wider implications of the findings:

DYG, which exhibits no or only weak inhibition of ovulation in normal dosage, can serve as an rec FSH adjuvant during ovarian stimulation. This finding suggests the possibility of a new application of DYG: as an appropriate alternative LH surge suppressor for hyperresponder patient in IVF.

Trial registration number:

Not applicable

O-241 Luteinizing hormone receptor deficiency in granulosa cells explains the retrieval of immature (germinal vesicle) oocytes after controlled ovarian stimulation

M. Casalechi[1], M.T. Pereira[1], I.K. Cavallo[1], C. Dela Cruz[1], F. Marcos dos Reis[1]

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Study question:

Why are immature (germinal vesicle, GV) oocytes retrieved after controlled ovarian stimulation (COS) for in vitro fertilization (IVF)?

Summary answer:

Follicles containing GV oocytes at the time of oocyte retrieval for IVF have almost undetectable luteinizing hormone receptor (LHR) mRNA levels

What is known already:

Following the LH surge, paracrine messages from granulosa cells induce the oocyte to resume meiosis and acquire full competence. However, some oocytes retrieved for IVF do not respond to this stimulus and remain at GV stage. Case reports have documented patients whose repeated IVF attempts yielded only GV oocytes despite of the correct use of gonadotropins for COS and ovulation triggering. Moreover, in typical IVF cycles about 8% of the retrieved oocytes are arrested in GV stage. Until now, there is no reasonable explanation to this failed meiotic resumption of some oocytes after COS.

Study design, size, duration:

This was a prospective cohort study including 39 women (26 to 42 years old) who underwent COS in a single IVF center. The participants were enrolled in the study from February to September 2018. The study was approved by the local IRB and all participants provided written informed consent.

Participants/materials, setting, methods:

Luteinized granulosa cells (GC) and cumulus cells (CC) were collected from 96 preovulatory follicles and the maturation stage of the oocytes was immediately evaluated. LHR gene expression levels were measured by quantitative reverse-transcription real-time PCR in GC and in CC surrounding each oocyte. The relative mRNA expression of LHR was compared between cells from follicles containing GV, metaphase I (MI) and metaphase II (MII) oocytes using Kruskal-Wallis analysis of variance.

Main results and the role of chance:

The qPCR results were homogeneous for amplification of the S26 reference gene. The duplicates of the samples were concordant and the melting curves (Tm) showed a single peak for LHR and S26 genes, demonstrating the reliability of the results.

LHR mRNA expression was detected in both CC and GC of follicles containing MII or MI oocytes, being four-fold more abundant in CC than in GC. LHR mRNA was nearly undetectable in both CC (fold change 0.25, $p<0.01$) and GC (fold change 0.24, $p<0.01$) from follicles containing GV oocytes.

Limitations, reasons for caution:

Samples were obtained from patients who underwent COS for IVF, hence we cannot extrapolate these findings to natural ovarian cycles. We did not measure proteins or perform functional studies, but the severe deficiency of LHR mRNA in GC and CC is likely to explain the oocyte unresponsiveness to LH.

Wider implications of the findings:

Until now, there was no reasonable explanation for the lack of oocyte maturation in response to COS. Our finding reveals a potential mechanism

for this condition and suggests that novel drugs acting beyond LHR should be developed to treat IVF patients with a high proportion of GV oocytes.

Trial registration number:

Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 66: BIOMARKERS OF OOCYTE AND EMBRYO HEALTH

Wednesday 26 June 2019 Haydn 3 10:00 - 11:45

O-242 Are human embryos able to modulate mitochondrial DNA (mtDNA) content before implantation?

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[4]*IVI-RMA Global, IVF lab, Valencia, Spain*

Study question:

To determine if the human embryo is able to modulate his mitochondrial DNA (mtDNA) content in response to an external stress before implantation.

Summary answer:

There is a high increase in mtDNA copy number in arrested cleavage stage embryos which are arrested and in blastocyst after vitrification and warm.

What is known already:

Mitochondrial dynamics are essential for stress responses. Mitochondria are able to respond to an acute cellular stress by different mechanisms: motility; fusion and fission processes; inter-organellar contacts; cristae remodeling; rebuilding, recycling, permeabilization and biogenesis; (Eisner V. et al., 2018). However, no evidences have been reported so far concerning the ability of human embryo to modulate mtDNA content before implantation occurs. The study of the mtDNA content in embryos that have suffered some kind of stress will shed light on this topic.

Study design, size, duration:

A prospective cohort study was performed with 35 fresh arrested cleavage stage embryos, 21 vitrified embryos on day 3 of development, 32 aneuploid blastocysts cultured 0 hours post-warm, 5 aneuploid blastocysts that were warmed and cultured 2 hours post-warm and 9 aneuploid blastocysts warmed and cultured 5 hours post-warm. All embryo stages were tubed in PCR tubes separately with 2,5 µl of PBS.

Participants/materials, setting, methods:

Q-PCR was performed with SurePlex DNA Amplification System (Illumina) using specific primers for the ATP8 and β-Actin genes to assess the total and per cell mtDNA copy number. Data was analysed by ANOVA test with Scheffé multiple comparison for categorical variables and linear regression for numerical variables.

Main results and the role of chance:

Arrested cleavage stage embryos had a significantly ($P<0.05$) higher total and per cell mtDNA copy number (Log_2 ATP8=34.20, Log_2 ATP8/β-Actin=23.10) compared to not arrested day 3 embryos (Log_2 ATP8=18.26, Log_2 ATP8/ β-Actin=5.46) and blastocyst stage embryos cultured 0 hours post-warm (Log_2 ATP8=16.39, Log_2 ATP8/ β-Actin= 3.15). The earlier the arrest occurred the lower mtDNA levels were found ($P<0.05$). Vitrification had a significant impact over the final quantities of mtDNA, reaching the higher levels 5 hours post-warm (Log_2 ATP8=17.81, Log_2 ATP8/ β-Actin= 5.17) compared to 2 hours (Log_2 ATP8=16.19, Log_2 ATP8/ β-Actin= 3.03) and 0 hours (Log_2 ATP8=16.39, Log_2 ATP8/ β-Actin= 3.15) post-warm ($P<0.05$). Then, the increase in mtDNA copy number observed in arrested embryos and after vitrification/warm process could indicate that human embryos are not only able to modulate the mtDNA content before implantation but also that embryos modify its mtDNA content to face a stress situation.

Limitations, reasons for caution:

All the analyzed blastocyst were aneuploid, so we need to check whether euploid human blastocysts will have similar behavior. Additionally, an increase in sample size is required to confirm these preliminary findings.

Wider implications of the findings:

As far as we know this is the first study evidencing how embryos are able to respond to stress situation modulating its mtDNA content, and that this can happen during its preimplantation period. This would be useful to do further studies to know how its implantation capacity would be affected.

Trial registration number:

Not applicable.

O-243 Gene Expression Profiling of Cumulus Cells Derived from Oocytes at Different Maturational Stages Reveals Marked Differences, Potentially Shedding Light on Pathologies of Maturation

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Study question:

Can transcriptomic profiling of cumulus cells (CCs) encapsulating germinal vesicle (GV) and metaphase-two (MII) oocytes, improve our understanding of pathologies of maturation and have implications on treatment decisions?

Summary answer:

Differences between transcriptomic profiles of CCs from GV and MII oocytes explain the functional gap between them, and have the potential to improve treatment outcomes.

What is known already:

CCs support growing oocytes and can be collected without compromising their integrity. Their transcriptomes mirror those of corresponding oocytes, and are considered as potential non-invasive biomarkers for oocyte quality. Trying to mature GVs that have been previously exposed to gonadotropins in the lab, is an inefficient process and often yields oocytes with lower developmental potential. Transcriptomes of CCs encapsulating oocytes of different maturity could better elucidate the functional gaps between them. Addressing these gaps could potentially be the first step in improving maturation rate in vitro, and the quality of the resulting oocytes.

Study design, size, duration:

Twelve consented patients undergoing IVF-ICSI at the CReATe Fertility Centre (Toronto, CA) were enrolled in the study. Cumulus cells were collected, and the corresponding oocyte maturation was assessed and recorded by an embryologist. RNA sequencing and differential gene expression and pathway analysis between CCs from mature MII and immature GV oocytes were performed.

Participants/materials, setting, methods:

cDNA libraries (Clonetech SMARTer-v4 and Nextera-XT) were sequenced using the NextSeq 550. Sequences were aligned to hg19, and differential expression was conducted. We then performed pathway analysis to elucidate differences in hallmark ovarian and cellular processes, and leading edge analysis to determine which genes were driving these differences. A comprehensive literature search revealed potential roles of each leading edge gene in the pathophysiology of defective oocyte maturation.

Main results and the role of chance:

Principal component analysis revealed that samples clustered primarily according to the maturation stage of the oocyte. Following differential expression, 795 genes were significantly up-regulated and 1034 were down-regulated between MIIs and GVs.

Mature oocytes expressed enhanced steroid metabolism, with up-regulation of *AREG* (which induces meiotic resumption, and progesterone production), enhanced *MAPK/ERK* signaling pathway, with up-regulation of *AREG*, *EREG* and *PTGS2* (which together mediate cumulus expansion and oocyte maturation, as well as corpus luteum formation and maintenance and reduce spindle abnormalities), and enhanced regulation of cell size with up-regulation of

BDNF, (which promotes oocyte maturity and follicular growth). Mature oocytes also expressed attenuated pathways including cell cycle regulation, with down-regulation of the pro-apoptotic and anti-angiogenic gene *SFRP4*, decreased assembly of pre-replication complex marked by down-regulation of *CDC6*, decreased mitotic spindle formation and chromosome segregation with down-regulation of *CDK1* (required for spindle migration and membrane protrusion leading to polar body extrusion) and attenuated extra-cellular matrix remodeling.

Limitations, reasons for caution:

Our findings are limited by the relatively small sample size and the inherent biological variability of human samples. To adjust for this, "significant differentially expressed genes" between MIIs and GVs were defined with stringent criteria, increasing the confidence in the validity of the findings.

Wider implications of the findings:

Data is slowly accumulating regarding the potential of modified incubation media for immature oocytes, mostly from non-human models. Our findings could further promote modifications for existing media, with the hope of improving maturation rates in the lab, as well as yielding MIIs with enhanced developmental potential in humans.

Trial registration number:

N/A

O-244 Pronuclear score significantly correlates with clinical pregnancy rates in top quality embryos

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[1]*IRCCS Ospedale Policlinico San Martino, UOS Physiopathology of Human Reproduction, Genova, Italy*

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Study question:

Is the pronuclear (PN) morphology an useful additional criterion to improve prediction of blastocyst development and clinical pregnancy?

Summary answer:

The PN score based on the recently standardized ESHRE system improves prediction of clinical pregnancy in cleavage-stage embryos with good morphology.

What is known already:

In assisted reproduction technology embryo competence is routinely evaluated on morphological criteria but their efficacy remains relatively low. Additional information could be obtained by evaluation of PN morphology. Up to now controversial results have been reported about correlation between the zygote morphology and the embryo competence. One of the main limitation of literature data is the use of different methods of PN classification. To this regard, in 2011 the ESHRE and Alpha Scientists in Reproductive Medicine defined three PN categories to standardize the zygote assessment. To date, no studies verified the efficacy and the reproducibility of such a recent classification.

Study design, size, duration:

We included 2799 zygotes from 731 women who underwent ICSI at our Center between 2014 and 2018. Zygotes were assessed 16-18 hrs after injection according to the Alpha consensus PN scoring system (1: symmetrical, 2: non-symmetrical, 3: abnormal). They were individually cultured. On day 2-3 1091 embryos were transferred into uterus, 1380 were cultured up to day 5-7, 213 arrested on day 2-3, 10 were cryoconserved on day 2-3; 105 zygotes did not cleaved.

Participants/materials, setting, methods:

Chi-square test was used to evaluate the relationship between the PN score and cleavage rate, quality of embryos, blastocyst development, clinical pregnancy rates. Clinical pregnancy was defined as viable pregnancy at 12 weeks. A multivariable logistic regression model was applied including the following characteristics: age of patient, PN score, embryo day 2-3 morphology grade. A P value <0.05 was considered significant. All analyses were carried out by R software, v. 3.4.1.

Main results and the role of chance:

A total of 2130 (76%) score 1, 594 (21%) score 2, and 75 (3%) score 3 zygotes were obtained. There was not a significant difference in day 2-3 morphology among the three PN score groups. Moreover, the zygote score did not always correlate with the embryo grade: only 58% top quality zygotes formed grade 1 embryos, and 51% poor quality zygotes (score 3) also became high quality embryos. A total of 1032 PN score 1-, 314 score 2-, and 34 score 3-derived embryos were placed in extended culture. We did not found any reduction of blastulation rates in score 2 (32%) and score 3 (50%) groups respect to score 1 (35%). A better clinical pregnancy rate for PN score 1- was found respect to score 2-3-derived embryos (14% and 7%, respectively, P=0.0101). Cycles with transfer of good quality cleavage-stage embryos from top quality zygotes had a significant higher clinical pregnancy rates than those with transfer of PN score 2-3-embryos (OR 0.42; 95% CI 0.21-0.77, P=0.008), even if the morphology of the embryos was good. Consistently, the PN score remained predictive of clinical pregnancy in the subgroup of embryos with top quality morphology grade (OR 0.43; 95% CI 0.21-0.68, P= 0.009).

Limitations, reasons for caution:

Although our findings could be interesting for the majority of laboratories conventionally performing discrete morphological assessment once a day by microscopic analysis, the main limitation of this study is its own not-morphokinetics nature. In fact, we did not evaluated the dynamics of pronuclear morphology and additional kinetic parameters.

Wider implications of the findings:

This is the first study applying the PN score system proposed by ESHRE. Although validation in randomized perspective studies is needed, our findings suggest that the PN score may be routinely included among criteria for embryo evaluation to provide patients with an increased chance of becoming pregnant.

Trial registration number:

N/A

O-245 Assessment of the oxidative stress levels in culture media as a biomarker of embryo cohort quality.

A. Mercader[1], L. Bori[2], L. Alegre[1], J.M. Zapata[2], M. Meseguer[1]

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[2]*IVIRMA Valencia, Research laboratory, Valencia, Spain*

Study question:

Are the oxidative stress levels measured in grouped cultured embryos spent culture media a biomarker of culture conditions, media quality or embryo quality?

Summary answer:

Optimum culture conditions linked with better pregnancy outcomes were related to oxidative stress levels.

What is known already:

Embryo's *in vitro* environment is known to affect their development and subsequent clinical outcome. The establishment of optimum culture conditions is therefore crucial aiming at the standardization of *in vitro* fertilization (IVF) protocols. The Thermochemiluminescence (TCL) Analyzer™ (Carmel Diagnostics, Israel) determines the oxidative status of the embryo's spent culture media assessing the adequacy of the embryo culture conditions, in terms of media, humidity, gas concentration, temperature, etc. The aim of the present study is to assess the use of a novel quality control device in IVF laboratories.

Study design, size, duration:

A prospective cohort study on 471 spent embryo culture media collected from 312 IVF cycles that were analysed from May 2017 to December 2018. Embryos were group cultured and monitored with time-lapse incubators: Embryoscope+® (Vitrolife) and Geri® (Genea). 15 µl/dish of embryo culture media were analysed through TCL Analyzer (Carmel Diagnosis).

Participants/materials, setting, methods:

The TCL Analyzer™ working mechanism consists on the heat-induced oxidation of biological fluids, leading to the production of light energy counted as photons emitted per second (cps). Cps amplitude is assessed in a 300-second period, where TCL parameters are obtained from: cps after 55 seconds (**H1**), 155 seconds (**H2**) and 255 seconds (**H3**). A smoothing algorithm (sm) was used to normalize data. Our results were statistically analysed by a multifactor Anova test.

Main results and the role of chance:

Even though both incubators allow cultivating 16 embryos in group, significantly higher oxidative stress was found in samples collected from Geri dishes than from ESD+ ones ($p<.05$). This might be due to the amount of culture medium in each dish (80µl and 180µl, respectively) and the impact over the concentration of free radicals. Samples from embryo cohorts cultured since blastocyst stage in single-step culture media (s-s) showed higher TCL parameters than those from embryos cultured in sequential culture media: **H1sm** (cps)= 93.24 for two-step vs. 98.75 for s-s, **H2sm** (cps)= 96.51 for two-step vs. 102.57 for s-s and **H3sm** (cps)= 104.89 for two-step vs 111.09 for s-s. In addition, samples provided from egg donor programs had higher oxidative stress values than those provided from cycles with autologous eggs. The mean and standard deviation of the TCL parameters were as follows (cps): 96.82 ± 39.34 for autologous eggs vs. 103.62±41.58 for donated eggs. Additionally, culture media from embryo cohorts that lead at least one ongoing pregnancy had significantly higher TCL parameters ($p<.05$). Culture media of embryo cohorts with no pregnancy showed an average of 92.08 ± 42.08 cps and 108.02 ± 39.88 cps for those with one or more pregnancy.

Limitations, reasons for caution:

There is a need to further assess the wide number of different independent variables in culture conditions that may be affecting the oxidative stress levels. Number of embryos cultured in each dish can vary depending on patient and may potentially affect the oxidation.

Wider implications of the findings:

TCL Analyzer™'s assessment has proven relationship with culture conditions (type of inclubator and type of media) embryo quality and reproductive outcome. Those values with higher oxidation were related with superior embryo quality and higher chances of a successful pregnancy.

Trial registration number:

Not applicable

O-246 Analysis of spindle morphology in MII oocytes matured in vitro from denuded GV and MI oocytes

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Study question:

How does the meiotic spindle morphology of MII oocytes from GVs and MI matured *in vitro* compare to that of MII oocytes matured *in vivo*?

Summary answer:

Spindles of MII from denuded GVs cultured *in vitro* show higher proportion of unfocused poles compared to those matured from MI or in vivo MII.

What is known already:

A functional spindle is required for chromosomal segregation during meiosis. GVs matured *in vitro* within the cumulus oophorous assemble spindles similar to *in vivo* matured MII oocytes. Maturation of GVs without cumulus cells (GV-MII) result in spindle abnormalities, chromosome misalignment and compromised developmental potential. Although aneuploidy rates in GV-MII are not higher than MII, disorganized chromosomes may contribute to compromised developmental potential. However, spindle morphology has not been quantitatively examined and no comparison with *in vitro* culture of MI oocytes matured to MII (MI-MII) has been reported

Study design, size, duration:

GV (n=109), MI (n=49), and MII oocytes (n=19) were retrieved from 30, 17 and 18 stimulated women, respectively, 20 to 35 years old. GVs and MI were denuded and cultured *in vitro* in G-2 PLUS medium until MII stage (approximately 30h and 8h, respectively). In vivo matured MII oocytes were stored vitrified, and warmed before processing.

Participants/materials, setting, methods:

All women were stimulated using a GnRH antagonist protocol, with a GnRH agonist trigger. Trigger criterion was 2 or more follicles ≥18mm; oocytes were harvested 36h later. Spindle microtubules were incubated with antibodies against alpha tubulin (T6199 Sigma-Aldrich at 1/100 dilution), chromosomes stained with Hoechst 33342 and samples subjected to confocal immunofluorescence microscopy (ZEISS LSM780). Images were collected and analyzed with ImageJ software; the Kruskal Wallis and Fisher's exact test were used for statistical analysis.

Main results and the role of chance:

Evaluation of spindle quality was based on measurements of the length of major, minor, proximal and distal axes, proximal/distal axis ratio, major to minor axis angle, spindle to cortex distance, spindle pole shape and spindle volume estimation as established in Cottichio et al 2013 (*Hum Reprod*). Of all matured oocytes, only properly oriented spindles (orthogonal orientation to the objective) were analyzed. All parameters were similar between groups, except for a) the proximal/distal axis ratio which was higher in MII oocytes and GV-MII as compared to MI-MII (Kruskal Wallis 3 way comparison, $p = 0.017$), and b) double flattened spindle poles (poles displaying a flat cross-segment >2µm with a barrel-like appearance), which were observed in 12/15 of the GV-MII (80%) compared to 6/20 of MI-MII (30%) and 5/19 of MII (26.3%) (Fisher´s exact test, $p = 0.0035$). We hypothesize that the loss of microtubule focus points at spindle poles may contribute to chromosome misalignment, delayed chromosome separation and compromised developmental potential.

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 the cumulus oophorus is a major factor in oocyte maturation and correlation with spindle dynamics has been inferred.

Wider implications of the findings:

The inability to organize focused poles could be an underlying cause of impaired quality of GV-MII. Microtubule motors (dynein-dynactin), pole components (NuMA) and minus-end binders may be imbalanced; investigating the localization of these factors in GV-MII in comparison to MII oocytes would offer a molecular understanding of the flat-pole phenotype.

Trial registration number:

not applicable

O-247 A novel mutation in the TUBB8 gene is associated with a complete oocyte maturation arrest: a case report about an infertile woman

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Study question:

Is there a new mutation in the tubulin beta eight class VIII *(TUBB8)* responsible of female infertility with an oocyte maturation arrest phenotype?

Summary answer:

We identified a new mutationsin *TUBB8 gene* associated with a total oocyte maturation arrest. This mutation was confirmed by a family analysis.

What is known already:

A genetic defect in the *TUBB8* gene has been shown to be responsible for oocyte maturation, fertilization and early embryonic developmental arrest. This gene encodes a special β-tubulin isotype that is the major component of the oocyte and early embryo spindle. Heterozygous or de novo mutations in the TUBB8 gene are associated mainly to human metaphase I (MI) arrest and some early embryonic development failure. Other homozygous or compound heterozygous mutations seem to be responsible of fertilization failure and early embryonic development arrest.

Study design, size, duration:

We have characterized a patient with 5-year history of primary unexplained infertility belonging to a consanguineous family from Tunisia and with infertile relatives. During 3 assisted reproduction attempts (conventional IVF, ICSI and IVM), the woman presented a total MI oocyte arrest for each oocyte retrieval after controlled ovarian hyperstimulation. We performed Sanger sequencing of the *TUBB8* gene in the patient and her family members. A spindle immunostaining was performed on some blocked MI oocytes.

Participants/materials, setting, methods:

The oocytes morphology was evaluated by light inverted microscope. Oocyte spindles were stained with anti β-tubulin antibody after fixation and membrane permeabilizing, then examined by confocal microscopy. Genomic DNA samples of the patient and her family's members were extracted from peripheral blood using Chemagic technique. The 4 exons composing *TUBB8* were amplified and sequenced using an Illumina NextSeq500. TUBB8 sequences was aligned by Human GRCh37/hg19 UCSC Genome Browser and analyzed by SIFT and Polyphen-2.

Main results and the role of chance:

In total, after 3 oocytes retrievals, 42 oocytes were collected for our 32 years-old patient. All (13, 10 and 19 oocytes) were arrested at MI. Six oocytes from the second oocyte cohort were immunostained to analyze the spindle structure. The spindles were very weakly visible and unstructured comparing to control.

The genomic analysis of the fourth exon found a missense homozygous variant c.935 C>T (**p.Thr312Met**) in the patient, her father and her two sisters. The same mutation was detected for the mother and the brother but with a heterozygous expression. According to biostatistics browsers SIFT and Polyphen-2, this variant is considered as deleterious and probably damaging. In ExAC browser, this variant is rarely described, only 3 times among 243004 alleles, and always as heterozygous.

According to the genomic analysis, the missense homozygous variant **p.Thr312Met** (unknown signification, Class III) located on the C-terminal domain of β-tubulin with a recessive autosomic father inheritance, can explain the patient infertility and her sisters infertilities (2 and 3 years respectively, but not attending an ART center yet). The detection of this variant may confirm the phenotypic arrest of oocyte maturation at MI stage, that has been verified by a molecular proof (the spindle immunostaining) too.

Limitations, reasons for caution:

The genomic analysis (DNA extraction DNA, TUBB8 sequencing) of blocked oocytes is not performed yet to detect the local expression of this variant.

A causal relationship between t mutation and the spindle assembly need to be established by microinjection of wild-type and mutant TUBB8 encoding RNAs on K.O. mouse oocytes.

Wider implications of the findings:

Yet, this case report brings a new insight about the role of *TUBB8* on meiosis and the possibility that a TUBB8 homozygous mutation can lead to a MI maturation arrest and not only heterozygous mutations are responsible of it.

Trial registration number:

None

O-248 Does the trophectoderm biopsy technique affect the result of the genetic analysis in PGT-A cycles?

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Study question:

Does the trophectoderm biopsy technique, pulling or flicking, affects the diagnostic rate, mosaicism rate or transferability rate displayed by the genetic analysis in PGT-A cycles?

Summary answer:

Pulling required more laser shots to obtain the same number of cells from the trophectoderm, and higher mosaicism rate was observed when pulling was performed.

What is known already:

Preimplantation genetic testing for aneuploidy (PGT-A) aims to find the best euploid embryo, improving live birth rate per transfer. The analysis of single day 3 cells has gradually led to trophectoderm (TE) biopsy, increasing the number of cells available, allowing the detection of mosaicism and being less harmful for the embryo.

Two techniques have been proposed: "pulling" or "flicking". Among 4-8 cells must be biopsied and the inner cell mass must be place far from the TE cells biopsied.

Although it has been demonstrated that TE biopsy does not harm the embryo, the biopsy technique may affect the genetic test.

Study design, size, duration:

144 PGT-A cycles were included in this retrospective analysis involving 421 blastocysts biopsied between January 2018-December 2018 by two expert embryologists[RB1] .

Assisted hatching on day 3 were performed; 212 blastocysts were biopsied using pulling and 209 using flicking; 244 were biopsied on day 5 and 177 on day 6; 273 blastocysts came from egg donation cycles (64.8%).

Each blastocyst was biopsied using pulling or flicking depending on the embryologist available to perform the procedure.

Participants/materials, setting, methods:

In the "flicking method", cells were aspirated and removed by a quick movement of the biopsy pipette against the holding.

In the "pulling" method, cells were suctioned and pulled away while laser pulses were applied.

Same embryologist performed biopsy and tubing, and registration of sample quality assessment, number of cells retrieved and number of laser shots (NLS).

Blastocysts were classified as miss-diagnosed or informative (euploid, aneuploid, transferable-mosaic, non-transferable-mosaic).

T-test, Chi-square and logistic regressions were performed.

Main results and the role of chance:

No significant differences were found between groups in: number of oocytes, oocyte age, sperm concentration, embryo quality (day 3) or blastocyst expansion and quality.

Regarding the biopsy technique, flicking and pulling reveals no differences in number of cells biopsied (5.58±1.5 vs 5.34±1.3, p=0.103), fragment visualization during tubing (97.1% vs 95.3%, p=0.322), amplification failure rate (4.3% vs 6.1% p=0.400) and transferability rate (55% vs 48%, p=0.177), respectively.

However, we found statistically differences in favor of the flicking method: mosaicism rate was higher in the pulling group (17.1% vs 9.5%; p=0.026). Moreover, the pulling method needed more shots than the flicking method (6.18±2.4 vs 3.51±1.9, p <0.0001) and also sample quality was better in flicking group (86.1% vs 78.3%, p=0.018).

A regression analysis was carried out to check which variables could affect the possibility that an embryo was transferable. The biopsy technique was the only influencing factor (p=0.019, OR=1.868, CI95% 1.109-3.146). Moreover, the biopsy technique turned out to be the most influencing factor in the possibility of being mosaic (p=0.026, OR=2.375, CI95% 1.11-5.076). Notwithstanding, when NLS appeared in the equation, biopsy technique disappeared as influencing factor, and NLS was the only one close to be statistically significant (p=0.051, OR=1.180, CI95% 0.999-1.394).

Limitations, reasons for caution:

These results came from an IVF laboratory with two embryologists, using the same micromanipulation and laser equipment. Further studies are needed to make consistent conclusions about the impact of biopsy technique, the influence of the NLS on the genetic analysis, and the correlation between NLS and mosaicism.

Wider implications of the findings:

In our study, both biopsy techniques resulted in similar euploidy rate, but flicking technique was proved to need less laser shots to obtain the same number of cells from the TE, resulting in better fragment quality. Biopsy by flicking also was associated to less mosaicism rate.

Trial registration number:

SELECTED ORAL COMMUNICATIONS

SESSION 67: ICSI AND SURGICAL SPERM RETRIEVAL

Wednesday 26 June 2019 Haydn 2 10:00 - 11:45

O-249 Men with fertilization failure linked to PLCZ1 mutations exhibit abnormal phospholipase C zeta pattern and overcome by ICSI with assisted oocyte activation

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Study question:

Does different *PLCZ1* mutations affect levels and localization patterns of the oocyte activation factor phospholipase C zeta (PLCz) and how to overcome fertilization failure?

Summary answer:

Three men with *PLCZ1* mutations exhibited abnormal localization in PLCz and no dynamic changes during capacitation and acrosome reaction, which overcame by ICSI with AOA.

What is known already:

PLCz is a sperm-related oocyte activate factor that is required to induce normal calcium oscillations after fertilization. Abnormal patterns of Ca^{2+} oscillations caused by infertile male sperm are associated with oocyte activation defects and fertilization failure. PLCz is localized to acrosomal and post-acrosomal regions and undergoes dynamic changes during capacitation and the acrosome reaction. Previous studies reported that two biallelic mutations of *PLCZ1* identified in two men were related to fertilization failure.

Study design, size, duration:

Twenty-eight couples with fertilization failure were collected from January 2014 to December 2018. Seven of twenty-eight infertile couples were tested by mouse oocyte activation test (MOAT) that ensure sperm-related activation deficiencies.

Participants/materials, setting, methods:

Four affected men and their parents from consanguineous families and three men from non-consanguineous families were subjected to whole-exome sequencing. After identifying possible causative genes, in silico and evolutionary conservation analysis was used to evaluate possible effects of candidate mutations on protein secondary structure. Immunofluorescence was used to analyze the protein levels of PLCz and acrosome status.

Main results and the role of chance:

In fertilization failure couples of China, about 25% (7/25) couples were caused by sperm factors. From seven men with definitive sperm factors, 3 patients were identified homozygous mutations in *PLCZ1* gene: c.T1048C (p.S350P), c.C736T (p.L246F) and c.C588A (p.C196X), which were highly conserved. These infertile men exhibited normal sperm counts, motility, and sperm morphological features. Though immunofluorescence of PLCz and Lectin, sperm from three *PLCZ1* mutations showed no dynamic changes during capacitation and the acrosome reaction. The sperm with p.S350P showed signal for PLCz in the post-acrosomal region, and sperm with p.L246F exhibited signal in opposite ends in equatorial region. The sperm with homozygous stop-gain mutation p.C196X exhibited no expression of PLCz. Among them, three patients were treated with ICSI with assisted oocyte activation (AOA) in the next cycle, and successful fertilization and embryo development was obtained (the rate of fertilization and good-quality embryo: 76.4% and 41.2%). Two of them got successfully pregnancy.

Limitations, reasons for caution:

Only a small number patient with *PLCZ1* mutations was available because of its rare incidence.

Wider implications of the findings:

This study expanded the mutation spectrum of *PLCZ1* gene and found the mutation frequency was 42.9% (3/7) in male-factors ICSI fertilization failure population in China. It uncovered the abnormal pattern of PLCz expression may effect the Ca^{2+} oscillations. It further provided a therapeutic and prognostic method for *PLCZ1* mutations patients.

Trial registration number:

None.

O-250 Prediction of sperm extraction in men with non-obstructive azoospermia (NOA):A machine learning perspective

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Study question:

Can clinical and biological variables-based machine learning support the prediction of the presence or absence of sperm in testicular biopsy in NOA patients?

Summary answer:

Our machine learning model was able to predict (AUC of 0.8) presence or absence of spermatozoa in patients with NOA.

What is known already:

Patients with NOA can conceive with their own biological gamete using intra-cytoplasmic sperm injection (ICSI) in combination with testicular sperm extraction. Testicular sperm retrieval is successful in up to 50% of men with NOA; however, to the best of our knowledge there is no existing model that can accurately predict the success of sperm retrieval in testicular sperm extraction (TESE). Moreover, machine learning was never used for this purpose.

Study design, size, duration:

A retrospective cohort study consisting of 118 patients who underwent TESE in a single IVF unit between the years 1995-2017 was conducted. All patients with NOA who underwent TESE procedure during their fertility treatments were included. To develop a random forest model(RFM) to predict presence or absence of spermatozoa in patients with NOA in comparison to a multivariable logistic regression mode(MvLRM).

Participants/materials, setting, methods:

We used univariable and multivariable binary logistic regression to try and predict the probability of successful TESE using a dataset from a retrospective cohort. In addition, we used the ensemble machine learning model (Gradient Boosting Trees) and evaluated its predictive performance using the leave one-out cross validation procedure. A cut–off value for successful/unsuccessful TESE was calculated with receiver operating characteristic (ROC) curve analysis.

Main results and the role of chance:

ROC analysis resulted in an AUC of 0.807± 0.064 (95%CI, 0.682-0.927) for the proposed RFM and 0.75 ±0.052 (95%CI 0.65-0.85) for the MvLRM to predict presence or absence of spermatozoa in patients with NOA. This RFM approach and the MvLRM yielded, respectively sensitivities of 78% and 97% and specificities of 75% and 25% A total of 77 (65.3%) men with NOA experienced successful TESE. FSH, LH, testosterone, semen volume, age, BMI, ethnicity, and testicular size on ultrasound were included in those models.

Limitations, reasons for caution:

This study is a retrospective cohort study with all associated inherent biases. This model was used only for TESE, since micro-TESE is not performed in our center

Wider implications of the findings:

This machine learning model can support the clinicians together with their patients when making decision concerning TESE in patients with NOA. The findings of this study should be confirmed with further largerand prospective studies

Trial registration number:

SOR-0053-17

O-251 The FSHB -211 G>T polymorphism as predictor for testicular sperm extraction success (TESE) in patients with unexplained azoospermia

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Study question:
The FSHB -211G>T single-nucleotide polymorphism (SNP) is known to impact spermatogenesis, however to which extent it also serves as predictor for sperm retrieval rates in TESE is unknown.

Summary answer:
In patients with unexplained azoospermia the FSHB -211 G>T polymorphism is significantly associated with positive sperm retrieval rate in surgical testicular sperm extraction.

What is known already:
In azoospermia, characterized by the lack of sperm in semen, the etiology for the phenotype remains most often unexplained. Patients are often referred to TESE followed by assisted reproductive techniques. The known non-invasive predictive parameters for sperm retrieval rate (SRR) like FSH level and testicular volume are inadequate to counsel azoospermic patients prior to surgery. Therefore, powerful non-invasive predictive markers are warranted.FSH plays a key role in initiation and maintenance of spermatogenesis. One well-studied single nucleotide polymorphism (SNP) within the FSHB gene (c.-211G>T) impacts endocrine feedback, testicular size and spermatogenesis. However, its impact on SRR is so far unknown.

Study design, size, duration:
In a monocentric, retrospective analysis we studied 1075 patients with azoospermia who had undergone TESE procedure at our centre. In 248 couples subsequent TESE-ICSI was performed. We evaluated the association of the FSHB -211G>T SNP (rs10835638), as well as two SNPs in the FSH receptor FSHR -29G>A (rs1394205) and FSHR c.2039A>G (rs6166) with reproductive hormones, testicular histopathology, sperm retrieval rate and pregnancy rate.

Participants/materials, setting, methods:
Patients were grouped upon the etiology of azoospermia: i) unexplained ii) genetic cause iii) obstruction. Histological evaluation was classified according to the highest achieved grade of spermatogenesis (complete vs. arrest vs. Sertoli cell only). TESE was considered positive if at least one spermatozoon was detected. Biochemical and clinical pregnancy were defined by serum hCG >30IU/L or fetal heartbeat. Logistic regression models were used to analyze the association of continuous and categorical variables with binary outcomes.

Main results and the role of chance:
We observed a significant association of FSHB -211G>T with reduced FSH levels in patients with unexplained azoospermia. For the histopathological analysis we found in the same cohort that T allele homozygosity was 8 times higher in patients with Sertoli cells only, than in patients having complete spermatogenesis ($p<0.05$). The FSHB -211G>T T allele count was significantly associated with reduced chances of positive sperm retrieval in TESE. For minor allele homozygosity (TT) Odds ratio was 0.20 (0.06 to 0.70, p=0.01). Interestingly, the association of the SNP with SSR could not be solely attributed to decreased FSH levels. Our results indicate an improvement of the predictive power (proportional reduction in error) by 7% for FSHB -211G>T in the group of unexplained azoospermia. In patients with azoospermia due to genetic (Klinefelter Syndrome, Y-Microdeletions) or obstructive disease the FSHB -211 G>T did not have an impact on testicular histopathology nor SRR. FSHR -29G>A and FSHR c.2039A>G were not associated with testicular histopathology or SRR in any etiology.61 out of 248 female partners obtained pregnancy after TESE-ICSI. FSHB -211G>T minor allele count was not associated with pregnancy rates ($p = 0.62$), and fertilization potential seems not to be reduced by this genetic variant.

Limitations, reasons for caution:
This analysis delineates FSHB -211G>T as a new, remarkably informative and non-invasive predictor for SRR. This effect could be mediated by an inadequately low FSH stimulus in T allele carriers, which might affect Sertoli cell proliferation and numbers earlier in life, however this remains to be elucidated in subsequent studies.

Wider implications of the findings:
In perspective, a calculator/score including the non-invasive parameters FSH, testicular volume and the FSHB haplotype can be considered to estimate the chances for sperm retrieval in azoospermic men.

Trial registration number:
Study funding: DFG Clinical Research Unit 326 Male Germ Cells

O-252 Heterozygous mutations in PLCZ1 are associated with fertilization failure after ICSI

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Study question:
Are heterozygous mutations in the phospholipase C zeta (PLCZ1) gene associated with fertilization failure after ICSI?

Summary answer:
PLCZ1 mutation screening in 35 patients with failed or low fertilization after ICSI resulted in the identification of six different mutations in twelve patients.

What is known already:
The oocyte activation process is initiated by sperm-oocyte fusion and is characterized by a series of calcium oscillations in the oocyte cytoplasm.Phospholipase C zeta (PLCZ1) is responsible for this series of calcium oscillations, which hallmarks the start of oocyte activation.Mutations in *PLCZ1* gene are described in some case reports to cause oocyte activation failure(OAF) and subsequent fertilization failure after ICSI. To obtain more insight in to the role of PLCZ1 in fertilization and to know the frequency of *PLCZ1* gene mutations in patients experiencing failed or low fertilization after ICSI, *PLCZ1* gene screening in larger cohort of patients is required.

Study design, size, duration:
Patients (n=35) with previously low or total failed fertilization after routine ICSI were enrolled in the study after obtaining written consent from 2014-present. Genetic screening for *PLCZ1* mutations was performed, next to evaluation of the activation potential of the sperm

Participants/materials, setting, methods:
Genomic DNA was extracted from the saliva samples. PCR was performed using primers designed to amplify all the 15 coding exons and the exon-intron boundaries. The amplified PCR products were sequenced using illumine MiSeq. A diagnostic test Mouse oocyte activation test (MOAT) was performed on the mouse oocytes by injecting patient sperm in to mouse oocytes and determine the number of activated oocytes, in comparison to a positive control

Main results and the role of chance:
PCR amplification and Illumina Sequencing of *PLCZ1* gene revealed six different mutations in twelve patients, which include four missense mutations D46N, H233L, S500L and R141H, one invariant splice site mutation c.136-1G>C and one protein truncating mutation K322∗. Three of the patients had experienced partial hydatidiform moles (PHMs) with their partners *in vivo*. Of the twelve patients, eight patients carried mutations in a heterozygous state which include four patients with S500L mutation, two with H233L mutation, one with R141H mutation and one patient with c.136-1G>C mutation. Two patients carried S500L mutation in a homozygous state. Two patients carried two mutations in each, one patient had D46N and S500L and the other patient had H233L and K322∗ in a compound heterozygous state.The activation potential of the sperm was classified in three groups according to the MOAT result; based on the percentage of mouse oocytes activated by patient sperm: MOAT1 group(0-20%), MOAT2 group(21-84%) and MOAT3 group(>85%).The patient with H233L and K322∗ mutations in a compound heterozygous state was classified in MOAT1 group and the patient with H233L mutation and R141H mutation showed normal activation potential (MOAT3 group). All the other patients were classified as MOAT2 group.

Limitations, reasons for caution:
We are now verifying further the functional effect of these mutations by making recombinant protein of these mutations and injecting them into oocytes

to evaluate its effect on activation rate, calcium pattern and embryonic developmental potential.

Wider implications of the findings:

From our study we show that most of the patient's sperm with heterozygous mutations cannot initiate oocyte activation and the frequency of PLCZ1 mutations in our patient cohort with OAF is as high as 34% and it is therefore recommended to do PLCZ1 screening for better management of OAF.

Trial registration number:

not applicable

O-253 Clinical results of couples with AZFc microdeletion using testicular sperm

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Study question:

To clarify the results of intracytoplasmic sperm injection (ICSI) using sperm of azoospermia factor (AZF) c microdeletion patients.

Summary answer:

Sperm was retrieved from 88.2% patients with AZFc microdeletion, however, fertilization rate was fairly low in ICSI using sperm from those patients.

What is known already:

Microdeletions of Y chromosome, especially in the AZF, are the most common known genetic cause involving spermatogenesis and three spermatogenesis loci in the Y chromosome long arm have been classified as AZFa, AZFb, and AZFc. Especially, AZFc microdeletion is the most commonly encountered Y microdeletion, comprising 60% of all AZF deletions type that range from oligozoospermia to complete azoospermia and patients with AZFc microdeletions have a chance to father babies through sperm extraction techniques in vitro fertilization with ICSI and there were few reports regarding ICSI outcome in the couples of AZFc microdeletion.

Study design, size, duration:

This study was retrospectively examined a total of 1222 patients underwent microdissection testicular sperm extraction (micro TESE), including 51 with AZFc microdeletion (36.1±5.1 years of age) and 649 with unexplained NOA (unexplained NOA; not including after orchidopexy, Klinefelter's syndrome, cryptozoospermia, mumps orchitis, etc) (38.4±6.1 years of age) a reproductive center from September 2013 to December 2018.

Participants/materials, setting, methods:

A total of 1077 azoospermic patients were examined genetic testing for AZF deletions by Promega Y Chromosome AZF Analysis System (version 2.0). A total of 45 patients with AZFc microdeletion who attempted ICSI cycles were studied. Outcomes were compared sperm retrieval rate (SRR), two pronuclei (2PN) oocytes, blastocysts development, good-quality blastocysts (Grade 3BB and above on day 5 by the Gardner scoring), and clinical pregnancy rate between AZFc microdeleted group and control group (unexplained NOA).

Main results and the role of chance:

We identified 111 Y chromosome microdeletions out of 1077 azoospermic patients (10.3%). Forty-five (4.2%) had AZFc deletion, and 65 of microdeletions were AZFa, AZFb, AZFb+c, or complete Yq deletions in azoospermia.

Sperm were retrieved 45 of 51 in AZFc deleted patients (88.2%) and 121 of 649 in unexplained NOA (18.6%). In first TESE cases, 87.1% were retrieved (27/31) in AZFc deleted patient similar to those of patients who previously underwent micro TESE were retrieved to 90.0% (18/20). Whilst in unexplained NOA, SRR in first TESE cases was significantly higher than patients who previously underwent micro TESE (21.2% vs. 11.4%). AZFc deleted group was significantly higher SRR than unexplained NOA ($P<0.001$) by micro TESE. As for the number of motile sperm that we injected for oocytes in ICSI, in AZFc microdeletion was 343/929 (34.9%) and unexplained NOA was 1796/2066 (86.9%) ($P<0.001$). There were significant differences for 2PN oocytes rates (41.3% vs. 53.9%), blastocyst development rates (14.8% vs. 45.1%) and good-quality blastocyst rates (9.4% vs. 18.7%) ($P<0.001$). Clinical pregnancy rates per embryo transfer cycles for AZFc deleted group (27.5%) were similar to those for unexplained NOA (29.7%). The number of healthy babies of the AZFc deleted couples was 15 so far.

Limitations, reasons for caution:

In this study we included both of cryptozoospermia and azoospermia. We did not show the data using ejaculated sperm with AZFc microdeletion. We need long-term follow-up of babies of the AZFc deleted couples.

Wider implications of the findings:

Sperm retrieval rates for the AZFc microdeleted patients were significantly higher than those of unexplained NOA patients with. However, couples with AZFc microdeletion seem to have lower ICSI outcomes than unexplained NOA.

Trial registration number:

None.

O-254 Effect of sperm processing time on reproductive outcomes after ICSI

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Study question:

Do sperm processing times, from obtaining the sample to fertilization, affect reproductive outcomes in ICSI cycles?

Summary answer:

Sperm processing times do not seem to affect fertilization rates (FR), pregnancy or live birth rates.

What is known already:

Sperm sample processing is necessary in IVF, but processing times differ both between and within facilities. While longer processing times are associated to increased sperm DNA fragmentation, intermediate processing times seem to be beneficial for capacitation, chromatin condensation and FR after ICSI; nevertheless, controversy on the appropriate processing time to maximize outcomes still exists. Most studies make use of manual annotation to record times, and do not report live birth rates as outcomes. We use an operator-independent radiofrequency-based time recording system and extensive statistical analysis to assess the effect of processing times on reproductive outcomes up to live birth.

Study design, size, duration:

Retrospective cohort study of 560 ICSI cycles carried out between 2012 and 2016. All cycles included patient's own fresh semen and oocytes either from partner or donor, followed by fresh embryo transfer (ET). A radiofrequency-based system was used to record sperm processing times: T1: from sample collection to swim-up; T2: swim-up to ICSI; T3: total time (T1+T2). We analyzed the effect of these times on FR, biochemical, clinical, ongoing pregnancy, and live birth (LB) rates.

Participants/materials, setting, methods:

Differences in processing times (T1, T2, and T3) between positive and negative pregnancy rates (biochemical, clinical, ongoing) and LB rates were tested by Student's t-test. The likelihood of pregnancy and LB was modeled by LOWESS regression and logistic regression, adjusting for man's and woman's age and BMI, semen parameters, number of oocytes, number and morphological score of transferred embryos, and day of ET. The effect of times on FR was modelled by generalized linear modelling.

Main results and the role of chance:

Mean male age was 42.5±7.1 years, while women were 33.15±9.65 years on average. Mean sperm concentration and motility were 45.2±72.5 million/ml and 34.1±19.7 %a+b, respectively, and semen processing times in hours were T1: 0.36±0.24 [range: 0.09-1.63], T2: 3.28±2.2 [0.23-10.7] and T3: 3.64±2.2 [0.42-11.2]. Biochemical, clinical, ongoing pregnancy and live birth rates overall were 42.1%, 32.5%, 29.5% and 29.5%, respectively. Neither T1, nor T2 or T3 were significantly different for patients who did achieved pregnancy (biochemical, clinical and ongoing) and LB compared to those who did not. LOWESS regression did not return relevant correlations between T1 and FR or reproductive outcomes, while T2 and T3 showed a slight positive tendency to higher ongoing pregnancy and LB upon time. However, multivariate analyses did not reveal a significant effect for any of the time intervals on LB rate: OR 1.003 (95%CI: 0.72-1.40) for T1, and OR 1.091 (95%CI: 0.85-1.41) for T2. The same results were found for biochemical, clinical and ongoing pregnancy. Similarly, generalized linear modelling analysis did not show a significant effect of sperm processing time on FR after ICSI ($p=0.27$ for T1, $p=0.54$ for T2).

Limitations, reasons for caution:

These results should not be extended to ICSI cycles using testicular spermatozoa or samples with severe semen parameters, such as motility <1% a+b or cryptozoospermia, which were excluded from the study.

Wider implications of the findings:

This is the first study evaluating the effect of sperm processing time on ICSI using an exact, radiofrequency-based, operator-independent system on a large cohort. Our results suggest that processing time, especially after swim-up, have undetectable effect on pregnancy and live birth rates after ICSI, within the time interval analyzed.

Trial registration number:

not applicable

O-255 Evidence that testicular sperm in infertile men has improved DNA integrity compared to ejaculated sperm

J. Ramsay[1], L. Vyas[1], E. Foster[1], A. Taylor[3], J. Nicopoullos[3], R. Faris[3], S. Minhas[1], S. Lewis[2]

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[2]*Examen, Examen, Belfast, United Kingdom*
[3]*Lister Hospital, Fertility clinic, London, United Kingdom*

Study question:

Is there any difference in DNA integrity between testicular sperm and epididymal sperm, compared with fertile donors

Summary answer:

This data provides novel and compelling evidence that testicular sperm from infertile men has no significant difference in comet score compared to fertile donors.

What is known already:

The prevalence of high DNA fragmentation occurs in male partners of unexplained infertile couples. DNA fragmentation in sperm can increase the risk of miscarriage and IVF/ICSI outcomes. There are multi-factorial causes of DNA damage and Comet is a gel electrophoresis test used to measure DNA fragmentation. It can be conducted in an alkaline or neutral solution and gives a percentage for DNA fragmentation.

Study design, size, duration:

Sperm DNA fragmentation was measured by alkaline Comet assay to assess double plus single stranded breaks and neutral Comet assay to determine double stranded breaks only. Main outcome measures included comparison of single stranded break (SSB) and both double stranded breaks (DSB) and SSB, in ejaculates and with testicular sperm. Average Comet scores, Low Comet scores and High Comet scores were determined. Comparisons made for ejaculated and testicular sperm from infertile men and fertile donors.

Participants/materials, setting, methods:

59 men with persistently raised DNA fragmentation from couples who had failed cycles of intracytoplasmic sperm injection (ICSI) with ejaculated sperm underwent testicular sperm extraction. Results were also compared with 76 fertile donors. In 10 infertile men ejaculate comparison was made between single stranded DNA abnormalities compared to single and double stranded abnormalities

Main results and the role of chance:

For total DNA damage ejaculate Average Comet scores (ACS) (%) from infertile men was 39.9+/- 1.3 versus 17.9+/-1.3 in testicular sperm and 14.8+/- 0.6 from ejaculates from fertile donors[sm1] .

Ejaculate Low Comet scores (LCS) (%) from infertile men was 34.7+/- 2.7 versus 77.3+/-2.4 from testicular sperm and 90.2+/- 1.0 from ejaculates from fertile donors.

Ejaculate High Comet scores (HCS) (%) from infertile men was 29.1+/-2.9 v 8.8+/-1.8 from testicular sperm and 1.9+/-0.4 from ejaculates of fertile (P<0.01) donors.

Comparison of infertile ejaculate with fertile ejaculate was significant for all 3 groups of scores (P<0.001).

The comparison of SSB only v SSB plus DSB in testicular sperm sdemonstrated ACS (%) 10.6+/-1.2 v 14.7+/-0.9; LSC (%) 91.3+/- 2.0 v 92.2+/-1.4; HSC (%) 4.4+/-1.2 v 1.89+/-0.5 (P>0.05).

The total sperm DNA damage in testicular sperm from infertile men is not significantly different from ejaculated sperm from fertile donors (P=0.08). However, DSB made up most DNA damage in testicular sperm and only half of the damage observed in ejaculated sperm.

Limitations, reasons for caution:

To understand the clinical utility of the test.

Wider implications of the findings:

This data provides novel and compelling evidence that testicular sperm from infertile men have the same high DNA quality as ejaculated sperm from fertile donors. DNA damage in testicular sperm is all double stranded compared to only half of DNA damage in ejaculated sperm in a subgroup.

Trial registration number:

Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 68: RECENT DEVELOPMENTS IN POOR OVARIAN RESPONSE

Wednesday 26 June 2019 Haydn 4 10:00 - 11:45

O-256 Cumulative live birth rates in women with poor ovarian response (POR) meeting the Bologna criteria: the importance of the phenotype

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[5]*IVI - Lisboa, Instituto valenciano de infertilidad, Lisboa, Portugal*

Study question:

Does reproductive outcome differ among the various types of women with poor ovarian response (POR) meeting the Bologna criteria?

Summary answer:

Live birth rates (LBR) and cumulative LBR differ significantly among Bologna POR patients

What is known already:

In 2011, the European Society of Human Reproduction and Embryology (ESHRE) elaborated on the definition of women with inadequate response to ovarian stimulation. This consensus definition—known as the Bologna criteria—was initially introduced with the primary objective of standardising the definition of POR. However, the Bologna criteria may have merged various patient categories with potentially different prognosis. Evidence regarding the reproductive outcome of different categories of patients is sparse.

Study design, size, duration:

This was a retrospective cohort analysis carried out at a university based tertiary centre aiming to evaluate cumulative LBR in different categories of Bologna POR. All Bologna POR patients who underwent ovarian stimulation for ART using a GnRH-antagonist protocol from 1st January 2011 until 31th December 2017 were included in the study.

Participants/materials, setting, methods:

Women were divided in four categories according to their Bologna criteria pattern: group A women ≥ 40 years with an abnormal ovarian reserve test; group B women ≥ 40 years with an abnormal ovarian reserve markers and one previous cycle with poor response; women in group C were ≥ 40 years and had one previous cycle with poor response; group D patients with an abnormal ovarian reserve test and one previous cycle with poor response.

Main results and the role of chance:

In total 846 cycles in 706 Bologna POR patients were included in the analysis: 310 cycles in group A, 169 in group B, 52 in group C and 315 in group D. There were significant differences in female age, antral follicle count, antimüllerian hormone, cycle cancellation rates and number of retrieved oocytes between the four groups. LBR and cumulative LBR differed significantly between groups and were highest in Group D (LBR: 7.4% (A) vs. 4.1% (B) vs. 5.8% (C) vs. 13.4% (D),

p =0.001 and CLBR: 8.3% (A) vs. 4.1 % (B) vs. 9.6% (C) vs. 16.8% (D) p<0.001). In particular, the p values for the unadjusted cumulative LBR between groups were as followed: Group B vs A (p=0.15), Group C vs A (p=0.9), Group D vs A (p<0.001), Group C vs B (p=0.2), Group D vs B (< 0.001), Group D vs C (p=0.08). A multivariate regression model accounting for relevant confounders demonstrated that the Bologna criteria pattern was an independent predictor of cumulative LBR (coefficients Group (A): reference group, Group (B): -0.6, Group(C): 0.05, Group (D): 0.8, p value <0.001). The number of oocytes retrieved was also significantly associated with cumulative LBR.

Limitations, reasons for caution:

The retrospective study design should be taken into consideration when interpreting these results.

Wider implications of the findings:

POR represent a heterogeneous population with distinct clinical prognosis. This is the first study evaluating cumulative LBR in the different Bologna criteria patterns.

Trial registration number:

Not applicable

O-257 The differences on the cumulative live-birth rates in poor ovarian responders using mild and controlled ovarian stimulation protocols:a randomized controlled study

X.P. Liu[1], B. Wang[1], T.T. Li[1], R. Huang[1]

[1]*Reproductive Medicine Center, 6th Affiliated Hospital-Sun Yat-sen University, Guangzhou, China*

Study question:

What is the difference on the cumulative live birth rate between mild ovarian stimulation and conventional controlled ovarian stimulation for poor ovarian responders?

Summary answer:

There were similar cumulative live birth rates between mild ovarian stimulation and conventional controlled ovarian stimulation for poor ovarian responders.

What is known already:

In in vitro fertilization (IVF) treatment a considerable proportion of women responsed poorly during conventional ovarian stimulation, facing with a low number of retrieved oocytes and available embryos for transfer. These poor responders have reduced pregnancy rates compared with normal responders. Poor ovarian response (POR) to controlled ovarian stimulation (COS) occurs in 9%–24% of women undergoing IVF. Treatment of this common condition remains a major challenge in assisted reproduction technology (ART). A number of studies have sought to determine the ideal protocol for women identified as poor responders, but most have failed to reach definitive conclusions.

Study design, size, duration:

A randomized, controlled, prospective study,conducted from September 2013 to September 2015 at the Reproductive Medicine Research Center, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.

Participants/materials, setting, methods:

One hundred and ninety-one patients who met the Bologna criteria of poor ovarian response were recruited in this study.

All women were prospectively randomized into two groups according to random number table. The patients in LA group were stimulated according to the letrozole/antagonist protocol (n=97), while the patients in HGS group were stimulated according to a high dose of Gn combined with GnRH agonist stop protocol (n=94).

Main results and the role of chance:

Demographic parameters including mean age, duration of infertility, body mass index (BMI), serum AMH, AFC and basal FSH level were similar between two groups before the initiation of stimulation protocols.Comparing with HGS group, both the stimulation duration and the total gonadotropin dose was significantly lower in the LA group (6.33±1.80 vs. 10.81±2.38 day, 939.43±264.52 vs. 3117.82±749.56 U, respectively; *P*<0.001). Likewise, the serum E_2 level at trigger was statistically different between two groups (563.69±465.06 vs. 1195.10±701.45 pg/mL, respectively; *P*<0.001). No significant difference was found between the two groups in the serum LH and P levels on the trigger day.A higher number of retrieved oocytes (2.77±1.89 vs. 4.01±2.81; *P*<0.001), number of 2 pronuclei (2PN) oocytes (1.74±1.52 vs. 2.53±2.01; *P*<0.001) and transferred embryos (1.50±1.41 vs. 1.97±1.61; *P*=0.038) was observed in the HGS group. While no statistically significant differences were noted with respect to the percentage of fertilized oocytes or the number of good-quality embryos.The cumulative live birth rate after one complete ART cycle including fresh and subsequent frozen-thawed cycles per allocated woman were similar in the LA and HGS group 19/97 (19.59%) and 17/94 (18.09%), respectively (*P*=0.791).

Limitations, reasons for caution:

The study has a smaller simple size.It was not powerful enough to detect the significant differences.

Wider implications of the findings:

Mild stimulation treatment can be a valid alternative to PORs instead of conventional controlled ovarian stimulation by using high dose of gonadotropins.

Trial registration number:

ChiCTR-TRC-13003454

O-258 The use of autologous platelet-rich plasma (PRP) versus antioxidants in women with low ovarian reserve undergoing assisted reproductive technology (ART): a comparative controlled pilot study.

P. Melo[1], C. Navarro[2], C. Jones[1], K. Coward[1], L. Coleman[2]

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[2]*Assisted Reproduction, Fertiaguerrevere Fertility Clinic, Caracas, Venezuela*

Study question:

Is PRP an effective treatment for low ovarian reserve in infertile women prior to undergoing ART?

Summary answer:

A 3-month course of intracortical ovarian injections of PRP significantly improves ovarian reserve and subsequent reproductive outcomes in infertile women.

What is known already:

Female age remains the principal limiting factor of reproductive success due to an inevitable loss of ovarian reserve as women become older. Antioxidant supplements are thought to delay ovarian senescence, although meta-analyses have not confirmed their effectiveness. PRP, a blood derivative containing platelets in much higher concentrations than circulating serum, is effective in treating nerve and muscle injuries, however there is a paucity of data regarding the regenerative properties of PRP in reproductive medicine. While encouraging results have been reported from *in vitro* and animal experiments, there are no controlled studies evaluating the impact of PRP on human ovarian reserve.

Study design, size, duration:

Prospective three-arm comparative study conducted in a private clinic between February 2015 and February 2018. Strict inclusion criteria (age≥38; FSH>12 mIU; estradiol<100 pg/mL; AMH<0.8 ng/mL; normal hysteroscopy) and exclusion criteria (previous pelvic inflammatory disease; known hyperandrogenism; tubal or male-factor infertility) were applied. A total of 115 women were allocated to one of the following groups for 3 months according to patient choice: monthly ovarian PRP injections (n=46); daily oral antioxidants (n=32); or no intervention (n=37).

Participants/materials, setting, methods:

Written consent was obtained from all participants. In all three arms of the study, day-3 baseline ovarian reserve was quantified with FSH, AMH, estradiol and antral follicle count (AFC) in cycle 1. Repeat day-3 ovarian reserve measurements were recorded 3 months later as primary outcomes. Details of subsequent ART treatment (timed intercourse, IUI or IVF/ICSI) were documented. Secondary outcomes included number of oocytes collected and fertilised; biochemical/clinical/ongoing pregnancy rates; and miscarriage and live birth rates.

Main results and the role of chance:

Mean age was 41.2±2.6 (*P*=0.88). Demographic characteristics and baseline ovarian reserve markers were similar between the 3 arms. At the 3-month follow-up, women treated with PRP had the largest improvement in ovarian reserve: AMH rose by 86% (versus a 15% increase in the antioxidant group and no change in the controls,*P*<0.001); FSH dropped by 39% (versus a 9% reduction in the antioxidant group and no change in the control arm,*P*<0.001);

circulating estradiol increased by 67% (versus no change in the antioxidant and control groups,$P<0.001$); and there were on average 86% more antral follicles on ultrasound, compared to an 18% increase in the antioxidant group and a 7% reduction in the controls ($P<0.001$). Among women who underwent IVF/ICSI following participation in the study (n=55), those previously treated with PRP yielded more than double the oocyte number of controls (5.2±1.9 versus 2.2±1.9 respectively, mean±SD,$P<0.001$), although fertilisation rates were similar between groups ($P=0.46$). Women conceiving with timed intercourse/IUI were more likely to achieve biochemical and clinical pregnancy following PRP (OR 1.26, 95% confidence interval [CI] 1.03-1.55), but not those who conceived with IVF/ICSI (OR 3.73, 95% CI 0.67-20.9) Rates of miscarriage ($P=0.16$) and live birth ($P=0.64$) were similar across the 3 study arms.

Limitations, reasons for caution:

The main limitation of this study was the lack of randomisation. However, in the absence of previous data attesting the safety of PRP injection into human ovaries, voluntary enrolment into one of the 3 groups was deemed appropriate. Randomised controlled studies would be required to corroborate our results.

Wider implications of the findings:

This is the first controlled study attesting the efficacy of PRP injections in inducing a resurgence of ovarian activity. Further research is required to investigate whether the increase in estradiol following PRP is sustained, and the potential cardiovascular and bone health benefits of PRP in women with premature ovarian insufficiency.

Trial registration number:

Ethical approval was granted by a local Institutional Review Board (IRB) and the Venezuelan Health Ministry (IRB reference number #0940).

O-259 Effectiveness of recombinant-LH supplementation (LHS) on reducing pregnancy loss (PL) for poor ovarian responders (POR) in IVF/ICSI cycles.

P. Arvis[1], N. Massin[2], P. Lehert[3]

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[2]*Centre Hospitalier Inter-communal Créteil, Amp, Creteil, France*
[3]*Faculty of Medicine, - the University of Melbourne, Melbourne, Australia*

Study question:

Our objective was to assess the effectiveness of LHS on pregnancy loss for POR, based on a large controlled study in a real world context.

Summary answer:

r-LH supplementation to rFSH (LHS) has a highly significant beneficial effect in reducing pregnancy loss among POR, positively correlated with the severity of POR.

What is known already:

PL is known to generate emotional burden for women, especially when pregnancy is attempted through ART. The detrimental effects of miscarriage are reported to endure a complex course of resolution. Moderate-quality evidence suggests little or no LHS benefit in rates of pregnancy loss (PL) during controlled ovarian stimulation (COS) for normo-responders. As to whether this benefit is limited to POR remains controversial. PL may be affected by the type (fresh/frozen) of the embryo transfer, baseline conditions and PL incidence during previous cycles undertaken by the patient. On the other hand, PL is suspected as varying among centers.

Study design, size, duration:

This non-interventional retrospective national multicentre controlled study compared rFSH+rLH to rFSH alone administered during COS, in POR patients defined by ESHRE Bologna criteria. We[P1] used an exhaustive registry collected from 12 IVF French centres during 2007–2016 period. Out of a total of 113,253 cycles, 11571 were found in POR associated with r-FSH or LHS treatment. Out of them a gestational[P2] sac was found for 2120 cycles (1112 r-FSH,1008 LHS).

Participants/materials, setting, methods:

The primary endpoint was the PL from all fresh/thawed embryos. The statistical model was a full factorial design of treatment by baseline severity, random factors being patient nested into site, and matched-subclass constructed from a ranked propensity score. Baseline severity was defined following the PROSPER score (mild, moderate and severe POR groups predicting PL). A non linear mixed model featuring logistic regression was used for PL

Main results and the role of chance:

The overall PL rate was 26.9%. the population of reference were cycles with fresh embryos, without previous PL observed in previous cycles, mild Prosper baseline score and treated with r=FSH Alone for which a PL rate of 24.2% ([19.5%,32.1%]) was found. However, this value was strongly varying among centres with a credibility interval of [21%,28.2%]. The PL rate was impacted by several baseline covariates: Higher PL for frozen embryos compared with fresh (OR=1.52 [1.15,2.03],p=.003), PL occurrence during previous cycle (OR=1.91, [1.53, 2.37, p<.001), and Prosper Score (OR=1.29/point [1.09,1.52],p=.002). Moreover, an important center effect was observed In adjusting for these covariates, a highly significant benefit was found for LHS compared with r-FSH alone (OR=66, [.52,.84],>p=.001)

Limitations, reasons for caution:

Potential biases might still exist due to non randomized treatment attribution selection, in spite of correction through propensity score and statistical adjustment.

Wider implications of the findings:

Whereas LHS appears to improve cumulative live-birth rate only for the most severe POR, LHS has a beneficial effect on reducing PL in the whole range of POR. As LHS has no effect on the oocytes yield, these findings suggest a beneficial effect of LHS on oocyte quality for POR.

Trial registration number:

None

O-260 Subcutaneous progesterone (25mg twice daily) for endometrial preparation in substituted cycles for oocyte donation: an International, multicenter, pilot study to correct a probable dose mistake

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[2]*Instituto de Medicina Reproductiva IMER-NextClinics, Assisted Reproductrion, Valencia, Spain*
[3]*Institute for Maternal and Child Health- IRCCS Burlo Garofolo, Department of Medical- Surgical- and Health Sciences- University of Trieste, Trieste, Italy*

Study question:

Are there outcomes differences, between subcutaneous progesterone (SP), using twice the recommended dose and micronized vaginal progesterone (MVP) in embryo transfer cycles to oocyte recipients?

Summary answer:

In the subcutaneous progesterone group, significant differences were observed in terms of higher ongoing pregnancy rate at 12 weeks of gestation and lower miscarriage rate.

What is known already:

The dose recommended by the manufacturer / official supplier of the aqueous subcutaneous progesterone is 25 mg per day.

A previous randomized, controlled trial, found no significant difference in ongoing pregnancy rate between the use of SP at the usual recommended dose (25 mg daily) and MVP (200 mg tid daily). However, a higher percentage of biochemical abortion was observed in the group of patients in whom 25 mg per day of SP was used.

To date, clinical outcomes using SP at double the recommended dose (25 mg x 2 daily) has not been assessed.

Study design, size, duration:

International, multicenter, prospective, pilot study. During one year, 149 oocyte recipients were included in the study to receive 25 mg twice daily SP (n=67) or 200 mg three times daily MVP (n=82).

Participants/materials, setting, methods:

The patients had medical and legal criteria to be included in an oocyte donation program. All followed standard treatment of endometrial preparation with oral estradiol valerate.

The oocytes used were frozen / thawed and in all cases fresh blastocysts were transferred.

Progesterone administration, in both groups, started on the day of thawing / ICSI of the oocytes.

The quantitative variables were assessed by t-test and anova test; Fisher test were used for qualitative variables.

Main results and the role of chance:

The mean age and BMI of the patients included in both groups was similar (SP group: 41.5 years & 24.1 Kg/m2; MVP group: 41,02 years & 23.6 Kg/m2). The treatment days of the endometrial preparation were also similar in both groups (15.9 and 16.2 days, respectively).

The mean of blastocysts transferred in both groups was similar (SP: 1.4 & MVP: 1.5). However, the implantation rate was significantly higher in the SP group (55.2%) than in the MVP group (35.2%).

We found a significantly higher ongoing pregnancy rate in the SP group (67,2%) than the MVP group (46,3%). The miscarriage rate in the SP group was 5,9% and in the MVP group it was 18,4% (p<0,005).

Limitations, reasons for caution:

As this is a pilot study, the limitations of this format should be taken into account ie. sample size, RCT design, etc. . .

Wider implications of the findings:

Despite our data show that the administration of 25 mg twice daily of subcutaneous progesterone results in higher implantation and pregnancy rates and lower rate of miscarriage, caution must be exercised in interpreting these promising results. Additional studies are needed to demonstrate its utility in substituted cycles.

Trial registration number:

not applicable

O-261 Is more always better? Female age modifies the oocyte number where maximal cumulative live birth rate per aspiration is observed: an analysis of 221,221 cycles

Y.J. Law[1], N. Zhang[1], C. Venetis[1], G. Chambers[1], K. Harris[1]

[1]University of New South Wales, Centre for Big Data Research in Health, Sydney, Australia

Study question:

Is the oocyte number where maximal cumulative live birth rate per aspiration (CLBA) is observed during assisted reproductive technology (ART) different based on female age?

Summary answer:

Maximal CLBAs were observed at ~25 oocytes in women aged 18-35, >30 oocytes in women aged 36-44, and ~9 oocytes in women aged ≥45.

What is known already:

Live birth rate per fresh or frozen/thaw embryo transfer (FET) has traditionally been the primary measure of ART success. However, with the introduction of highly efficient embryo cryopreservation methods, CLBA encompassing live delivery outcomes from the fresh and all subsequent FET following a single ovarian stimulation and oocyte collection is increasingly viewed as a more meaningful measure of treatment success. There is growing evidence suggesting that larger oocyte yields are associated with higher CLBA. Whether this association is uniform across female ages has not yet been properly explored and warrants further investigation.

Study design, size, duration:

This is a large retrospective population-based cohort study using data from the Australian and New Zealand Assisted Reproduction Database (ANZARD). Overall, 116,677 women undergoing 221,221 autologous oocyte aspiration cycles between January 2009 to December 2015 were included in the analysis. All fresh and FET resulting from the associated aspiration cycle were included in the analysis until one live birth occurred or all embryos were used. Cycles where no oocytes were retrieved were excluded from analysis.

Participants/materials, setting, methods:

CLBA was defined as at least one liveborn baby at ≥20 weeks gestation resulting from an ART aspiration cycle. Generalised estimating equations were used to account for the clustered nature of data. Univariate and multivariable regression analysis was performed to identify and adjust for factors known to independently affect CLBA. An interaction term between female age and the number of oocytes retrieved was introduced to assess the effect-modifying role of female age.

Main results and the role of chance:

The number of oocytes retrieved remained a significant predictor (P<0.001) of CLBA after adjusting for female age, parity and cycle count. Compared to the reference group of 10-14 oocytes retrieved, the adjusted odds of achieving a live birth (i.e. CLBA) increased with the number of oocytes retrieved:

With 1-3, 4-9, 15-19, 20-24 and ≥25 oocytes, the adjusted odds are 0.21 (95% CI: 0.20-0.22), 0.56 (95% CI: 0.55-0.58), 1.38 (95% CI: 1.34-1.43), 1.75 (95% CI: 1.67-1.84) and 2.10 (95% CI: 1.96-2.25) respectively.

The CLBA for women aged <30 and 30-34 years appeared to reach a plateau at 73% after retrieval of 25 oocytes. The CLBA of women aged 35-39 and 40-44 years continued to increase with higher oocyte yields, reaching 68% and 40% respectively when 30 or more oocytes were retrieved. The CLBA of women aged >45 peaked at around 9 oocytes, remaining consistently below 5%. Trends in CLBAs and oocyte numbers differ by age, with the rate of increase in CLBA per additional oocyte retrieved being lower in older age groups.

Limitations, reasons for caution:

Despite this study being in complete ascertainment of ART cycles across two countries, ovarian stimulation protocols and oocyte quality parameters were not collected by ANZARD and could not be adjusted for. It cannot be excluded that the observed association may be due to the inherent characteristics of the population examined.

Wider implications of the findings:

This study demonstrates that female age modifies the number of oocytes retrieved where maximal CLBAs are observed. Younger women require fewer oocytes to achieve maximal CLBA. Furthermore, there is limited benefit in stronger ovarian stimulation protocols to retrieve more oocytes in older women as maximal CLBA decreases with advancing age.

Trial registration number:

Not applicable.

O-262 the cycle scheduling impacts the cytokine profile in cervical mucus: oral contraceptive pills (OCP) for 14 days vs. pretreatment with estrogen in luteal phase

B. Martazanova[1], N. Mishieva[2], M. Martynova[2], V. Vtorushina[2], L. Krechetova[2], A. Abubakirov[2]

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Study question:

Does the cycle scheduling affect the cytokine profile in cervical mucus and impact pregnancy rate in fresh GnRH antagonist cycles?

Summary answer:

OCP is associated with depression of VEGF, FGF basic, IL-6, LIF, IL-17A, IL-12p70, GM-CSF levels in cervical mucus and lower pregnancy rates in fresh cycle.

What is known already:

According to Cochran database the OCP pretreatment is associated with a lower ongoing pregnancy rate than no pretreatment; however the pathogenesis of this is still unknown. Also controversy exists around estrogen pretreatment: some studies show that estrogen priming improves the implantation rate. According to other data, estrogen does not impact cycle outcome. Uterine secretomics was used for a non-invasive analysis of the in-vivo milieu encountered by the embryo; previous studies showed that soluble regulators of implantation could be detected in cervical mucus. However, there is limited data about cervical mucus cytokine profile and IVF treatment success after cycle priming.

Study design, size, duration:

This prospective randomized study included 94 normal responder patients. Group 1 (n=31) received OCP (2 mg cyproterone + 0.035 mg ethinylestradiol/day) for 14 days, and stimulation was started on day 5 after discontinuing the OCP. Group 2 (n=30) received 4 mg/day oral estradiol valerate from day 25 for 5-10 days, stimulation was started next day after estrogen stopping. Group 3 (n=33) did not receive pretreatment, stimulation was started on day 2-3 of the cycle.

Participants/materials, setting, methods:

Inclusion criteria: age <35 years; basal FSH<12 IU/ml; regular cycle, AMH 1.0-3.5 ng/ml. Exclusion criteria: uterine fibroids, deep endometriosis, severe male infertility, PCOS; reduce ovarian reserve, bacterial vaginosis.

Concentrations of 29 key regulatory cytokines, chemokines and growth factors were measured with immunofermental assay. Cervical mucus samples were collected via velvet endocervical flocked swab («Copan», Italy) after cervix was cleansed on oocyte pick up day (OPU), on OPU+3 and ET days.

Main results and the role of chance:

Concentrations of 2 an angiogenic factors (VEGF, FGF basic), 4 proinflammatory cytokins (IL-6, LIF, IL-17A, IL-12p70) and 1 growth factor (GM-CSF) showed significant changes after cycle scheduling: *VEGF* (223.15±188.34 vs. 1032.39±245.23 pg/ml, *p*=0.019), *IL-12p70* (32.1±25.5 vs. 143.98±37.0 pg/ml, *p*=0.024), *FGF basic* (6.3±0.7 vs. 34.7±12.5 pg/ml, *p*=0.046), GM-CSF (146.28±139.14 vs.653.13±156.30, *p*=0.027), and *IL-17A* (25.9±11.7 vs. 63.9±25.2, *p*=0.044) levels were significantly lower in group I compared to group III on OPU day. Concentrations of *VEGF* (383.09±212.97 vs. 1839.71±271.49, *p*=0.031), *IL-12p70* (55.7±25.9 vs. 250.94±74.0, *p*=0.028), *LIF* (13.6±3.3 vs. 41.8±4.6, *p*=0.023), and *IL-6* (740.11±229.97 vs.1271.66±29, *p*=0.048) were significantly lower on ET day in group I compared to group III. *FGF basic* (6.3±0.7 vs. 33.5±14.7, *p*=0.017) *and IL-17A* (25.9±11.7 vs. 87.6±33.6, *p=0.045*) on OPU day, plus *IL-6* (740.11±229.97 vs. 1605.75±225, *p*=0.012) levels on ET day were lower in group I compared to group II. Embryological characteristics were similar in all groups. Pregnancy rate per ET were comparable between groups (35.5% (11/31) vs. 50.0% (15/30) vs. 51.5% (17/33) in group I, II and III, respectively). However, ongoing pregnancy rate were significantly lower in group I compared to other groups (19.4% (6/31) vs. 46.7% (14/30) vs. 48.5% (16/33) in group I, II and III, respectively, *p***=0.04, *p****=0.036).

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:

The study was funded by an internal grant from the Kulakovs National Medical Research Centre of Obstetrics, Gynaecology and Perinatology of the Russian Ministry of Healthcare.

Trial registration number:

N/A

SELECTED ORAL COMMUNICATIONS

SESSION 69: PREGNANCY LOCATION AND OUTCOME

Wednesday 26 June 2019 Strauss 1+2 10:00 - 11:45

O-263 To analyze the incidence of ectopic pregnancy in fresh embryo transfer compared with frozen-thawed embryo cycles in a tertiary infertility centre in India.

K.D. Nayar[1], S. Gupta[1], M. Singh[1], M. Gupta[1], R. Gahlot[1], G. Kant[1], A.J.S. Gulati[1], D. Nayar[1]

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Study question:

Is there is a difference in rate of ectopic pregnancy in assisted reproductive technology(ART) cycles in fresh or frozen-thawed embryo transfer?

Summary answer:

The incidence of ectopic pregnancy is greatly reduced in frozen thawed embryo transfer when compared to fresh embryo transfer cycles.

What is known already:

In vitro fertilization–embryo transfer (IVF-ET) is one of the major risk factors for an ectopic pregnancy (EP). Some authors believe that it might be due to increased incidence of tubal disease in such patients or stimulation of uterine contractions at the time of transfer or dysfunction of uterine musculature due to disturbed hormonal status. Recent research has suggested that frozen-thawed ET (FET) is associated with a greatly reduced incidence of EP compared with fresh transfers. This study was undertaken to evaluate the incidence of ectopic pregnancies in fresh and frozen embryo transfer at a tertiary fertility centre in India.

Study design, size, duration:

A prospective cohort study was carried out between 1st January 2016 to 31st December 2018.

Participants/materials, setting, methods:

A total of 671 fresh cycles and 767 frozen thawed ET cycles occurred during the duration of the study. Main outcomes measured were incidence of Ectopic pregnancy with fresh IVF-ET compared with frozen-thawed ET cycles, clinical pregnancy rate, and rate of ectopic pregnancy per embryo transfer.

Main results and the role of chance:

For the 671 fresh IVF-ET cycles, 286 patients had clinical pregnancies and 20 patients had ectopic pregnancy and out of 767 FET cycles, 375 patients had clinical pregnancy and 10 patients had ectopic pregnancy. The clinical pregnancy rate in fresh IVF –ET cycles was 42.62% and in FET cycles was 48.89%, which was statistically significant(p= 0.02). The incidence of an ectopic pregnancy per embryo transfer was 2.23% (20/671) for the fresh group and 1.04% (10/767) for the FET group ; the difference was statistically significant (p=0.042). Majority of patients with ectopic pregnancies had tubal factor as a cause of infertility, Fresh ET group 55% and frozen ET group 60%. There was a statistical significant difference in ectopic pregnancy rate in fresh vs. FET cycle when day 3 embryos were transferred (13 vs. 5; p= 0.05) but the difference was not significant when day 5 embryos were transferred in the fresh and FET groups (7 vs. 5; p=0.60).

Limitations, reasons for caution:

The study was a prospective cohort study at a single centre, larger prospective trials with an increased number of patients are needed to confirm our findings.

Wider implications of the findings:

Frozen thawed ET is associated with significantly lower rates of ectopic pregnancy compared with fresh cycles. These findings suggest that increased chance of ectopic pregnancy is due to disturbed hormonal milieu of ovarian stimulation. Freeze all strategy followed by FET would decrease the incidence of ectopic pregnancies in ART.

Trial registration number:

MCDH/2016/45

O-264 In-vivo intrauterine oxidation-reduction potential determines uterine receptivity

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[4]University of Manchester, Maternal and Fetal Health Research Centre- Division of Developmental Biology and Medicine- School of Medical Sciences- Faculty of Biology-Medicine and Health, Manchester, United Kingdom

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Study question:

Can the measurement of in-vivo intrauterine pH and/or oxidation-reduction potential (ORP) determine the alterations of uterine endometrium for implantation and evaluate prospective uterine receptivity?

Summary answer:

In-vivo intrauterine ORP, but not pH, can detect the alteration of uterine endometrium for implantation and evaluate prospective uterine receptivity electrophysiologically in mouse and human.

What is known already:

Uterine receptivity is associated with various glycosylation changes that affect negative charge density at the luminal epithelial cell surface.

Study design, size, duration:

To prove our basic concept, we assessed in-vivo intrauterine pH and ORP during the early stages of pregnancy in naïve mice, as well as in an implantation failure model mice (n = 21). Then, to investigate whether in-vivo intrauterine ORP could be used to predict pregnancy in women, a prospective cohort study was conducted for patients, undergoing ART treatment (n = 52).

Participants/materials, setting, methods:

The implantation failure model mice were prepared by local and transient suppression of Stat3 in the uterus. The patients who had received a frozen-thawed single embryo transfer in a programmed, hormonally controlled cycle were enrolled in this study. The in-vivo intrauterine ORP was measured 3 times during the treatment cycle, at cycle days 9-10 (time [T] 1), 1 day before progesterone administration (T2) and immediately before the embryo transfer (T3).

Main results and the role of chance:

Sulfation and sialylation changes were observed in the luminal epithelial glycocalyx. There was no change in the in-vivo intrauterine pH between post-coitus Days 2 and 6, while ORP was significantly higher compared to the day before (during early stage of pregnancy in the naïve mouse). One day before implantation began, intrauterine ORP was significantly decreased in the implantation failure model mice compared with the naïve ($P < 0.001$, Student's t-test) and control ($P < 0.001$, Student's t-test) mice. In women there was no significant difference in age and BMI between the pregnant and non-pregnant groups. The intrauterine ORP values at T1 were significantly lower in the pregnant group than in the non-pregnant group ($P < 0.001$, Student's t-test). Receiver operator characteristic (ROC) curve analysis of intrauterine ORP as a predictor of non-conception showed an area under the ROC curve of 0.96 (95% confidence interval 0.92–1.00) in the mouse study and 0.80 (95% CI: 0.61-1.00) in the human clinical study.

Limitations, reasons for caution:

Embryo biopsy for preimplantation genetic testing for aneuploidies could not be performed in this study due to the policies of the Institutional Ethics Committee.

Wider implications of the findings:

The measurement of intrauterine ORP may help to prospectively evaluate uterine receptivity in women. This includes the measurement of intrauterine ORP as part of a frozen–thawed embryo transfer strategy may improve the efficiency of ART.

Trial registration number:

clinical trial No. 813; No. 2013-06-1

O-265 Endometrial microbiota and pregnancy outcome of IVF patients in Japan: an analysis using species level resolution 16S rRNA gene amplicon sequencing

K. Kyono[1], T. Hashimoto [1], N. Okuyama[1] , Y. Nagai[2] , Y. Sakuraba[2]

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[2]*Varinos Inc.-, Provide services of next-generation genomic testings, Tokyo, Japan*

Study question:

What kind of species comprised of the endometrial communities detected in the endometrium of IVF patients who achieved successful ongoing-pregnancy or miscarriage?

Summary answer:

Lactobacillus iners-dominated and L. *crispatus*-dominated groups accounted for one-third of the delivered/ongoing-pregnant cases. Nearly half of the miscarried cases showed L. *crispatus*-dominated endometrium.

What is known already:

Recent reports have suggested that human uterine microbiota are related to implantation success, and that non-*Lactobacillus*-dominated microbiota was associated with adverse pregnancy rates [Moreno et al. 2016]. Also, in our previous study, pregnancy rate of IVF was higher in patients with ≧80% *Lactobacillus*-dominated endometrial microbiota compared to that with < 80% *Lactobacillus* [Kyono et al. 2018]. Although specific species in vaginal microbiota are reported to be related to infertility [Campiscinano et al. 2017] and preterm birth [DiGiulio et al. 2015], there are no studies relating the composition of endometrial microbiomes at the species level to miscarriage.

Study design, size, duration:

This is a retrospective, non-comparative single-center study carried out from August 2017 to June 2018. 117 IVF patients had their endometrial bacterial status (genus level) examined from August 2017 to March 2018. 56 patients achieved pregnancy (53 by single frozen-thawed blastocyst transfer, 3 by natural conception) by the end of June 2018, and for those 56 cases, bacterial status at the species level was analyzed by remnant samples. Last follow-up was January 2019.

Participants/materials, setting, methods:

Endometrial fluid samples were collected using an IUI catheter. The species-level bacterial profiles were analyzed using next-generation sequencing technologies. The V1-V2 region of 16s rRNA gene was amplified and sequenced by MiSeq platform, and data was analyzed by inhouse QIIME using SILVA and V1-V3 16S rRNA reference database for vaginal microbiome. Clinical pregnancy was defined as confirmed gestational sac in the uterine cavity. Ongoing pregnancy was defined as the pregnancy having completed ≥20 weeks gestation.

Main results and the role of chance:

Of the 56 patients who achieved pregnancy by the end of June 2018, the sample quality was sufficient for endometrial species composition analysis in 49 patients (47 by single frozen-thawed blastocyst transfer, 2 by natural conception). Of the 49 pregnancies, 28 delivered, 10 were ongoing, and 11 miscarried, as of January 2019. 48 cases were Japanese.

7 cases received prebiotic/probiotic therapy before bacterial analysis. The average age of delivered/ongoing cases was significantly lower than that of miscarried cases (35.2 ± 4.2 vs 38.4 ± 3.0 years old, $P<0.05$).

12 cases (10 delivered/ongoing, 2 miscarried) showed non-*Lactobacillus*-dominated (NLD) (<80%) endometrium prior to embryo transfer. All 7 cases with NLD endometrium delivered at term. Among the 21 delivered cases with *Lactobacillus*-dominated (LD) (≥80%) endometrium, only one case delivered preterm, due to pregnancy-induced hypertension at 35 weeks pregnant.

The bacterial communities were clustered into six main groups, dominated by I: *Lactobacillus crispatus*, II: L. *iners,* III: L. *gasseri*, V: L. *jensenii*, IV and VI: non-*Lactobacillus* dominated. Groups I and II accounted for 28.9% and 31.6% of the delivered/ongoing cases, while group I accounted for 45.5% of the miscarried cases. There was no correlation between the percentage of dominant species and pregnancy outcome.

Limitations, reasons for caution:

The limitations of this study are short follow-up period, limited study numbers, not analyzing other aspects of gynecological histories, such as bacterial vaginosis or endometriosis. The endometrial species composition should have been compared with patients with LD endometrium who did not achieve pregnancy but was not analyzed in this study.

Wider implications of the findings:

This may be the first report analyzing endometrial microbiota at the species level regarding pregnancy outcome of IVF patients in Japan. Although this study has limitations, a specific *Lactobacillus* spp. in the endometrium detected prior to implantation may not be a strong biomarker of miscarriage.

Trial registration number:

Not applicable.

O-266 Regulation of PI3K/Akt pathway in idiopathic recurrent spontaneous miscarriage during window of implantation

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Study question:

Is PI3K/Akt pathway associated with idiopathic recurrent spontaneous miscarriage(IRSM) during window of implantation?

Summary answer:

PI3K/Akt pathway dysregulation with down-regulation of glucose receptors and enhanced inflammation mediated by PI3K via IKK/NFκB pathway were observed in endometrial tissue of IRSM women.

What is known already:

Akt signaling promotes proliferation and remodeling of the endometrium with an increased expression during embryo implantation in mice model. It is also reported that phospho Akt (p-Akt) is essential for endometrial decidualization and implantation of fertilized eggs in rats. PI3K/AKT signaling pathway is associated with follicular implantation during cellular proliferation induced by

estrogen and *progesterone*. PI3K/Akt pathway also regulates glucose metabolism for preparation of the epithelium and stroma in embryo implantation during successful pregnancy. PI3K/Akt pathway has a pivotal role in implantation, placentation and maintenance of normal pregnancy by enhancing trophoblast proliferation and invasiveness.

Study design, size, duration:

This study, approved by the internal review board, was conducted at the Institute of Reproductive Medicine, Kolkata in collaboration with the School of Medical Science & Technology,IIT Kharagpur, India between October 2017 and June 2018. Twenty infertile women with history of 3 or more previous IRSM and 20 women with azoospermic male partners (controls) were enrolled. Histology with immunoblotting techniques were used to study endometrial tissues collected from these subjects during window of implantation.

Participants/materials, setting, methods:

Eligible IRSM patients and controls were recruited and treated with estradiol valerate &vaginal progesterone for 10 days following which endometrial thickness (ET) was determined by serial USG. Endometrial tissue was collected when ET $\geq$ 6.5mm during window of implantation. Histological studies by hematoxylin and eosin staining and immunohistochemistry and quantitative estimation of p-PI3K (p85, p110), p-Akt (Ser473, Thr308), GLUT3, GLUT4, IKK and NF$\kappa\beta$ proteins was done using immunoprecipitation and immunoblotting techniques.

Main results and the role of chance:

We found the expression of p-PI3K (p85)(2.73-fold, $p<0.001$), p-PI3K (p110)(2.86-fold, $p<0.001$),p-Akt (Ser473)(1.98-fold, $p<0.001$) and p-Akt (Thr308) (1.68-fold, $p<0.01$) to be significantly reduced in the endometrial tissue of women with IRSM. Levels of both the glucose receptors, GLUT3 (2.46-fold, $p<0.001$) and GLUT4 (4.49-fold, $p<0.001$) were also down-regulated in these patients, possibly mediated by the PI3K-dependent pathway. IKK (3.43-fold, $p<0.001$) and NF$\kappa\beta$ (2.75-fold, $p<0.001$), two key proteins associated with inflammation, were also significantly increased in IRSM cases. IKK aggravated inflammatory response via NFκB seems likely. Immunohistochemistry was used to localize the insulin signaling proteins, including p-PI3K (p85), p-PI3K (p110),p-Akt (Ser473), p-Akt (Thr308), GLUT3 and GLUT4 in tissues of both the groups. While a reduced number of immunostained cells was observed for p-PI3K (p85),p-PI3K(p110), p- Akt (Ser473), p-Akt(Thr308) and GLUT4, the expression of IKK and NfkB were found to be significantly higher in women with IRSM. This study strongly suggests that PI3K/Akt pathway is dysregulated in the endometrium of women with IRSM during window of implantation.

Limitations, reasons for caution:

Glucose and insulin level estimation in the endometrial tissue to be performed. Correlation between the altered proteins and IVF outcome parameters, such as miscarriage rate and pregnancy rate could provide useful insight to the clinicians.

Wider implications of the findings:

Targeted intervention of PI3K/Akt signaling pathway specifically in the endometrium could be a promising treatment for the effective management of IRSM.

Trial registration number:

NA

O-267 Mitotic arrest deficient 1 like 1 (MAD1L1) and MAD2L1 gene variations in products of conception with aneuploidy

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Study question:

Is the frequency of mutations in MAD1L1 and MAD2L1 genes in products of conception (POC) with aneuploidy higher than the highest reported frequency of these mutations?

Summary answer:

We found two novel deleterious mutations in these genes of POC with aneuploidy. Some of known mutations had higher frequency than the highest reported frequencies.

What is known already:

Chromosomal abnormality certainly aneuploidy is a main cause of spontaneous abortion. Although aneuploidy is associated with advanced maternal age it is frequent in young women. Spindle assembly checkpoint (SAC) complex has a critical role in correct chromosome segregation. Several genes are involved in SAC, such as *MAD1L1*, *MAD2L1* and BUB1. A study has reported decrease in the level of BUB1 MAD2 proteins in clinical samples of spontaneous miscarriages. The probable role of deletion of the last part of MAD2L1 gene in fibroblasts of the patients with a history of trisomic abortion has also been reported.

Study design, size, duration:

An ongoing descriptive study of 100 POC samples with aneuploidy was started in February 2018. At the time of submission, study of 40 cases has been completed.

Participants/materials, setting, methods:

To detect aneuploidy, POC of mothers younger than 36 years were analyzed using quantitative fluorescence polymerase chain reaction (QF-PCR) and/or array comparative genomic hybridization (aCGH). Those with aneuploidy were enrolled in genotyping. Areas of interest of *MAD1L1* and *MAD2L1* were genotyped using PCR and Sanger sequencing. Frequency of the observed SNVs were compared with the highest population minor allele frequency (MAF) using Chi Square. Pathogenicity of already unreported SNVs was predicted using seven predictor tools.

Main results and the role of chance:

We found some unreported SNVs that their deleterious/neutral confidence according to predictSNP is mentioned below: Q34H of MAD2L1 is deleterious (87%), F629I of MAD1L1 is deleterious (51%) and V621A of MAD1L1 is neutral (60%). We observed rs10260386, rs1481591257, rs10257349, rs372373978, rs752408355, rs1639921, rs376061507, rs62442486, rs74431414 SNVs with following allele frequencies and P values of comparison with the highest population MAF in *MAD1L1* gene: A:0.7/G:0.3 ($P<0.0001$), G:0.9875/A:0.0125 ($P=0.8222$), T:0.4/C:0.6 ($P=0.2816$), G:0.9875/0.0125 ($P=0.8222$), A:0.9875/G:0.0125 ($P= 0.1775$), T:0.89/C:0.11 ($P<0.0001$), T:0.71/C:0.29 ($P<0.0001$), A:0.71/G:0.29 ($P<0.0001$) and G:0.9625/T:0.0375 ($P=0.9092$), respectively. In exons 4 and 2 of *MAD2L1* gene, we observed rs758373815, rs752146697, rs78047690 and rs1168908864 SNVs with following allele frequencies and P values of comparison with the highest population MAF: T:0.97/G:0.03 ($P=0.7402$), G:0.92/A:0.08 ($P=0.0069$), G:0.94/A:0.06 ($P=0.1295$) and T:0.975/C:0.025 ($p=0.822$), respectively.

Limitations, reasons for caution:

This is an ongoing project. Completion of experiments and analyses might change some SNVs frequencies.

Wider implications of the findings:

Finding of deleterious SNVs in POCs, certainly, those with high confidence in genes contributed in chromosome segregation could lead to the selection of healthier embryos for transfer through preimplantation genetic testing.

Trial registration number:

N/A.

O-268 Molecular analysis of Products of Conception (POC) provides comprehensive understanding of the chromosomal causes of clinical miscarriage

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Study question:
Is the POC analysis with NGS (Next Generation Sequencing) +STRs (Short Tandem Repeats) providing relevant information for the cause of clinical miscarriage?

Summary answer:
POC analysis with NGS+STRs and multi-dissection allows to rule out maternal cell contamination (MCC) providing the chromosomal constitution in 99% of the cases

What is known already:
Spontaneous miscarriages are one of the main complications in first trimester pregnancies occurring in 20-25% of clinically recognized pregnancies. Aneuploidies are the cause in more than 50% of the cases, mostly due to autosomal trisomies and monosomy X (1). This percentage can be higher than 60% in assisted reproduction treatments (ART) (2). Classic cytogenetic techniques require tissue culture, and growth failure can occur from 10 up to 60% of the cases (3). Also, MCC frequently mask the results and could give a false negative normal female result

1. Hassold et al., 1980.
2. Campos-Galindo et al., 2015.
3. Fritz et al., 2001.

Study design, size, duration:
Prospective observational study that includes 2,531 POC analyzed from January 2014 to December 2017. Results were performed using either array Comparative Genomic Hybridization (aCGH) (n = 675) or NGS (n = 1,856) after STR analyzed to rule out MCC and/or polyploidy. Maternal age, gestational age and origin of oocytes were investigated

Participants/materials, setting, methods:
POC samples were received on saline sterile solution and dissected in three portions. Then, maternal DNA (provided in a blood sample in EDTA tubes) was compared versus fetal DNA from the dissected samples performing SRTs to rule out MCC and/or polyploidy. When MCC was ruled out, aCGH was performed using array 24sure BAC (Bacterial Artificial Chromosome) (Illumina Inc., San Diego, CA, USA) or NGS with Ion ReproSeq PGS Kit (ThermoFisher Scientific, Waltham, MA, USA)

Main results and the role of chance:

1. Informativity rates and the percentage/distribution of abnormal results was similar with aCGH and NGS. Informative results were obtained in 99% of POC, being 12.8% of them MCC identified by STRs analysis. From the informative cases, 53.5% were abnormal (39.7% trisomies, 5% monosomy X, 4.1% complex abnormal, 2.5% polyploidies, 1.2% deletions/duplications (del/dup), and 1% autosomal monosomies). It is important to point out that 21 autosomal monosomies were identified for chromosomes 4, 13, 15, 21 and 22. As expected, the percentage of abnormal POC increases significantly ($p<0.05$) with female age: 50% in women $\leq$35 years; 65% in women 36-40 years, and 70% in women >40 years. Regarding gestational age, between 9-12 weeks the percentage of abnormal results was 65%, but after 12 weeks decreased to 34% ($p<0.0001$). When the patients miscarried with own oocytes (either with spontaneous pregnancy or after ART) the percentage of abnormal results was 54.5% versus 29.7% with donated oocytes ($p=0.001$). Interestingly, the percentage of sex chromosome monosomy was 9.1% in miscarriages with own oocytes versus 36.8% with egg donation ($p=0.001$), highlighting the potential higher contribution of aneuploidies of paternal origin in egg donation from young donors

Limitations, reasons for caution:
The resolution of the genetic platform allowed to detect del/dup $\geq$6Mb. Mosaicism was not considered

Wider implications of the findings:
This is the largest number of POC analyzed by NGS technology. Together with STRs analysis, we can provide results in 99% of the cases. With the use of more accurate technology we have been able to detect for the first-time monosomies for chromosomes 4 and 15 in POC

Trial registration number:
Not applicable

O-269 The implications of unsuccessful IVF/ICSI cycles for future placental development

C. Sacha[1], K. James[2], A. Harris, L[1], J. Yeh[1], I. Souter[1], D. Roberts, J[3]
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Study question:
Is a history of multiple failed IVF/ICSI cycles associated with abnormal placentation in nulliparous women?

Summary answer:
Nulliparous women with multiple prior failed IVF/ICSI cycles may have an increased risk of marginal placental cord insertion and chronic decidual inflammation when pregnancy occurs.

What is known already:
IVF/ICSI pregnancies are at increased risk of abnormal placentation compared to spontaneous pregnancies. However, the mechanisms leading to that increased risk—whether it is the hormonal environment, the assisted reproduction procedures themselves, or an unidentified endometrial or uterine factor—remain unclear.

Study design, size, duration:
A cohort of 1147 live births with placental pathology arising from autologous IVF/ICSI cycles between 2004 and 2017 was retrospectively reviewed.

Participants/materials, setting, methods:
We compared placental pathology between nulliparous women who underwent their first, second, or third or greater IVF/ICSI cycle with fresh transfer at a large academic fertility center. An experienced placental pathologist categorized placental pathology from electronic reports as anatomic, infectious, inflammatory, and/or vascular. We used Pearson's chi-squared test and multivariate logistic regression models for analysis, controlling for maternal age, IVF diagnosis, gestational age at delivery, and number of fetuses.

Main results and the role of chance:
There were 477 nulliparous women with pathology available who underwent a fresh embryo transfer, including 303 undergoing a first attempt, 117 undergoing a second attempt, and 57 undergoing their third or greater attempt. The median singleton placental weights based on standard reference values were 430g in the first attempt group, 459g in the second attempt group, and 430g in the third or greater attempt group (corresponding to 25%ile for each), but 34%, 29% and 39% of placentas, respectively, were at the 10%ile or lower ($p = 0.34$). In unadjusted models, there were no differences in infectious or vascular pathology between women who underwent a first, second, or third or greater cycle. However, an increasing number of prior failed IVF attempts seemed to be associated with a significantly greater incidence of anatomic placental pathology, specifically marginal cord insertion (8.6% vs. 13.7% vs. 19.3%, respectively, $p = 0.04$), and inflammatory pathology, specifically chronic plasma cell deciduitis (1.3% vs. 5.1% vs. 8.8%, respectively, $p = 0.005$). When adjusting for possible confounders, the trend towards a higher incidence of chronic deciduitis with increasing number of failed IVF attempts remained significant (attempt $\geq$ 3 vs. first attempt, OR=6.7, CI 1.7-26.9, $p = 0.007$).

Limitations, reasons for caution:
Our findings are limited the retrospective design of the study and by the relatively small number of nulliparous women with three or greater cycles, thus limiting the power to compare the incidence of some placental pathologies.

Wider implications of the findings:
This data indicate that nulliparous women with multiple prior failed IVF/ICSI attempts may have an altered implantation environment that leads to increased placental inflammation and altered cord insertion when pregnancy occurs. Our

findings highlight the need for a greater understanding of how infertility and assisted reproduction impact the placenta.

Trial registration number:

Not applicable

INVITED SESSION

SESSION 70: BURNING QUESTIONS IN POLYCYSTIC OVARY SYNDROME

Wednesday 26 June 2019 Mozart 12:00 - 13:00

O-270 Is AMH guilty of the dysregulation of the reproductive axis in women with Polycystic Ovary Syndrome?

D. Dewailly [1]

[1]*INSERM U1172 Jean-Pierre Aubert Research Centre, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Lille, France*

Abstract text

Is AMH guilty of the dysregulation of the reproductive axis in women with Polycystic Ovary Syndrome?

Didier Dewailly, France

Until now considered as a pathology affecting only the ovaries, the PCOS would also modify the activity of neurons housed in the heart of the brain, in the hypothalamus by causing their overexcitation. The culprit would be the anti-Müllerian hormone (AMH). AMH is a hormone secreted by the ovaries, known to play a crucial role in sexual differentiation and gonadal function. Women with PCOS have 2 to 3 times higher AMH levels. In addition, the severity of the PCOS phenotype is correlated with the AMH level. On the other hand, in more than half of the cases of PCOS, abnormally high levels of LH are found without it being possible to say whether this increase is the cause or consequence of PCOS. In our group, we found that AMH had extra-gonadal effects and was notably able to increase the activity of GnRH neurons (1). It has been observed that around 50% of GnRH neurons (murine and adult human) have AMH type 2 (AMHR2) specific receptors. The combination of several in vitro and in vivo experiments has shown that AMH increases the pulsatile secretion of GnRH-dependent LH through central action. In the end, the increase in AMH concentration would cause a real chain reaction: the hypothalamic neurons would start to secrete more GnRH, which would then increase the production and pulsatility of LH by the anterior pituitary. These results raise the innovative hypothesis that the abnormally high levels of AMH found in patients with PCOS could either be at the origin or contribute to the hormonal and gonadal disorders encountered in this pathology. Indeed, the increase in pulsatility of LH is known to aggravate hyperandrogenism of ovarian origin. In addition, hyperandrogenism combined with the intra-gonadal action of AMH disrupts follicular growth at all stages, selection of the dominant follicle and therefore ovulation.

However, the exact pathophysiology of PCOS remains unknown at this time. Indeed, family studies have revealed the heritability of PCOS, but all the mutations identified do not explain the high prevalence of PCOS suggesting the intervention of environmental/epigenetic components at the origin of this syndrome. Recent results from our group suggest that high maternal AMH level may be the fetal trigger for neuroendocrine alterations that occur in PCOS later in life. While it was known that AMH levels decreased significantly in women during pregnancy, we demonstrated for the first time that in PCOS women, AMH levels remained significantly higher during pregnancy compared with controls. We also demonstrated that recombinant AMH administered intra-peritonaly in mice at a dose of 0.12 mg/kg/day between 16.5 and 18.5 gestation days was able to have a programming effect on female progeny, leading to the occurrence of perinatal hyperandrogenism and lean PCOS phenotype in adulthood (PAMH model) (2). Finally, we showed for the first time that the bioactive form of peripherally injected AMH could act directly on the brain via the windowed capillaries located at the median eminence of the hypothalamus. Androgenization and propensity to PCOS in PAMH model would be the consequence of a central action of AMH on GnRH neurons causing their hyperexcitation resulting in an increase in ovarian steroidogenesis via an increase in LH in mothers and/or of an inhibition of placental aromatase activity resulting in an increase in bioavailable testosterone both in mothers and fetuses. These results are consistent with data from the literature that showed a decrease in placental aromatase activity in PCOS women.

1- Cimino I et al. Nat Commun 2016;7:10055

2- Tata B et al. Nat Med 2018; 24:834-846

O-271 Should we go for prevention of long term health consequences?

J.S. Tapanainen [1]

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Abstract text

PCOS is a common condition in fertile-aged women, with a prevalence of 10-18%. Women with PCOS have an increased risk for adverse metabolic derangements. This presentation focuses on these metabolic alterations and their role in increasing the risk for cardiovascular diseases (CVD) in affected women. They commonly present with overweight/obesity (up to 70%), glucose intolerance, prediabetes, dyslipidemia, chronic inflammation, hypertension and metabolic syndrome (MetS). Although most of these are known to increase the prevalence of CVD it is still unknown whether this is also the case in PCOS. Why is that? The risk of CVD-related morbidity is greater in the presence of multiple risk factors, and PCOS can be considered often as a mild/moderate metabolic disorder. Furthermore, the studies have differed in study designs and PCOS phenotypes. More importantly, high-quality follow-up studies are lacking, and the oldest women with reliable diagnosis of PCOS are not more than 70-75 years old.

Prevention of weight gain and healthy lifestyle behaviors should be recommended as first-line intervention, as the weight gain often starts at childhood/adolescence. Individuals with overweight have an increased prevalence of T2DM, hypertension and dyslipidemia, and especially visceral fat increases CVD risk. Most quidelines identify diet and exercise as essential approaches for CVD risk reduction. Reducing the use of unsaturated fatty acids and use of fruits, vegetables, fish and lean meats are the key for controlling weight and dyslipidemia. Weight reduction diminishes PCOS symptoms and weight loss of 5-10% yields significant clinical improvements.

Insulin resistance and T2DM (3-5 fold risk) are increased in PCOS. Several studies and meta-analyses have concluded that PCOS is associated with an increased risk of these disorders, independently of BMI. Thus, the detection of glucose metabolism abnormalities is important as T2DM is a major risk factor for CVD, and approximately 65% of diabetes-related mortality is due to CVD. It is notable that elevated blood glucose is a late manifestation of insulin resistance. Also individuals with prediabetes are considered to have an increased risk for CVD. Up to 75% of women with PCOS fulfill the criteria for MetS. Metformin has been used in PCOS for ovulation induction and to improve metabolic disturbances, but duration of the studies has been weeks to months, and long-term data are lacking. Nevertheless, metformin seems to lower fasting glucose, helping in weight control and to some extent it also improves lipid profile. In nondiabetic population (mean age 51) with high metabolic risk, metformin therapy reduces the incidence of T2DM, but lifestyle intervention is even more effective.

Women with PCOS present atherogenic lipoprotein profile, which carry a significant risk for CVD. As in other adverse metabolic alterations in PCOS, the first-line approach to is the implementation of lifestyle changes. In PCOS statins have been shown to lower testosterone levels either alone or in combination with oral contraceptives but have not shown to improve clinical hyperandrogenic symptoms. Statins reduce total and LDL cholesterol levels, but seem to increase glucose intolerance in some women. Although long-term studies of statins on clinical CV outcomes are lacking in PCOS, there is strong evidence in general population that they are effective for both primary and secondary prevention of CVD.

In conclusion, although there are no long-term data on the morbidity for CVD in PCOS, it is advisable to perform metabolic and cardiovascular assessment in women with PCOS in order to prevent CVD and conditions leading to CVD. Especially individuals at increased risk (obese, family history) should be identified already at early adulthood. This means targeted screening, life style changes and medication on the basis of generally accepted criteria and individual risk assessment of glucose metabolism disorders and CVD.

INVITED SESSION

SESSION 71: CAN IVF INFLUENCE HUMAN EVOLUTION?

Wednesday 26 June 2019 Haydn 1 12:00 - 13:00

O-272 Yes, IVF can influence human evolution

H.I. Hanevik [1]

[1] *Sykehuset Telemark HF, Fertilitetsavdelingen Sor, Skien, Norway*

Abstract text

As all other animal species, humans are influenced by evolution. We change. In large, well-studied human populations, some recent changes in physiology and behavior were due to evolutionary effects [1]. The most common mechanism of evolution is natural selection, where selection pressures are applied on available phenotypes. All the necessary requirements for natural selection are present in IVF clinics, and in this talk I argue that IVF influences human evolution.

Natural selection also occurs in non-assisted human reproduction of course, but selection pressures in IVF are very different compared to the natural situation. Take for example the sprinter spermatozoon that wins the race in IVF. This spermatozoon swims very short distances compared to the long-distance specialist that fertilizes the oocyte in nature. ICSI is an extreme variant of this, where the spermatozoon barely has to move at all. The ICSI spermatozoon also avoids the hassle of finding its way to the oocyte by a probably chemotactic mechanism.

This and other examples concerning the systematically different selection pressures that are applied on oocytes, embryos and even couples wanting to conceive in IVF programs compared to the natural situation, suggest that IVF influences human evolution. If so, this is another case of cultural and technological advances inducing changes in the human genome. A previous, well-known example of such gene-culture co-evolution is the relationship between lactase expression and milk consumption in human populations. In comparison, the evolutionary implications of systematically different selection pressures in IVF could be even more influential, as evolutionary theory underlines that selection works through differential reproductive success and not through differential survival.

The purpose of this talk is not to judge or impose a set of norms on IVF, but rather to draw attention to the fact that IVF is not just a treatment for infertility, but also a technological intervention at the point in a human life cycle where natural selection operates at its strongest. Although IVF is a great medical achievement, it circumvents a range of pre- and post-zygotic reproductive barriers. It increases the reproductive fitness of subfertile couples by technologically removing several naturally occurring selective barriers and by altering other such barriers. In accordance with the basic principle of evolution, the subsequent generations will thus be genetically and epigenetically adapted to an environment in which reproduction is increasingly dependent on technological intervention.

1. *Measuring selection in contemporary human populations.* Nat Rev Genet, 2010. **11**(9): p. 611-22.

O-273 No, IVF cannot influence human evolution – but may change fate for many families

INVITED SESSION

SESSION 72: NURSES/MIDWIVES INVITED SESSION: PATIENT EDUCATION

Wednesday 26 June 2019 Haydn 3 12:00 - 13:00

O-274 Reaching ethnic minorities with a comic booklet to raise awareness of fertility

S. Gameiro[1], **E. El Refaie**[2], **B.B. De Guevara**[3], **A. Payson**[4]

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[2] *Cardiff University, School of English- Communication and Philosophy, Cardiff, United Kingdom*

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Abstract text

Infertility can affect any women or men in their reproductive years. The most recent statistics show that in the UK 8% of infertile people are non-white, with 51% of these being Asian or Asian British, 26% Black or Black British, 12% Chinese or other ethnic groups, and 11% Mixed (HFEA, 2006). Research suggests that some people from minority ethnic or religious groups perceive pressure to conceive from their communities, experience social costs when they are unable to have children, and stressful interactions with the fertility healthcare system while attempting to conceive. Nonetheless, there is a lack of available guidance about the support needs and preferences of ethnic and religious minority infertile patients.

This study was based on a one-day drawing workshop to collect visual (artwork produced by participants) and textual (all conversations and discussions during the workshop) data about the participants' views and experiences of infertility and their fertility care needs. Participants were nine adult women with a minority ethnic or religious status living in Wales, UK, who were experiencing or had experienced infertility in the past. The workshop comprised five activities: 1) small and large group discussion of infertility-related drawings, 2) powerpoint-based lecture consisting on an introduction to the basics of drawing objects and people and 3) thoughts and feelings, 4) free drawing session and 5) group sharing. Audio recordings of the workshop were transcribed verbatim. Textual data was analysed with thematic analysis. Risk for bias was addressed via individual coding by two authors followed by joint discussion and presentation and discussion of results with the research team and participants.

Forty-one themes were identified and grouped into 8 distinct higher order themes. These themes described the emotional, relational and social burden of infertility experienced by women, which they perceived to result from their communities' highly pronatalistic attitudes and stigmatization of infertility. Themes also captured women's coping strategies, their overall positive evaluation of their fertility health care, their desire for more infertility education (for themselves and their communities) and for socio-culturally and inter-personal sensitive care.

Results from this study support the view that women from ethnic and religious minority backgrounds perceive highly pronatalistic attitudes and stigmatization of childlessness and infertility from within their community, to which they attribute (most of) their infertility related stress. Women were critical of such attitudes, considered that fertility education was needed to overcome these, and put forward concrete proposals on how to implement it. Women's overall evaluation of their fertility health care was positive. Nonetheless, they desired more socio-culturally and interpersonally sensitive fertility care and recommended for fertility staff to be trained in these topics. Women also desired more infertility education (e.g., biological causes). Overall results suggest that these women present high levels of resilience and effective coping strategies (with religious coping being highly prevalent) in the face of infertility and the personal and social adversity it creates.

The sample was small and recruited in collaboration with a local charity, which may mean that all participants were well integrated in their communities. Analysis focused on capturing commonalities in participants' experiences and this may sometimes result in homogenizing diverse experiences.

In conclusion, more education about the infertility experiences of minority ethnic and religious groups at the community and healthcare deliver level can be beneficial for women. Together with our workshop participants we co-produced the comic booklet 'Thorns and Flowers - Infertility experiences of Black and Minority Women' (https://www.cardiff.ac.uk/psychology/about-us/engagement/thorns-and-flowers) that is available online and can be used by infertile people, fertility clinics and other stakeholders to introduce conversations and discussions on this topic.

O-275 Patient's information needs

M. Bulmanska-Wingett [1]

[1] *Stowrzyszenie na Rzecz Leczenia Nieplodnosci i Wspierania Adopcji Nasz Bocian- Association for the Infertility Treatment and Adoption Our Stork- Fertility Europe, Patient, Petaling Jaya, Malaysia*

Abstract text

The presentation reviews infertility patients' information needs and preferences as well as the actual situation and practice in the matter. As the internet is one of the main sources of patients' expertise on infertility and methods of its treatment, providing accurate and thorough information to patients is exceptionally important. In the era of easy access to all sorts of data, helpful as well as harmful, at times of massive information chaos delivering reliable data has became a true challenge. Not only does the availability of qualititative and verified information help to make the right choices in terms of existing medical procedures and reaching for professional help within a reasonable time but vastly helps to cope with burdens of treatment and infertility itself by increasing level of understanding of one's unique situation and suggested medical solutions. From both formal and legal points of view, access to proper information is an emanation of patients' fundamental rights and therefore indisputable.

A survey conducted among infertility patients throughout European countries delivers intelligence on their preferences in terms of content, the right form and manner of delivering and the right timing of providing information as well as proves quite common deficiencies in the field of quality and intelligibility of information offered to patients. It focuses on relation patient - health care provider although it also investigates briefly other sources of information available and their importance to patients. There are reported cases of biased medical advice based on personal beliefs of health care providers rather than on evidence-based medicine as well as cases of bias towards certain groups of patients present due to their specific features or life choices, i.e. age, gender, weight, education, sexual orientation etc. The presentation aims at delivering accurate and complete information on practice in terms of informing and including patients in the process of their own diagnosis and/or treatment in various European countries. It also aims at delivering the analysis of observed problems and seeking suggestions for desired solutions from the patients perspective. It will also indicate the areas for much-needed improvement in access to reliable information based on patients preferred sources of obtaining information and for common efforts of health care providers and patients' organizations towards providing knowledge and effective tools for critical verification of commonly and easily available information. The importance of providing easy access to reliable, qualitative and intelligible information is a factor vastly influencing not only patients well being during treatment but also the actual outcome of treatment when taking into account timing and limitations reflected by various factors, e.g. age or progressing severity of accompanying medical conditions.

INVITED SESSION

SESSION 73: ENDOMETRIOSIS, INFLAMMATION AND THE IMMUNE SYSTEM

Wednesday 26 June 2019 Haydn 2 12:00 - 13:00

O-276 Inflammatory changes in endometriosis

B. McKinnon [1]

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Abstract text

Endometriosis, characterised by the growth of endometrial tissue outside the uterine cavity, is often described as an inflammatory disease. Alterations in immune cells and inflammatory mediators have been reported in the eutopic endometrium of women with endometriosis and the inflammatory response to the growth of ectopic tissue alters the peritoneal environment. The inflammatory changes associated with endometriosis however are both varied and complex and their consequences are heavily reliant on both temporal and spatial context. An understanding of this context is required to fully appreciate the inflammatory changes associated with the disease.

To place inflammatory changes into context requires an understanding of both disease progression, and the contribution of various biological compartments and components. While the underlying aetiology of endometriosis remains unclear there is an increasing acknowledgement that the disease has a natural temporal progression starting with subtle changes in the eutopic endometrium that are transferred to the peritoneal cavity through the appearance of endometrial cells at various anatomic locations and eventually progresses towards large fibrotic lesions. Moreover, although characterised and ultimately diagnosed by the presence of endometrial epithelial and stromal cells there are significant roles for other cells including macrophages, natural killer cells, platelets and others. Inflammatory changes accompany all of these stages and are both mediated and influenced by all of these components.

Endometriosis therefore is a complex disease that involves multiple components all of which evolve over time. To better understand the role of inflammation in endometriosis context is paramount. In this presentation the inflammatory changes in endometriosis will be discussed through the paradigm of context, assessing its influence on the natural course of the disease and the components that contribute to its aetiology. A deeper understanding of inflammation within a temporal and spatial context may lead to better design and application of both current and novel treatments.

O-277 The association between endometriosis and other inflammatory diseases

SELECTED ORAL COMMUNICATIONS

SESSION 74: NEW ASPECTS IN REPRODUCTIVE ENDOCRINOLOGY

Wednesday 26 June 2019 Mozart 14:00 - 15:30

O-278 Measuring LH Pulsatility in Patients with Reproductive Disorders Using a Novel Robotic Aptamer-Enabled Electrochemical Reader (RAPTER)

W. Dhillo [1], **S. Liang** [2], **A. Kinghorn** [3], **M. Voliotis** [4], **J. Prague** [1], **J. Veldhuis** [5], **K. Tsaneva-Atanasova** [4], **C. McArdle** [6], **R. Li** [7], **A. Cass** [8], **J. Tanner** [2]

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Study question:

Could the emerging technology of electrochemical aptamer-based sensing offer a generalizable approach for LH pulsatility measurement in humans?

Summary answer:

The RAPTER system provides a new approach for LH pulsatility determination by reliably and immediately calculating varying LH concentrations within distinct patient cohorts.

What is known already:

Assessment of LH pulsatility is important for the clinical diagnosis of patients with reproductive disorders, but is not routinely available in the clinic because of the need for frequent blood sampling coupled with expensive serial immunochemical analysis.

The emerging technology of electrochemical aptamer-based (E-AB) sensing could potentially offer a generalizable approach for LH pulsatility measurement in humans.

E-AB sensors take advantage of the intrinsic properties of nucleic acid aptamers to specifically bind to a molecular target and undergo a reversible conformational change which can be measured through the electrochemical signal response.

However, there is currently no aptamer-based LH sensing technology.

Study design, size, duration:

Here, we report the development and application of a novel antibody-free DNA aptamer-mediated electrochemical analysis to accurately assess LH pulsatility in patients with reproductive disorders.

Through selective evolution of ligands by exponential enrichment, single-stranded DNA oligonucleotides (aptamers) were identified that bind specifically to LH (and that do not bind to FSH).

The aptamers were integrated into E-AB sensors on a robotic platform (which we term RAPTER) to enable sensitive, rapid and repeatable detection of LH.

Participants/materials, setting, methods:

We next determined if the RAPTER system could measure LH pulsatility in clinical samples from patients with reproductive disorders. We obtained 441 serum samples from 3 patient cohorts - young females with regular menstrual cycles, menopausal women, and women with hypothalamic amenorrhoea.

Blood sampling was performed every 10 minutes for 8 hours in these patients and samples were analysed using both the current gold standard LH immunochemical assay and RAPTER.

Main results and the role of chance:

We performed a Bland-Altman analysis and plotted the linear regression to compare the results obtained.

The RAPTER assay showed an almost perfect correlation with the clinical immunochemical assay (R2= 0.94).

Bayesian Spectrum Analysis was used to determine the effective LH pulse interval from both datasets (clinical assay and RAPTER assay).

We are able to distinguish 3 patient cohorts based on the time interval ranges.

Limitations, reasons for caution:

We have provided proof of principle that LH pulsatility measurement can be accurately carried out using aptamer based technology in patients with reproductive disorders. The next steps will be to convert this technology into a wearable device which can continuously sense LH in freely moving patients.

Wider implications of the findings:

The RAPTER system can determine LH pulsatility by reliably and immediately calculating varying LH concentrations within distinct patient cohorts. This has the potential to transform the clinical care of patients with reproductive disorders as LH pulsatility could be measured easily in clinical practice to aid correct diagnosis.

Trial registration number:

not applicable

O-279 Melatonin ameliorates mitochondrial injury by enhancing SIRT1 expression and inhibiting PINK1/Parkin-mediated mitophagy in granulosa cells of classic polycystic ovary syndrome

S. Yi[1], M. Zhu[1], J. Meng[1], H. Sun[1], J. Zhou[1]

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Study question:

Does melatonin affect mitochondrial function by regulating the expression of Sirtuin1 (SIRT1) in granulosa cells (GCs) of classic polycystic ovary syndrome (PCOS)?

Summary answer:

Melatonin ameliorates mitochondrial injury by upregulating SIRT1 expression and inhibiting PINK1/Parkin-mediated mitophagy.

What is known already:

Recent researches suggest that chronic inflammation and oxidative stress caused by mitochondrial damage in granulosa cells of PCOS patients are closely related to pathophysiological changes of PCOS, and SIRT1 plays an important role in maintaining the normal function of mitochondria. Melatonin has been suggested to improve the pregnancy outcomes in PCOS patients, though the underlying mechanism remains largely undetermined.

Study design, size, duration:

Granulosa cells of classic PCOS patients diagnosed according to the 1990 NIH criteria and dihydrotestosterone (DHT)-treated KGN cells were used to investigate the molecular mechanisms in vitro. DHT-induced PCOS mouse model was performed to examine the roles of melatonin on granulosa cells. This is an experiment study lasted ten months.

Participants/materials, setting, methods:

GCs of classic PCOS patients, DHT-treated KGN cells and DHT-induced PCOS mouse were used as study models. Western blotting, quantitative RT-PCR, JC-1 dye, identification of mitochondrial DNA and transmission electron microscope (TEM) were used to determine the mechanism of mitochondria function changes induced by melatonin.

Main results and the role of chance:

The results of western blotting analysis showed that PINK1/Parkin-mediated mitophagy level was significantly increased in GCs in classic PCOS patients compared with control group. We found that mitochondrial membrane potential assessed was decreased ($p<0.05$) and the mtDNA content in GCs were significantly lower in classic PCOS than control group ($p<0.01$). The expression of SIRT1 mRNA and protein levels in GCs were significantly lower in classic PCOS group (both $p<0.01$). But supplementation of in-vitro culture medium with 100pM melatonin for 24h could enhance the expression of SIRT1 protein in GCs of PCOS ($p<0.05$). After 500nM DHT treatment for 24h in KGN cells, SIRT1 protein level was significantly decreased by ($p<0.001$), the PINK1/Parkin-mediated mitophagy level was significantly decreased, mitochondrial membrane potential and mtDNA content were significantly decreased (both $p<0.01$). At the same time, co-treatment with 100pM melatonin for 24h significantly increased SIRT1 protein level ($p<0.05$), inhibited PINK1/Parkin-mediated mitophagy, enhanced mitochondrial membrane potential ($p<0.05$) and increased mtDNA content ($p<0.05$). The results of western blotting assay and TEM showed that induction of PINK1/Parkin-mediated mitophagy was significantly enhanced in GCs collected from mice subjected to DHT administration. In contrast, reduced mitophagy level in GCs of PCOS mouse was observed following melatonin administration.

Limitations, reasons for caution:

The mechanism of melatonin ameliorates mitochondrial injury mediated by SIRT1 and the change in ovarian function of PCOS mouse should be further evaluated.

Wider implications of the findings:

Our results suggest that melatonin ameliorates mitochondrial function by enhancing SIRT1 expression and inhibiting PINK1/Parkin-mediated mitophagy. These finding may subsequently provide novel potential therapeutic regimens for patients with PCOS.

Trial registration number:

This work was supported by the National Natural Science Foundation of China (81771537).

O-280 Evaluation of the hormone Dehydro-epiandrosterone sulphate (DHEAS) as a potentially compelling 'oocyte-related factor' in mammalian oocyte activation: A paradigm shift?

B.N. Chimote [1], N.M. Chimote [1]

[1]Vaunshdhara Fertility Centre, Embryology- Endocrinology, Nagpur, India

Study question:

Is the lipid-soluble steroid hormone dehydro-epiandrosterone sulphate (DHEAS), the elusive oocyte-related factor that regulates mammalian oocyte activation by controlling Ca^{2+} release from inner mitochondrial stores?

Summary answer:

The **oestro-androgen** DHEAS is the 'oocyte-related factor' that influences fertilization, embryo development and pregnancy outcomes potentially by regulating oocyte activation via maintenance of calcium homeostasis.

What is known already:

Sperm-factor PLCζ is largely implicated in oocyte-activation (OA). However, fertilization-failure despite using PLCζ activated sperm; observation of OA-fertilization-live-birth using **PLCζ null sperm;** and obtaining oocyte calcium-signatures, suggests involvement of oocyte-factors. The androgen DHEAS, peculiarly also behaves as an oestrogen. Synthesized by ovarian cumulus/-granulosa cells, DHEAS acts via putative oocyte receptors/anion exchangers. In certain human cell-types, DHEAS regulates the same calcium-pumps which maintain calcium-oscillations and cause OA in mice. Altered DHEAS levels have been demonstrated in women with hampered fertilization/embryo development/pregnancy outcomes. DHEAS thus promises to be the hitherto elusive, potential oocyte-related factor that regulates oocyte-activation and affects fertilization/embryo development/pregnancy outcome.

Study design, size, duration:

Prospective Randomized study (July 2016-November 2018) of n=625 women (age:25-35years, BMI:18-28kg/m^2) undergoing antagonist stimulation protocol IVF-ICSI followed by day5 blastocyst-transfer for tubal-factor/unexplained infertility/previous 3-4 failed intra-uterine-insemination cycles or with previous 2-3 attempts of low/failed IVF/ICSI fertilizations. Patients with adrenal patho-physiology and/or male factor were excluded. Three DHEAS groups: Low (**A:**<95 µg/dL), Average (**B**:95-270 µg/dL), High (**C**:>270 µg/dL), were classified as per previously established thresholds of baseline D3serum-DHEAS, measured by radioimmunoassay. Follicular-fluid DHEAS was also measured.

Participants/materials, setting, methods:

Three groups, divided randomly into sub-groups, received following interventions: **A1** (lower control/no supplementation), **A2** (3 months oral micronized DHEAS 75mg/day); **B** (normal controls/no intervention); **C1** (higher control/no treatment), **C2** (3 months metformin treatment 1500mg/day). Anticipating 20-25% cycle-cancellations/dropouts/no response, a pre-treatment sample size n=125 per sub-group, assured 80-85% power to study. Intra-group, Intergroup differences in fertilization-cleavage-blastocyst formation, clinical pregnancy, live-birth, early miscarriage rates were calculated by Student's t-test/ANOVA, odds-ratio, correlation-coefficient, as applicable, using Graphpad PrismVI software.

Main results and the role of chance:

A2-Group women whose DHEAS levels **increased to optimal/average** after 3 months oral DHEAS supplementation (n=98) showed significantly improved fertilization (80 vs.55%;p<0.0001), cleavage (78.5 vs.53%;p<0.0001), blastocyst formation (43 vs.22%;p=0.002), live-births (35vs.15%;p=0.0006), reduced early miscarriages (3.5vs.9%;p=0.008), compared to no-supplementation A1 group (n=107).

Metformin treatment is known to lower DHEAS levels in women with raised baseline DHEAS, whereas it has no effect on women with normal baseline DHEAS. Indeed, **Group-C2** women whose DHEAS levels **reduced to optimal/average** after 3 months metformin treatment (n=105) displayed significantly enhanced fertilization (79vs.53%;p<0.0001), cleavage (76vs.52%;p=p=0.0005), blastocyst formation (40vs.20%;p=0.0001), live-births (31vs.14%; p=0.0003), reduced early miscarriages (4vs.11%;p=0.0005) compared to no-supplementation C1 group (n=109).

As expected, post-treatment improved parameters in A2 and C2 groups were comparable with the results observed in the normal-control/average B-group women (Fertilization 88%, Cleavage 87%, Blastocyst-formation 48%, Live-births 39%, early miscarriage 2.5%; p=ns).

Follicular-fluid DHEAS levels were also classified into **Low-I** (<760ng/ml), **Medium-II** (760-1850ng/ml) and **High-III** (>1850ng/ml) groups. All evaluated parameters in A1 and C1 groups were comparable with Low-I and High-III groups respectively whereas those in A2, C2 and B groups corroborated with Medium-II group. Fertilization rates strongly correlated with Follicular-fluid DHEAS levels (Pearsonr=0.78). Odds of fertilization, embryo development and live-births increased post-treatment in both A2 and C2 groups.

Limitations, reasons for caution:

Instead of directly measuring Ca^{2+}oscillations to evaluate OA, we indirectly assessed fertilization/embryo development/pregnancy outcomes, since ethical considerations prevented use of oocyte-destructive methods. Measuring Ca^{2+}oscillations in mice is impractical as results cannot be extrapolated to humans. Future plan involves measuring Ca^{2+}oscillations in donated human oocytes by epi-fluorescence microscopy using radiometric dyes.

Wider implications of the findings:

This study sets new paradigms by introducing DHEAS as a **potential first-messenger oocyte-related factor** in OA. Results suggest that since not all poor-responders have lower thresholds, nor all PCOS women have higher thresholds of DHEAS; treatment modality should be based on baseline serum DHEAS levels rather than providing empirical treatment.

Trial registration number:

Not Applicable

O-281 Effect of MVT-602, a potent kisspeptin receptor agonist, on luteinizing hormone (LH) concentrations in healthy pre-menopausal women undergoing a minimal controlled ovarian stimulation (COS) protocol

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Study question:

What is the effect of MVT-602 on luteinizing hormone (LH) concentrations in healthy women undergoing a minimal COS protocol?

Summary answer:

Peak LH concentrations occurred 20 hours after MVT-602 following minimal COS achieving an endocrine milieu similar to the preovulatory phase of the menstrual cycle.

What is known already:

Kisspeptin-54, the endogenous peptide ligand of the kisspeptin receptor (KISSR), is known to be responsible for GnRH release from the hypothalamus. Kisspeptin is therefore considered to play an essential role in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis. In a previous Phase 1 study, MVT-602, a potent KISSR agonist, showed increases in LH and follicle-stimulating hormone (FSH) after administration of MVT-602 to healthy premenopausal women in the follicular phase of their menstrual cycle. The peak post-MVT-602 LH concentrations occurred later than those observed using native kisspeptin-54 (Dhillo et al).

Study design, size, duration:

A randomized, double-blind, placebo and active comparator-controlled, parallel-group, phase 2a study conducted May and October 2018. Seventy-five women (<36 years, body mass index 18.0 to 30.0 kg/m^2) underwent minimal ovarian stimulation (initially 100 IU rFSH [follitropin alfa] and 0.25 mg GnRH agonist [ganirelix]). Once the dominant follicle reached ≥17 mm, women were randomized to single dose MVT-601 (0.1, 0.3, 1 or 3 µg), triptorelin 0.2 mg or placebo (3:1:1 ratio).

Participants/materials, setting, methods:

Follicular growth was monitored by transvaginal ultrasound (TVUS) beginning on day 3-5 of their menstrual cycle. Once the dominant follicle reached ≥13 mm, minimal stimulation with rFSH and GnRH commenced. The trigger agent (MVT-602, triptorelin, or placebo) was administered once the dominant follicle reached ≥ 17 mm, 12 hours following the last ganirelix dose. Hormone levels were measured every 2 to 8 hours for 48 hours post-dose, then daily until discharge

Main results and the role of chance:

After MVT-602, mean peak LH concentrations of 50 IU/L occurred at approximately 20 hours (range: 16 to 36 hours) after all doses of MVT-602 and returned towards baseline by 60 hours post-dose. In contrast, mean LH concentrations reached a higher amplitude after triptorelin (150 IU/LH) and occurred sooner (approximately 12 hours post-dose). The proportion of subjects who achieved a maxiumum LH concentration > 50 IU/L increased with increasing dose of MVT-602. Changes in FSH concentrations followed a similar pattern to those observed for LH. Estradiol levels after MVT-602 administration were approximately 2000 pmol/L or less in subjects whose pre-dose baseline values were 1000 pmol/L or less. In all MVT-602 dose groups, progesterone concentrations rose from approximately 50 hours post-dose and continued to increase up to 120 hours post-dose. Administration of MVT-602 was generally well tolerated in this study with no apparent dose-dependent trends in safety parameters and, as expected, no ovarian hyperstimulation was observed.

Limitations, reasons for caution:

A minimal stimulation protocol was used to avoid unethical unnecessary stimulation of healthy women who were not seeking conception. Further research is indicated in subfertile women treated with a full COS protocol to establish the safety and efficacy of MVT-602 as a trigger of oocyte maturation in IVF treatment.

Wider implications of the findings:

The LH profile after MVT-602 is of lower amplitude and longer duration than GnRH agonist. However, the LH profile was still a shorter duration than following hCG suggesting MVT-602 could offer an ideal novel agent to trigger oocyte maturation during IVF treatment, that balances the risks of over and under-stimulation.

Trial registration number:

2018-001379-20

O-282 Granulosa cell signaling-based bioassay in vitro for personalized stimulation in ART

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Study question:

Is the individual response to gonadotropins predictable?

Summary answer:

Granulosa cells (hGLC) from normo-responder women display higher cAMP and progesterone production *in vitro*, as well as estradiol levels *in vivo*, than sub-responders.

What is known already:

The various stimulation protocols do not provide the best stimulation setting and some patients may be subjected to inadequate stimulation resulting in clinical treatment failure. It is expected that hGLC discarded samples donated by normo-responder and sub-responder women undergoing assisted reproduction (ART) feature specific follicle-stimulating hormone (FSH) receptor (FSHR) expression and response to gonadotropins in vitro, suggesting they may be used for predicting the response *in vivo* of normo- *versus* sub-responder women.

Study design, size, duration:

hGLC samples from anonymized donor women undergoing oocyte retrieval for ART are collected during a 30-months period (2017-2019). Cells are purified, cultured, genotyped and treated by gonadotropins *in vitro* and intracellular signaling endpoints are collected. Data obtained by cells from 105 normo-responder women are compared to those from 105 sub-responders, which are identified as patients providing ≤4 oocyte retrieved. Experiments are performed under the local Ethics Committee permission (n°2018/0080377, 16/07/2018) and donor written consent.

Participants/materials, setting, methods:

hGLC donor women undergone ART without endocrine abnormalities and infectious diseases. hGLC samples are genotyped for FSHR polymorphisms known to modulate the response to FSH (rs6166, rs6165, rs1394205) and stimulated by increasing doses (nM range) of recombinant FSH (Gonal-F, Merck KGaA, Darmstadt, Germany). cAMP and progesterone are measured by immunoassays and dose-response curves performed. Results from samples will be used as reference Control Group and/or compare different sample populations, together with clinical data.

Main results and the role of chance:

To date, 76 samples resulted to be eligible for *in vitro* stimulation. 3-h cAMP data from normo- *versus* sub-responder women resulted in different potency, as demonstrated by 50% effective concentrations (EC50) *in vitro* (FSH EC50 normo-responders=5.8±4.9 nM; EC50 sub-responders=2.4±9.8 nM; $p<0.05$; t-test), and different *plateau* levels, indicating phenotype-specific efficacy (cAMP *plateau* normo-responders=37.34±5.3 pmol/ml; cAMP *plateau* sub-responders=15.69±4.2 pmol/ml). 24-h basal progesterone production of cells from normo-responder women is about 1.3-fold higher than that of cells from sub-responders. Most importantly, both the groups of data confirmed that FSH elicit phenotype-specific response, depending on the normo- or sub-responder *status*. Indeed, FSH EC50, and progesterone basal and *plateau* levels are higher in cells from normo-responders versus sub-responders ($p<0.05$; t-test). Distribution of FSHR genotypes was evaluated by contingency analysis. No different allele frequencies and *FSHR* expression levels between normo- versus sub-responders were found (Chi square and t-test; $p>0.05$; n=55), demonstrating that each the two groups are genetically homogeneous. Estradiol levels are higher in normo- than sub-responders (2026±903.5 *versus* 1375±898.9 pg/ml; t-test; $p>0.05$; n=49). We demonstrate that normo-responders display higher sensitiveness to FSH than sub-responders, revealing association between intracellular signaling, estradiol production and follicle growth.

Limitations, reasons for caution:

To date, patient recruitment is still ongoing and about one hundred individuals should be required to achieve proper statistical power.

Wider implications of the findings:

This study may predict the individual response to personalize ART protocol, in case of stimulation cycle planning after previous failures. Given the different in-vitro response between cells from normo- vs sub-responders, we expect that this in-vitro assay could lead to future clinical trials that may confirm in vitro results.

Trial registration number:

not applicable

O-283 Pharmacokinetics and pharmacodynamics of a new recombinant human chorionic gonadotropin (rhCG) in healthy women and men after single and multiple subcutaneous administration

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Study question:

Do pharmacokinetic and pharmacodynamic characteristics differ between rhCG expressed in a human cell line and rhCG expressed in a Chinese hamster ovary (CHO) cell line?

Summary answer:

The pharmacokinetics of the two rhCG preparations were different, causing higher exposure and a higher pharmacodynamic response for hCG expressed in a human cell line.

What is known already:

In current clinical practice, urinary or recombinant hCG is administered for treatment of infertility either during ovarian stimulation or to trigger final follicular maturation. The new rhCG (FE 999302) currently in development is produced by a human-derived cell line, PER.C6. The amino acid sequence of the α- and β-chains are identical to that of rhCG expressed by CHO cells (choriogonadotropin alfa, Ovitrelle®), but the glycosylation profile differs between the two rhCG preparations.

Study design, size, duration:

Part 1 treated five female cohorts with single ascending doses from 4 to 256 µg of FE 999302 or placebo (N=35). Part 2 treated two female cohorts with

Table I LIF T>G(rs929271) gene X population/semen parameters

	LIF Genotype						
	TT	TG	GG	*P*	TT	TG+GG	*P*
n	152	172	40		152	212	
Age(years)	38.0±6.4	38.1±5.9	38.2±5.3	0.98	38.0±6.4	38.1±5.7	0.86
BMI(Kg/m^2)	28.7±4.4	28.4±3.9	27.7±5.6	0.11	28.7±4.4	28.3±4.3	0.29
Abstinence(days)	3.4±1.9	3.2±2.3	3.0±1.7	0.12	3.4±1.9	3.2±2.2	0.40
pH	8.1±0.3	8.1±0.3	8.1±0.3	0.74	8.1±0.2	8.1±0.3	0.49
Volume(ml)	2.8±1.4	2.8±1.3	3.1±1.8	0.76	2.8±1.4	2.8±1.4	0.94
Concentration(mlx10^6)	64.1±48.1	63.7±56.4	55.7±44.4	0.60	64.1±48.1	62.2±54.3	0.38
Progressive motility(%)	53.0±17.2	54.0±16.1	51.2±14.4	0.60	53.0±17.2	53.5±15.8	0.38
Total motility(%)	59.4±17.0	61.8±16.1	57.6±13.2	0.17	59.4±17.0	61.0±15.8	0.55
Normal sperm(%)	**0.8±0.8[a]**	**0.6±0.8[a]**	**0.6±0.7[a]**	**0.03[a]**	**0.8±0.8**	**0.6±0.8**	**0.03**
Leukocytes(x10^6/ml)	0.4±0.56	0.5±0.9	0.3±0.2	0.75	0.4±0.6	0.4±0.8	0.65
Vitality(%)	61.5±16.1	63.5±14.1	59.8±19.3	0.70	61.5±16.1	62.8±15.1	0.67
DNA fragmentation(%)	13.7±7.6	13.5±8.0	14.1±7.6	0.78	13.7±7.6	13.6±7.9	0.77
Apoptosis(%)	19.2±7.2	19.6±8.2	21.2±10.0	0.79	19.2±7.2	19.9±8.6	0.68
CMA3 positivity(%)	**52.5±17.5[a,b]**	**57.7±16.1[a]**	**59.7±17.3[b]**	**0.02[a]/0.04[b]**	**52.5±17.5**	**58.1±16.3**	**0.01**
Abnormal MMP(%)	26.8±16.3	25.9±17	23.2±19	0.53	26.8±16.3	25.3±17.4	0.42

multiple ascending doses of 8 or 16 µg FE 999302 or placebo for 10 days via daily injections (N=16). Part 3 administered single 125 µg injections of both FE 999302 and rhCG expressed in a CHO cell line to 33 males in a randomised crossover design.

Participants/materials, setting, methods:

Women and men were pituitary-suppressed by contraceptives and triptorelin, respectively and all rhCG injections were administered subcutaneously in the abdominal wall. Serum hCG and testosterone levels (men only) were assessed at pre-dose and up to 264 hours. Pharmacokinetic parameters of hCG and pharmacodynamic parameters of testosterone were calculated using non-compartmental methods. Analysis of serum hCG and testosterone concentrations was performed by means of a validated immunoassay, and validated liquid chromatography and mass spectrometry method, respectively.

Main results and the role of chance:

In women, the area under the curve (AUC) and the peak serum concentration (C_{max}) increased consistently with dose proportionality following single and multiple doses of FE 999302. The apparent clearance (CL/F) was approximately 0.5 L/h, the mean terminal half-life approximately 45 hours and the apparent distribution volume (Vz/F) approximately 30 L. After single administration of rhCG in down-regulated men, mean AUC was 1.5-fold greater for FE 999302 than for rhCG expressed in CHO cells. Mean C_{max} was similar for the two preparations. In accordance with the differences in AUC, the apparent clearance (i.e. the ratio CL/F) was lower for FE 999302 (CL/F 0.5 vs 0.8 L/h), respectively, explained by a longer terminal half-life (47 vs 32 hours). The apparent distribution volumes (Vz/F) were comparable. The concentrations of testosterone (reflecting production of testosterone as a response to hCG exposure) reflected the pharmacokinetic profiles with a slight delay, resulting in 59% higher AUC with FE 999302. The pharmacokinetic parameters for FE 999302 were comparable in men and women at doses of 125 and 128 µg, respectively.

Limitations, reasons for caution:

Differences between rhCG expressed in a human cell line and in a CHO cell line were assessed in men only. However, since the PK differences of gonadotropins between men and women are known to be limited, differences similar to those found in men would also be expected in women.

Wider implications of the findings:

Due to pharmacokinetic differences, the therapeutic dose(s) for rhCG expressed in a human cell line are likely to be different from those of rhCG expressed in a CHO cell line.

Trial registration number:

EudraCT Number 2015-004628-75

SELECTED ORAL COMMUNICATIONS

SESSION 75: BASIC SCIENCE OF ANDROLOGY

Wednesday 26 June 2019 Haydn 1 14:00 - 15:30

O-284 Association between leukaemia inhibitory factor (LIF) gene polymorphism (rs929271) and sperm quality and sperm DNA integrity

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Study question:

Is there an association between leukaemia inhibitory factor (LIF) thymine (T)>guanine (G) (rs929271) gene polymorphism and sperm quality and sperm DNA integrity?

Summary answer:

Males carrying the G allele(TG/GG genotypes) exhibited decreased counts of sperm with normal morphology and impaired sperm protamination when compared to males carrying the TT-genotype.

What is known already:

LIF is a cytokine that plays a critical role in the human reproductive process. Changes in the expression of the LIF gene have been associated with infertility. A higher prevalence of mutations near the start codon of exon 1 and exon 3 was observed in infertile women.The single nucleotide polymorphism (SNP) T>G (rs929271) located in the untranslated region 3′ of the LIF gene has been associated with use of fertility medication, human embryo survival, and implantation and pregnancy outcomes after ART. However, the influence of LIF gene polymorphisms on semen quality is not usually analysed.

Study design, size, duration:

The study enrolled 364 men from couples submitted to infertility assessment. Individuals with genital tract disorders/azoospermia were excluded. The semen analysis results derived from one sample of semen of each enrolled man were recorded (WHO criteria/morphology:motile sperm organelle morphology examination/MSOME). For DNA integrity analysis, the proportions of DNA fragmentation (by TUNEL assay), abnormal chromatin packaging/underprotamination (by chromomycin A3/CMA3), abnormal mitochondrial membrane potential (MMP/by MitoTracker Green), and apoptosis (by annexin-V) were recorded.

Participants/materials, setting, methods:

DNA was extracted from peripheral blood samples taken from each participant and the LIF T>G SNP (rs929271) was genotyped by real-time PCR. The men were divided into three genotype groups according to their results: TT; TG; or GG. Potential confounders (age, abstinence, smoking, drinking alcohol, and varicocele) were also observed. The level of significance was set at $P<0.05$. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.

Main results and the role of chance:

No correlation was observed between LIF T>G and age, abstinence, smoking, drinking alcohol, or varicocele. The LIF genotypes carrying the G allele were associated with higher proportions of spermatozoa with abnormal chromatin packaging and lower proportions of normal spermatozoa. There was no association between LIF genotypes and other semen parameters (Table 1).

Limitations, reasons for caution:

A possible limitation is the cross-sectional nature of the data. Furthermore, the study was conducted with couples seeking fertility treatment and might therefore be biased toward infertility. Differences in the genetic backgrounds of various ethnic populations might also be considered.

Wider implications of the findings:

The results indicated that a simple genetic variation (LIFT>G rs929271 gene polymorphism) apparently affects semen quality. Additional validation of the analysed SNP (increasing the number of cases) is required to confirm these findings and provide information about clinical implications

Trial registration number:

Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

O-285 The role of sperm surface galectin-3 in zona pellucida binding and its association with fertilization in vitro

P.C.N. Chiu[1], S. Mei[1], P. Chen[2], C.L. Lee[1], W. Zhao[2], Y. Wang[1], K.K.W. Lam[1], P.C. Ho[1], W.S.B. Yeung[1], C. Fang[2]

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Study question:

Is sperm surface galectin-3 involved in spermatozoa-zona pellucida (ZP) interaction?

Summary answer:

Galectin-3 is transferred to sperm surface by extracellular vesicles (EVs) during post-testicular maturation and plays a critical role in sperm-ZP binding after capacitation.

What is known already:

Human spermatozoa leaving the testis can fertilize an oocyte only after post-testicular maturation and capacitation. These processes involve modification and reorganization of molecules on the sperm plasma membrane, resulting in acquisition of the binding ability of the spermatozoa to the ZP of the oocyte. Defects in the binding are major causes of male subfertility. Galectin-3 is a secretory lectin well known for its action on cell adhesion and surface receptor trafficking/clustering. In the male reproductive tract, galectin-3 protein has been identified in Sertoli cells, epididymis, seminal plasma, ejaculated spermatozoa and EVs.

Study design, size, duration:

The expression of galectin-3 in seminal plasma/spermatozoa and its association with fertilization rate in clinical assisted reproduction were studied. The roles of galectin-3 on sperm function were assessed by functional blocking anti-galectin-3 antibody and galectin-3 competitive carbohydrate substrate.

Participants/materials, setting, methods:

Spermatozoa were obtained from semen samples from normozoospermic men. Human oocytes were obtained from an assisted reproduction program. EVs were isolated from the cell-free seminal plasma by sequential centrifugation and microfiltration. The localization and expression of galectin-3 was studied by immunostaining, Western blotting, ELISA, immunogold transmission electron microscopy and flow cytometry. Sperm functions including motility, viability, acrosome reaction and ZP-binding capacity were assessed by standard assays.

Main results and the role of chance:

The acrosomal region of ejaculated and capacitated spermatozoa possesses strong galectin-3 immunoreactivity, which is much stronger than that of epididymal spermatozoa. Expression of galectin-3 can also be detected on seminal plasma-derived EVs which can be transferred to the sperm surface. Blocking of sperm surface galectin-3 function by specific antibody against galectin-3 or competitive carbohydrate substrate reduced the ZP-binding capacity of human spermatozoa. Galectin-3 level in seminal plasma-derived EVs was positively associated with fertilization rates.

Limitations, reasons for caution:

The mechanisms by which galectin-3 regulates spermatozoa-ZP binding have not been depicted.

Wider implications of the findings:

Seminal plasma galectin-3 may be a biomarker for male infertility, relating to spermatozoa-ZP binding capacity. In addition, modulation of the surface galectin-3 content of the spermatozoa of subfertile men may help to increase fertilization rate in assisted reproduction.

Trial registration number:

not applicable

O-286 Sperm membrane Glucose regulated protein 78 (GRP78) crosstalk with activated alpha-2-macroglobulin (α2M*) chaperones sperm motility

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Study question:

What is the role of sperm membrane-bound Glucose regulated protein 78 (GRP78) in sperm function?

Summary answer:

Alpha-2-macroglobulin (α2M) secreted by Sertoli cells, upon activation, binds to sperm surface GRP78 during epididymal transit thereby facilitating sperm motility via actin reorganization.

What is known already:

GRP78, a resident endoplasmic reticulum (ER) chaperone is expressed on the cell surface in exceptional conditions as seen in cancer cells and macrophages. α2M from blood plasma upon activation (α2M*) reportedly binds surface GRP78 regulating macrophage motility. We have previously reported membrane localization of GRP78 on rat and human sperm and have shown that its tyrosine phosphorylation significantly increases during epididymal maturation. In human sperm, we have reported GRP78 phosphorylation to be significantly reduced in asthenozoosperm as compared to normozoosperm. Literature suggests significantly low levels of seminal plasma α2M in asthenozoosperm.

Study design, size, duration:

α2M presence, its co-localization and interaction with sperm surface GRP78, cofilin pathway proteins and intracellular calcium levels [$(Ca^{2+})_i$] in testicular (immature) sperm [Tsp] and caudal epididymal (mature) sperm [Cdsp] were determined. Upon incubation of Tsp with α2M*, the influence of this interaction on i) $[Ca^{2+}]_i$ levels ii) Membrane GRP78 tyrosine phosphorylation iii) Activation of downstream cofilin signaling pathway using specific inhibitors iv) Flagellar F-actin v) Cofilin translocation vi) motility changes in sperm was studied.

Participants/materials, setting, methods:

Cdsp and Percoll purified Tsp from adult Holtzman male rats were used. Western blot (WB) analyses performed for α2M, GRP78, cofilin pathway proteins. Surface- α2M and GRP78 determined by IIF and ELISA. Fura-2AM employed for $[Ca^{2+}]_i$ assays. Interaction studies and membrane-GRP78

phosphorylation status determined by Immunoprecipitation. Sperm- head and tail fraction proteins analyzed by WB in cofilin translocation studies. Sperm motility studied under light microscope. F-actin status and cofilin abundance demonstrated by IF. (n≥ 3)

Main results and the role of chance:

α2M expression was observed on the head, midpiece and principal piece of mature sperm (Cdsp). A weak and speckled localization was observed on immature sperm (Tsp). It co-localizes and interacts with membrane GRP78 in Cdsp but not in Tsp. The key proteins involved in cofilin pathway are present in both Tsp and Cdsp, however the pathway is more active in Cdsp than Tsp. $[Ca^{2+}]_i$ levels were significantly higher in Cdsp compared to Tsp. Treatment of Tsp with α2M* significantly increased $[Ca^{2+}]_i$ levels and activated the cofilin signalling pathway significantly. Cofilin-, LIMK-, Rac-, Cdc42-, & PAK phosphorylation, GRP78 tyrosine phosphorylation in lysate obtained from Tsp incubated with α2M* increased significantly as compared to Tsp treated with α2M. Significant increase of F-actin was observed with significant decrease of cofilin (actin severing protein) in the flagella, when Tsp were exposed to α2M*. This increase was abrogated in presence of PI3K inhibitor (LY294002) and tyrosine kinase inhibitor (Genistein) whereas Ras FTase inhibitor (Manumycin A) had no effect. Thus, profiles observed with mature sperm (Cdsp) could be re-created when immature sperm (Tsp) were exposed to α2M*. The basal motility levels of Tsp was maintained upon exposure with α2M* as compared to Tsp incubated with α2M.

Limitations, reasons for caution:

Rat Tsp and Cdsp used as representation of immature- and mature sperm respectively, due to difficulty in obtaining testicular biopsies from fertile individuals to obtain immature sperm required to address the research question. Evidences that α2M is largely in native form in testis and as α2M* in epididymis, is indirect.

Wider implications of the findings:

α2M interacts with sperm surface GRP78 during epididymal transit, increases GRP78 phosphorylation thereby activating cofilin pathway which prevents flagellar F-actin depolymerization. As F-actin is required for maintaining sperm motility, our finding has significant implication as our previous reports show reduced GRP78 phosphorylation and altered actin based motility pathway in asthenozoosperm.

Trial registration number:

Not applicable

O-287 Aberrant vitamin A and K signaling in the local testis microenvironment and their impact on extracellular matrix (ECM) composition

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Study question:

To study pathogenic features of the somatic testicular microenvironment associated with idiopathic germ cell aplasia.

Summary answer:

Impaired vitamin A and K signaling in human Sertoli and Leydig cells are associated with an unbalanced composition of testicular ECM and germ cell aplasia.

What is known already:

Several studies have established the relevance of the somatic microenvironment, including the ECM, in supporting spermatogonial stem cell differentiation. Indeed, germ cells of an infertile man or an aged mouse show to retain their capability of generating functional spermatozoa for a long time when, upon transplantation into a healthy or younger recipient's testis, they mature into functional spermatogonia. Conversely, spermatogonial stem cells from young mice transplanted into atrophic testicles are unable to promote and support spermatogenesis. Moreover, the testis contains 102 proteins ascribed to the ECM, whose role in supporting human spermatogonial stem cell survival was recently described.

Study design, size, duration:

Cross-sectional study carried out at a single tertiary referral center for male infertility. 20 testicular specimens from infertile men affected by idiopathic non-obstructive azoospermia, undergoing micro testicular sperm extraction (microTESE). Primary Sertoli cells with normal karyotype and phenotype were also used.

Participants/materials, setting, methods:

Proteomic analysis of the ECM from testicular specimens with positive vs. negative sperm retrieval. Gene ontology enrichment was used to identify upstream regulators based on the 11 deregulated ECM proteins, which were validated by immunohistochemistry and quantitative PCR. Continuous variables were expressed as medians and interquartile range.

Main results and the role of chance:

Germ cell aplasia was characterized by an increased signaling of the retinoic acid in Sertoli cells, and associated with decreased expression of the basal membrane markers Nidogen-2 and Heparan sulfate proteoglycan-2. In vitro stimulation of Sertoli cells with retinoic acid down regulated the expression of Nidogen-2 and Heparan sulfate proteoglycan-2, but not Laminin-4 and Laminin-5. The immature phenotype of Sertoli cells in the testis parenchyma with germ cell aplasia was further demonstrated by the high systemic level of the Anti-Mullerian Hormone. Decreased levels of the interstitial matrisome-associated Factor-IX and its regulator vitamin K epoxide reductase complex subunit 1 (VKORC1) were, rather, coupled with decreased signaling of vitamin K in Leydig cells. An altered expression of 8 further ECM proteins was also found, including Laminin-4 and Laminin-5. Peripheral levels of the two vitamins were within the reference range in the two cohorts of iNOA men.

Limitations, reasons for caution:

Limited number of patients from a single center. Further studies are needed to better define the causative mechanism of vitamin A and K dysfunction in the testis of men with complete germ cell aplasia.

Wider implications of the findings:

Our findings open new perspectives to look for the dysregulating mechanisms of the metabolic cycle of vitamin A and vitamin K in the Sertoli and Leydig cells, respectively, such as epigenetics silencing, post-transcriptional modifications, detrimental effect of commensal microbiome, eventually shedding light on male azoospermia.

Trial registration number:

Not aapplicable

O-288 High-throughput differential proteomics identifies altered mitochondrial and proteasomal sperm function in repeated fertilization failure

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Study question:

Do sperm samples from patients who suffered repetitive fertilization failure (FF) after ICSI present alterations in their protein contents?

Summary answer:

Sperm samples producing FF after ICSI show several alterations in the expression of mitochondrial and proteasomal proteins.

What is known already:

Unexplained fertilization failure, occurring in 1-3% of ICSI cycles, results in both psychological and financial burden for the patients, and it is often challenging for the clinicians because its causes remain largely unknown. Although altered PLCz function can cause FF, other mechanisms are also likely to be involved. Mass spectrometry is a powerful technique to identify and quantify proteins across samples, however, no study so far has used it to dissect the proteomic signature of sperm with FF after ICSI. We aim at identifying target mechanisms and pathways that could explain FF aetiology and, potentially, predict its recurrence.

Study design, size, duration:

We compared the sperm protein profile of 12 men undergoing ICSI according to fertilization rates by high-throughput proteomics (4 patients with >2 consecutive FF cycles and 8 patients with fertilization rates >75%). Differential

expression of proteins was validated by Western Blot and functional analysis of mitochondrial and proteasomal activity was determined by FACS analysis of JC-1 staining and AMC-peptide fluorimetric assay, respectively. The study period was 24 months.

Participants/materials, setting, methods:

Sperm samples were processed by 50% density-gradient, lysed, and protein extracts were used individually for TMT-labelling, followed by two-dimensional liquid chromatography and tandem mass-spectrometry (2D-LC-MS/MS) to identify and quantify proteins. Conventional (t-test on the relative quantification values) and novel approaches (stable-protein pairs) were carried out to analyze quantitative proteomic data to assess altered proteins in repetitive FF patients. Those altered proteins were validated in 11 samples by other techniques.

Main results and the role of chance:

A total of 1400 proteins were identified with at least one unique peptide and 232 proteins were considered for further analysis using our quantification strict criteria (<1% FDR, ≥2 PSM per unique peptide, <50% variation among samples). Four proteins presented lower abundance (FMR1NB, FAM209B, RAB2B, PSMA1) in the FF group compared to the control group ($p<0.05$), while five mitochondrial proteins had higher abundance in the FF group (DLAT, ATP5H, SLC25A3, SLC25A6, FH). Stable-protein pairs analysis identified 146 correlations comprising 28 proteins in controls, while in the FF samples >50% of correlations were lost, mainly for proteins related to mitochondrial function (i.e. PDHA2, PHB2, ATP5F1D). Altered abundance of DLAT (member of the pyruvate dehydrogenase complex) and PSMA1 (essential for proteasome function) in patients with repetitive FF was validated by Western-Blot. The mitochondrial function assessment revealed that FF patients have a slightly lower percentage of sperm containing active mitochondria (71.98 ± 20.2) compared to controls (59.12 ± 12.9) *but not reaching* statistical significance. Moreover, proteasome activity was lower in FF samples compared to controls (0.206 ± 0.08 vs. 0.352 ± 0.18 proteasome activity units, respectively). Altogether, these results suggest that mitochondrial and proteasomal functions could be altered in patients with repeated FF.

Limitations, reasons for caution:

Although strict criteria were used to select the couples in the study, an oocyte contribution to FF cannot be completely ruled out. Repeated FF cases are rare, and 4 cases were included. However, this is in line with other studies to identify molecular causes of infertility by high-throughput proteomics.

Wider implications of the findings:

This is the first proteomic analysis of sperm from patients with repeated FF after ICSI. The differentially expressed proteins and the dysregulation of mitochondrial and proteasomal functions could help to identify diagnostic/prognostic markers of FF and further dissect the paternal molecular contribution to successful fertilization.

Trial registration number:

not applicable

O-289 Mapping the genital tract: Using male gamete genomic integrity to guide reproductive outcome

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Study question:

Does sperm chromatin fragmentation (SCF) vary in different areas of the male genital tract, and is it useful for enhancing reproductive treatments?

Summary answer:

SCF decreases closer to the seminiferous tubule, suggesting that the utilization of surgically retrieved spermatozoa enhances implantation and clinical pregnancy rates.

What is known already:

Sperm genome integrity plays a crucial role in male reproduction. Physiological DNA severing is a prerequisite for supercoiling and nuclear compaction during spermiogenesis. The sequential process is highly conserved and ordained by DNAse and ligase mechanisms. If proper repair fails, suboptimal spermatozoa are picked up by the epididymis and phagocytized. When this downhill mechanism fails, leakage of abnormal spermatozoa may manifest in the ejaculate. Higher SCF can also be attributed to reactive oxygen species hindering the gametes during their permanence in and transit through the male genital tract.

Study design, size, duration:

In a preliminary assessment, clinical outcome was compared between men with normal (n=200) and abnormal (n=122) SCF. A total of 83 consenting men with abnormal ejaculate SCF who had failed to conceive with ICSI utilizing ejaculated spermatozoa agreed to undergo surgical retrieval in order to assess the genomic integrity of their spermatozoa at different levels of the genital tract. Some of these men (n=45) underwent 78 ICSI cycles utilizing surgically retrieved spermatozoa.

Participants/materials, setting, methods:

Ejaculated specimens were screened by terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL) assay using a commercially available kit, or SCSA. For TUNEL, a minimum of 500 spermatozoa were counted per specimen using fluorescent microscopy with a threshold of 15% as normal. Surgical samples were analyzed by TUNEL assay on specimens isolated from the vas deferens, epididymis, and testes before being cryopreserved in multiple vials for subsequent ICSI cycles.

Main results and the role of chance:

In the preliminary assessment, ICSI outcomes utilizing ejaculated spermatozoa were compared between men with normal (n=200, 9.3±3%) and abnormal (n=122, 22.6±9%) SCF. While fertilization and clinical pregnancy rates (CPR) did not differ between the 2 groups, the implantation rate was significantly impaired (P=0.04) in men with abnormal DNA fragmentation.

Surgical mapping showed that SCF decreased from 32.9±20.0% in the ejaculate to 20.0±9.6% in the vas deferens, 15.8±7.7% in the epididymis, and 11.5±5.7% in the testis.

In ICSI cycles utilizing ejaculated spermatozoa (n=35) with fragmented chromatin (n=85), the fertilization, implantation, and clinical pregnancy rates were 68.2%, 7.6%, and 13.9%, respectively. Of those clinical pregnancies, 20% resulted in a miscarriage.

For the men (n=45) who failed to conceive using ejaculated spermatozoa, frozen epididymal or testicular spermatozoa with normal SCF were used for a subsequent ICSI cycle (n=78). For this cycle, the fertilization rate was 66.9%, the implantation rate was 19.6% (P = 0.001), the clinical pregnancy rate was 40.0% (P < 0.001), and the clinical pregnancy loss fell to 14.3%.

Limitations, reasons for caution:

Surgical retrieval of spermatozoa is an invasive approach that carries a surgical risk. It should only be considered after extensive patient counseling and should be limited to cases in which other approaches have failed. This study utilizing surgically retrieved spermatozoa to treat this form of male-factor infertility is still preliminary.

Wider implications of the findings:

Topographic mapping of SCF demonstrated that sperm DNA integrity was progressively compromised as the gamete advanced through the male genital tract. Surgical retrieval of spermatozoa may be beneficial in men who struggle to achieve pregnancies due to compromised genomic integrity of their gametes.

Trial registration number:

N/A

SELECTED ORAL COMMUNICATIONS

SESSION 76: UPDATE ON EMBRYO DIAGNOSTIC TECHNIQUES

Wednesday 26 June 2019 Haydn 3 14:00 - 15:30

O-290 Predicting the euplody status of the embryos by using evolutionary algorithm in IVF PGT cycles – A 10793 embryos review

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Study question:
Can evolutionary algorithms, model ensembling, and synthetic features be used to evaluate euploidy rates in autologous IVF PGT-A cycles?

Summary answer:
Evolutionary algorithms can be used to evaluate euploidy rates in autologous IVF PGT-A cycles and define factors affecting euploidy rates with high accuracy.

What is known already:
The number of chromosomal aneuploidies in preimplantation embryos progressively increases with advancing maternal age. Euploidy rates may vary significantly and can be affected by clinical as well as IVF laboratory protocols.

Study design, size, duration:
2052 cycles of IVF PGT-A treatment between January 2013 and January 2019 were included in the study (average age 36.4 ± 4.5). A total of 10793 embryos were analyzed (5.2±2.9 per case). 175 clinical factors (age, BMI, AMH, FSH, AFC, gravidity history, etc.) and 145 morphological and kinetic parameters (number of eggs, number of 2PNs, embryo morphology, euploidy rate, biopsy day, etc.) were recorded for each PGT-A cycle.

Participants/materials, setting, methods:
From 320 original features, 38719 synthetic features were created (by Weight of Evidence for columns, Encoding of categorical levels of the feature, Cross Validation Target Encoding, Time series, etc.), tested, and 331 features were selected for each PGT-A cycle. 40 statistical models were trained and ensembled in the final model. Predictive accuracy was evaluated by 10-fold cross-validation and AUC value (ROC curve). In order to select the most relevant features, the evolutionary algorithm was used

Main results and the role of chance:
Analysis of the combined predictions from multiple weak learners (generalized logistic regression, random forest, gradient boosting, etc.) processed by Generalized Model Stacking produced a predictive performance of AUC = 0.7106 ± 0.0072, Logloss = 0.619 ± 0.006, GINI = 0.414 ± 0.019. Sensitivity (true positive rate) of the final model was 0.821 and specificity (true negative rate) was 0.771. In order to evaluate precision and recall of the model, F-measure was calculated (harmonic mean) – 0.732 ± 0.008. The probability of normal chromosomal status was calculated for each embryo and ranged from 0.133 to 0.946 (baseline prediction – 0.536). All contributing variables were evaluated: the variables of highest importance affecting euploidy rates in the ensembled model were the maternal age, morphological characteristics of the blastocysts, and time when blastocysts became available for biopsy (0.372, 0.233, and 0.118 respectively).

This statistical model can be used to: a) estimate euploidy rates in non-PGT-A cycles and determine the number of embryos to transfer based on unique patients' input parameters and priorities; b) define, rank, and evaluate the factors affecting euploidy rates in IVF PGT-A cycles.

Limitations, reasons for caution:
Retrospective study and heterogeneity of patients included.

Wider implications of the findings:
Analysis of data proved that euploidy rates can be accurately estimated from the large clinical datasets in order to create a reliable model and make predictions on prospective data with high accuracy.

Trial registration number:
Not applicable.

O-291 Segmental aneuploidies detection shows mosaic patterns from different biopsies of human blastocyst with consequent reduced predictive diagnostic value compared to whole chromosome aneuploidies

L. Girardi[1], V. Romanelli[1], D. Cimadomo[2], L. Rienzi[2], F.M. Ubaldi[2], M. Serdarogullari[3], O. Coban[3], N. Findikli[4], K. Boynukalin[4], M. Bahceci[4], C. Patassini[1], M. Poli[1], C. Rubio Lluesa[5], C. Simón[5,6,7,8], A. Capalbo[1]

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Study question:
Does NGS-based PGT-A provide reproducible results in detecting whole chromosome and segmental aneuploidies in multifocal trophectoderm (TE) and ICM biopsies of the same embryo?

Summary answer:
NGS-based PGT-A confirms consistent results for whole chromosome aneuploidies in multifocal biopsies, while recurrent mosaic patterns are observed for segmental aneuploidies lowering its predictive value.

What is known already:
The introduction of highly sensitive NGS platforms for PGT-A applications concurred with an increased detection of segmental aneuploidies. Whilst whole chromosome aneuploidies are common features of human preimplantation embryos, it is not yet clear whether the detection of segmental alterations represents a true biological finding or a technical variation in the analytical process. Recent studies focused on understanding whether segmental aneuploidies derive from mitotic or meiotic events, however, definitive data are still lacking. To further contribute on the characterization of segmental aneuploidies and their biological significance, here we evaluated multifocal portions of TE tissues and Inner Cell Mass (ICM).

Study design, size, duration:
Following technical validation on cell lines carrying segmental aneuploidies, a cohort study, blinded to the geneticist, was performed between January 2018 and October 2018. 55 embryos known to carry segmental aneuploidies, either in concomitance (n=24) or not (n=31) with whole chromosome aneuploidies were subjected to re-biopsy. PGT-A results from the second biopsies were compared with the original clinical results in order to define the uniformity of segmental alterations in different TE sections.

Participants/materials, setting, methods:
After initial PGT-A analysis and genetic counselling, patients who wanted diagnostic confirmation for embryos carrying segmental aneuploidies >15Mb were enrolled. Embryos were warmed, allowed to re-expand and subjected to a second TE biopsy. An additional subset of 14 donated embryos was subjected to dissociation and ICM isolation. Each biopsy was processed using Ion ReproSeq PGS kit and sequenced on Ion S5 platform. Sequencing data were blindly analyzed with Ion-Reporter software and compared with original diagnosis.

Main results and the role of chance:
Regarding whole chromosome aneuploidies, comparisons between PGT-A results obtained from the first and second TE biopsy showed a concordance rate of 96.36% (n=53/55;95%CI=87.47-99.56) per sample and 99.84% (n=1263/1265;95%CI:99.43-99.98) per chromosome. Sensitivity per chromosome was 94.29% (n=33/35; 95%CI=80.84-99.30) and specificity 100% (n=1230/1230;95%CI=99.70-100.00). Regarding segmental aneuploidies, technical validation on cell lines resulted in 100% concordance (n=12/12;95%CI=73.5-100.0). Subsequently, overall PGT-A results showed that only 53.97% (n=34/63;95%CI=40.64-66.61) of segmental alterations were confirmed in the second TE biopsy. In this group of results, 30.16% (n=19/63;95%CI=19.23-43.02) of paired samples showed the same alteration, suggesting a meiotic origin. Of the remaining results, 23.8% of the alterations (n=15/63;95%IC=13.98-36.21) showed a different aneuploidy pattern. In detail, 11.11% (n=7/63;IC95%=4.59-21.56) carried the reciprocal segmental aneuploidy of the same chromosome fragment and 12.7% (n=8/63;IC95%=5.65-23.50) showed the corresponding whole chromosome aneuploidy. These findings suggest that a percentage of TE segmental alterations are present in a mosaic constitution, consistent with mitotic origin. Discordant results, defined as the absence of any other alteration on the chromosome affected by the segmental aneuploidy, were 46.03% (n=29/63; IC95%=33.39-59.06). Considering ICM samples, showing segmental aneuploidies in their corresponding clinical TE, 7 out of 13 showed concordant karyotype whilst in 6 of 13 samples the segmental alteration was not confirmed.

Limitations, reasons for caution:
The presence of equally-represented reciprocal aberrations in the second biopsy specimen could have reduced the concordance rate of segmental aneuploidies detection. The preliminary dataset of ICM examined is a further

limitation that we are filling up. The clinical value of embryos showing segmental abnormalities shall be evaluated in future studies.

Wider implications of the findings:

These data are important for patient counselling in cases when the embryo carries only segmental alterations, practitioners should inform patients that they might be mosaic. Concomitantly, the high rate of intra-blastocyst concordance observed for whole chromosome aneuploidies confirms their high clinical predictive value and diagnostic robustness of NGS for PGT-A.

Trial registration number:

not applicable

O-292 The influence of ploidy status on the expression of DNA repair and cell cycle checkpoint genes in human preimplantation embryos

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Study question: Does ploidy status (euploid or aneuploid) of human preimplantation embryos affect the expression of DNA repair and cell cycle checkpoint genes in these embryos?

Summary answer: Expression of some DNA repair and cell cycle checkpoint genes including *GADD45A* and *CDKN1A* can be affected by ploidy status of human preimplantation embryos.

What is known already: In order to maintain the genetic integrity, human somatic cells established complex DNA repair and cell cycle checkpoint systems to respond to genetic errors. In human preimplantation embryos, a similar system responds to genetic errors, resulting in the loss or the arrest of embryonic cells when repair fails. However, in the early stage of development, cell cycle checkpoints allow rapid cell division despite the presence of genetic errors in embryos. Currently, how DNA repair and cell cycle checkpoint systems are regulated in human preimplantation embryos remains unclear.

Study design, size, duration: This is a prospective cohort study. Between 2016 and 2018, 114 human blastocysts were analysed in order to compare expression of DNA repair and cell cycle checkpoint genes between euploid embryos and aneuploid embryos. Among these embryos, 20 embryos were identified as euploid and 94 embryos were identified as simply aneuploid (<5 chromosomes were lost or gained) by trophectoderm biopsy followed by clinical aneuploidy screening using next-generation sequencing or array comparative genomic hybridisation.

Participants/materials, setting, methods: Before gene expression analysis, embryo and couple -related baseline data were collected from the fertility clinic. Gene expression of 16 genes including *GADD45A*, *PARP1*, *CDKN1A*, *BRCA2*, *RB1*, *TP53BP1*, *MPG*, *CREBBP*, *ERCC4*, *TUFM*, *FANCA*, *TP53*, *NF1*, *BRCA1*, *CHEK2*, and *XRCC6* was examined via qPCR. Correlations between ploidy status of embryos and expression of these genes were identified via multiple linear regressions, where all results were adjusted for baseline data of embryos and couples.

Main results and the role of chance: Both of *GADD45A* and *CDKN1A* are DNA damage response genes regulated by *TP53*. Results of this study showed that the expression of *GADD45A* (B = 14.487, *P* = 0.000) and *CDKN1A* (B = 16.726, *P* = 0.006) was positively correlated with the total number of chromosomes wholly lost or gained in blastocysts. The expression of *GADD45A* (B = 1.651, *P* = 0.039) and *CDKN1A* (B = 3.027, *P* = 0.028) was also positively correlated with the maternal age of embryos. This result indicated that human blastocysts responded to aneuploidy via upregulation of the expression of *GADD45A* and *CDKN1A*. When ploidy status was controlled for in linear regressions, the positive correlations between the maternal age and the expression of the two genes still existed. This indicated that embryos also responded to factors that were only advanced maternal age –related but not aneuploidy-related. In addition, this study identified that gene expression of many genes including *GADD45A*, *PARP1*, *RB1*, *MPG*, *CREBBP*, *TUFM*, *FANCA*, *BRCA1*, *CHEK2*, and *XRCC6*, was DNA-dose-dependent. In other words, for a gene A located on chromosome B, trisomy B and monosomy B lead to significantly increased and decreased expression of gene A in human preimplantation embryos, respectively.

Limitations, reasons for caution: This study was not a randomised trial and only limited samples were analysed. As qPCR is not a high-throughput analysis method, only a few genes were analysed. We plan to reanalyse all the samples via RNA-seq using next-generation sequencing in order to identify more related genes.

Wider implications of the findings: By RNA profiling of DNA repair and cell cycle checkpoint genes, quality of human preimplantation embryos can be assessed indirectly. An RNA-analysis-based method for human preimplantation embryo selection can be established in future, and infertile couples may benefit from the new method.

Trial registration number: Not applicable

O-293 Clinical outcome of balance chromosome rearrangement carrier diagnosis in preimplantation human embryos by MicroSeq technique

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Study question:

Is microdissecting junction region (MicroSeq) a reliable method to distinguish between balanced carrier and non-carrier embryos in preimplantation genetic testing (PGT) cycles for balanced chromosome rearrangements (BCRs)?

Summary answer:

Combination of MicroSeq and breakpoint junction adjacent informative single nucleotide polymorphisms (AISNPs) analysis of BCRs is powerful for distinguishing between carrier and noncarrier balanced embryos.

What is known already:

Preimplantation genetic testing for structural chromosome abnormalities (PGT-SC) is widely applied in chromosome balanced rearrangement (CBR) carriers to increase the chance of a successful live birth. MicroSeq has been proven to be a powerful tool to distinguish between balanced/euploid carrier and non-carrier embryos in preimplantation genetic testing cycles for balanced translocation carriers.

Study design, size, duration:

This was a prospective study for 746 BCR carrier couples who requested for preimplantation genetic testing for structural chromosome abnormalities with a informed consent. The cohort patients were treated with the combination of a next-generation sequencing (NGS) based MicroSeq and PCR breakpoint junction region adjacent informative single nucleotide polymorphisms analysis of BCRs derivatives to distinguish between balanced/euploid carrier and non-carrier embryos in our IVF clinics between October 2014 and December 2018.

Participants/materials, setting, methods:

This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. A total of 746 carrier couples (527 reciprocal translocation, 197 Robertsonian translocation, and 22 inversion) were recruited and participated in the two-phase study. In phase I, the rearrangement breakpoint and AISNPs were characterized by NGS and the microdissecting junction region for all 746 carriers. In phase II, AISNPs of junction region was used for linkage analysis to identify the truly normal embryos.

Main results and the role of chance:

MicroSeq can distinguish normal embryos and embryos with BCRs in all 746 cases. Trophectoderm biopsy was performed in 3883 blastocysts derived from 866 PGT-SC cycles. The signals of 3513 blastocysts were detected. Among them, 1351 blastocysts were diagnosed to be chromosomal balance with comprehensive chromosome testing. 690/1351 (51.07%) blastocysts were identified to be carriers and 661/1351 (48.93%) to be normal. The proportions of normal karyotype embryos obtained in reciprocal, Robertsonian and inversion carriers' couples were 16.8% (424/2523),

23.6%(208/883) and 29.9%(32/107), respectively. The proportions of carriers karyotype embryos obtained in reciprocal, Robertsonian and inversion carriers' couples were 17.2% (435/2523), 25.7% (227/883) and 28%(30/107), respectively. Cumulative 523 FET cycles (185 cycles with transfer of carrier embryos and 338 cycles with transfer of noncarrier embryos) were performed and resulted in 332 clinical pregnancies (120 from carrier embryo transfer and 212 from noncarrier embryo transfer). To date, 83 patients had prenatal diagnoses and 54 of them (65.1%) were confirmed normal fetus.

Limitations, reasons for caution:

These are preliminary results and the final clinical outcomes of the remaining 249 PGT-SC cycles are still not obtained. Further randomized clinical trials are needed to confirm the clinical benefit of MicroSeq for PGT-SC patients.

Wider implications of the findings:

The combination of MicroSeq and PCR breakpoint AISNPs analysis of BCRs derivatives is a universal, reliable, and accurate strategy for distinguishing between carrier and noncarrier balanced/euploid embryos. The method has potential application in clinical PGT-SC cycles for patients with BCRs.

Trial registration number:

Not applicable.

O-294 Parallel analysis of copy number aneuploidies and ploidy level by NGS and SNP array on single biopsy provides accurate results and improves PGT-A applications range

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Study question:

Can aneuploidies and ploidy level be effectively tested on the same embryo biopsy specimen?

Summary answer:

The combined use of NGS and SNP's B-Allele Frequency (BAF) was successfully validated for chromosome copy number and ploidy level assessments on single biopsy specimens

What is known already:

The inability to determine embryo ploidy level is a limitation of current NGS protocols. The development of a complementary PGT-A strategy combining ploidy assessment with aneuploidy testing using a single biopsy specimen expands the range of PGT-A applications. In particular, this approach improves PGT-A diagnostics for male patients showing high rate of diploid spermatozoa and couples with recurrent triploid conceptions. Additionally, it rescues euploid embryos derived from abnormally fertilized zygotes (1PN and >2PN). This strategy could be highly beneficial for those patients that show high rates of abnormal oocyte fertilization (AFO) and those with poor response to ovarian hyperstimulation protocols

Study design, size, duration:

Prospective blinded validation study. The effectiveness of our in-house algorithm at determining ploidy levels was evaluated by performing BAF analysis on nine embryo rebiopsies processed using SNP arrays (five 2PN and four AFO-derived embryos: two 1PN, one 3PN and one 2.1PN). Additionally, the accuracy of SNP-array and NGS-based combined analysis was evaluated by performing both PGT-A and PGT-SR on individual WGA products produced by 23 rebiopsied samples and 12 cell lines with known karyotype

Participants/materials, setting, methods:

SNP-array was performed by HumanKaryomap-12 kit on NextSeq550 (Illumina). Ploidy assessment algorithm was based on the genome-wide BAF obtained: expected BAFs were 1, 0.5 or 0 for diploids, 1 or 0 for haploid and 1, 0.66, 0.33 or 0 for triploids, as determined by the frequency of each allele for a specific locus investigated. PGT-A was performed by NGS ReproSeq kit on IonTorrent S5 (ThermoFisher) on the same MDA product used for SNP-array

Main results and the role of chance:

SNP-array data analysis with our custom-made algorithm confirmed the ploidy status in 100% of the samples with known ploidy (9/9,95%CI,65.5-100.0). Karyotypes obtained through NGS on the same MDA product used for SNP-array, showed high concordance with known expected karyotypes. In detail, we obtained PGT-A results on 91.3% of rebiopsies (21/23,95%CI,72.0-98.8), whilst 8.7% failed to amplify (2/23,95%CI,1.3-2.8). Per amplified sample, 85.7% karyotypes were confirmed (18/21,95%CI,64.5-95.9). The unconfirmed results, 14.3% (3/21,95%CI,4.1-35.5) showed previously undetected segmental aneuploidies. At the chromosome level, copy number of 99.4% of chromosomes were confirmed (480/483,95%CI,98.1-99.9), whilst 0.6% (3/483,95%CI,0.1-1.9) showed additional segmental aneuploidies. In parallel protocol validation experiments on cell lines, both NGS and SNP produced consistent results in all samples (6 NGS + 6 SNP, 12/12; 100.0%, 95% CI, 71.8-100.0). Hence, the lack of amplification or chromosome copy number confirmation in embryonic specimens is likely due to intrinsic biological variability of the sample rather than to analytical variability. These results demonstrate the effectiveness of our combined protocol to assess embryo ploidy status, both in regards of the algorithm developed, and the possibility of effectively employing SNP-array and NGS using the same WGA product generated from a single embryo biopsy specimen

Limitations, reasons for caution:

Ploidy assessment protocol was not tested to distinguish 3N triploids from 4N tetraploids. Furthermore, parents can be tested at their discretion. If parents were not tested, the parental origin of each chromosomal set could not be determined; therefore, in these cases, uniparental heterodisomy cannot be excluded in 2N diploid embryos

Wider implications of the findings:

The improved PGT-A scheme will allow extending the application of PGT-A to male patients with high rate of diploid sperm and couples with recurrent triploid conception. Importantly, the possibility of evaluating ploidy status of embryos derived from AFO, will allow clinical use of viable embryos otherwise considered non-transferable in IVF

Trial registration number:

none

O-295 Accuracy of mosaicism calling in PGT-A may be affected by cell numbers in biopsy specimens.

J. Wei[1], C. Zhang[1], X. Qin[1], C.J. Davidson[2], A. Harris[3], Z. Rosenwaks[1], K. Xu[1]

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[2]Thermo Fisher Scientific, research and development, South San Francisco, U.S.A.

[3]Thermo Fisher Scientific, research and development, Carlsbad, U.S.A.

Study question:

Would cell numbers of biopsy specimens influence the accuracy of diagnosis of various types of mosaicism in PGT-A?

Summary answer:

Comparing to a 5-cells model mimicking typical biopsy specimens, a larger sample size model of 10-cells tends to provide more accurate diagnostic result of mosaicism.

What is known already:

Recently, the application of Next Generation sequencing (NGS) in PGT-A has greatly enhanced the reporting of mosaicism. However, the clinical and diagnostic significance of mosaic profiles on NGS-based PGT-A results is still not well defined. This is mainly due to lack of systematic studies of contributing factors in the NGS-based PGT-A workflow that affecting the accuracy of mosaicism reporting, such as technique of laser ablation, biopsy size, methods of whole genome amplification, NGS platforms and mosaic calling algorithm.

Study design, size, duration:

From September to December 2018, an optimized NGS-PGT-A protocol to detect both aneuploidy and mosaicism was developed and validated using 4 Coriell cell lines with known karyotypes (46,XY; 47,XX,+21; 46,XY,del(4P) and 47,XY,+13). To test the effect of biopsy sample size to the sensitivity and specificity of the protocol, two models were used: 5-cells model mimicking typical biopsy specimens and 10-cells model. Precise number of cells were picked under dissection microscope and loaded into PCR tubes.

Participants/materials, setting, methods:

Cells from two cell lines were pooled with defined combinations to generate samples with known mosaic profile of 0%, 20%, 40%, 60%, 80% and

100%. A total of 96 samples, with 4 replications per combination were analyzed. A ReproSeq™ PGS protocol using Ion Chef™ and S5 sequencer was employed for whole genome amplification, library preparation and sequencing (Thermofisher). Ploidy and mosaicism were assessed using ReproSeq Mosaic PGS w1.1 pipeline with either 2Mb or 0.5Mb baseline.

Main results and the role of chance:

To evaluate full chromosome mosaic calling, 46,XY and 47,XX,+21 cell lines were used. While mosaic profiles of Chr21 in 10-cells samples mostly equaled expected values at all tested combinations, those of 5-cells samples revealed an approximately 30% mosaic mixture instead of value expected for both 20% and 40% groups. Meanwhile, in both models, mosaic profiles of ChrX showed 10-14% less than expected values at 40%, 60% and 80% groups and mosaic profiles of ChrY demonstrated a 10% overestimation at 40% and 60% groups. Moreover, mosaic calling pipeline failed in both models to consistently detect 80% mosaic loss of ChrY, which resulted in either 100% or 65% loss.

For assessing segmental chromosome mosaic calling, 46,XY,del(4p) and 47,XY,+13 cell lines were analyzed. Mosaic profiles of Chr13 in both 10-cells and 5-cells samples were closely matched expected values at all tested combinations. In contrast, mosaic calling pipeline failed to detect 20% segmental mosaic loss of Chr4 (27 Mb) in both models. In 10-cells model the pipeline could accurately detect 40% and 60% segmental loss of Chr4, but show a 15% overestimation at 80% groups, while in 5-cells model, it displayed 9%, 15% and 17 % overestimation at 40%, 60% and 80% groups, respectively.

Limitations, reasons for caution:

The sample size in this study is rather small. To have more comprehensive understanding of the effect of cell numbers in biopsy specimens on mosaic calling, more cell lines harboring either full and/or segmental changings in various chromosomes should be included. Also further validation in clinical embryo biopsy is necessary.

Wider implications of the findings:

Accuracy and sensitivity of mosaic calling may be affected by biopsy cell numbers and vary among different chromosomes. Segmental mosaic calling is less accurate than full chromosome mosaic calling. Algorithm of mosaic calling needs to be further modified and optimized. This study provides useful information for establishing NGS-based PGT-A guidelines.

Trial registration number:

None

SELECTED ORAL COMMUNICATIONS

SESSION 77: PREDICTING PREGNANCY OUTCOMES

Wednesday 26 June 2019 Haydn 2 14:00 - 15:30

O-296 Correlation between Anti mullerian (AMH) levels and miscarriage in preimplantation genetic testing for aneuploidy (PGT-A) cycles

S. Seshadri[1], X. Vinals Gonzalez[1], R. Odia[1], J. Ben Nagi[1], E. Yasmin[2], C. Maison[1], A. Capotescu[1], W. Saab[1], P. Serhal[1]

[1]CRGH, Assisted Conception Unit, London, United Kingdom

[2]UCLH, Reproductive Medicine, London, United Kingdom

Study question:

Can AMH be used as a predictor for miscarriage risk after transfer of euploid embryos?

Summary answer:

AMH levels were found to correlate with miscarriage after the transfer of an euploid embryo and irrespective of maternal age.

What is known already:

PGT-A improves time to pregnancy and reduces risk of miscarriage. Miscarriage risk remains after transfer of euploid embryos. AMH is thought to be useful for predicting pregnancy loss. The association between AMH and miscarriage rates has been equivocal with some studies demonstrating a link whilst others have refuted an association. A study concluded that AMH levels are inversely associated with the risk of miscarriage in women trying to conceive naturally and women with diminished ovarian reserve are at an increased risk of miscarriage. A meta-analysis concluded that AMH has clinical utility in counselling women undergoing fertility treatment regarding pregnancy rates.

Study design, size, duration:

Retrospective study involving 454 PGT-A cycles was conducted at the Centre for Reproductive and Genetic health (CRGH), UK from January 2016 to December 2017. Trophectoderm biopsy was performed on day 5/day 6 blastocysts and PGT-A was performed using next generation sequencing (Cooper Genomics). 7. *Female age was grouped according to HFEA categories (UK). The AMH levels was grouped in: <5 , 5-15, 15-25 and > 25 pmol/l.*

Participants/materials, setting, methods:

Female age was used to group the sample according to the Human Fertilisation and Embryology Authority (HFEA) categories into: <35 years (71/454), 35-37 years (90/454), 38-39 years (79/454), 40-42 years (137/454) and >42 (77/454). Number of euploid embryos, euploidy rate and relevant cycle parameters (i.e oocytes collected, embryos biopsied) was annotated for each of the categories. Statistical analysis was done using SPSS version 23 and statistical significance was set at the 5% level.

Main results and the role of chance:

5446 eggs collected, 1948 blastocysts biopsied. 546 euploid embryos available for transfer (28.0%), 1339 were aneuploid (68.7%) and 63 (3.3%) no result. Mean age for all the patients in the cohort was 38.6 ± 4.1 years. A negative correlation seen between female age (in years) and euploidy rate (%): <35 (51.04%), 35-37 (44.02%), 38-39 (24.01%), 40-42 (13.72%) and >42 (7.23%). AMH levels had no effect on euploidy rate (p=0.08) and only female age was contributing in the differences. Univariate analysis shows that there is a statistical difference in age in the different AMH groups (p<0.001), but this is only seen when comparing low with optimal groups (post-hoc test; p=0.01). A total of 137 single euploid embryo transfers were performed: 105 were pregnant (76.6%) and 32 were not (23.2 %). AMH levels were lower in the miscarriage group (p-value 0.001; 25.4 ±14.65 in LB vs 14.24 ±15.59 in miscarriage). The miscarriage rates for the different groups of AMH levels were 72.7%,56.5%,21.3% and 17.6% respectively. There was an inverse relationship between AMH and miscarriage risk i.e for a woman of an AMH level <5, the risk of having a miscarriage (OR 5.614 95%CI 1.22-25.67; p=0.026)compared to the group with a normal AMH.

Limitations, reasons for caution:

Larger studies (well-designed trials) with an increased number of biopsied embryos are needed to confirm our findings with analysis. The trials designed should also standardise the PGT-A technique being used as different laboratories have different reported sensitivity assays.

Wider implications of the findings:

This is the first study that has analysed the correlation between AMH and miscarriage rate where euploid embryos were transferred. AMH levels could be a predictive tool for miscarriage in ART cycles where PGT-A is performed. AMH levels has an important clinical utility for patient counselling in PGT-A cycles.

Trial registration number:

not applicable

O-297 Design of novel experience-based embryo transference scoring system (TSS) to predict final reproductive outcome, in terms of pregnancy and healthy live births

A. Palomar[1], J.V. Martinez[2], M. Balasch-Bernat[3], I. Molina Botella[2]

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[2]Hospital Universitari i Politecnic La Fe, Unit of Assisted Reproduction, Valencia, Spain

[3]Facultat de Fisioterapia. Universitat de Valencia., Department of Physiotherapy., Valencia, Spain

Study question:

To predict final reproductive outcome of double embryo transferences (DET) of cleavage embryos by means of proposed transference scoring system (TSS) based on individual scores of each embryo.

Summary answer:

TSS succeeded in sorting DET in five different categories according to likelihood of reproductive success in terms of both, pregnancy (PR) and live births (LB).

What is known already:

Despite the increasing tendency of transferring a single embryo (SET), DET are not scarce in most of IVF Units. Since the implementation of ARTs, the constant interest in selecting the top-quality embryo has been pursued; but fewer efforts have been addressed to determine which combination of embryos would lead to reproductive success, that can be defined as the achievement of pregnancy and the delivery of a healthy baby. Therefore, there is an urgent need to develop scoring systems that rank the final reproductive outcome when DET are performed, particularly when no top-quality cohorts are considered.

Study design, size, duration:

Cohort study held in HUiP La Fe from 2008 to 2018 including 7613 day-2 DET. TSS is described as a cumulative transference score based on partial scores of each embryo transferred. Partial scores were obtained using an experience-based embryo scoring system based on blastomeres number and grade, which referred to both, fragmentation's degree and blastomeres symmetry, developed in a previous study in a cohort of 11278 day-2 embryos where implantation rate was the primary outcome.

Participants/materials, setting, methods:

To design TSS, each transference was assigned a cumulative score created after combining partial scores of each embryo transferred. Logistic regressions were performed to rank transference scores according to PR and LB, respectively. Chi-square tests were used to compare differences regarding PR and LB among groups of transference scores exhibiting different PR and LB. Transference scores which did not show differences in terms of PR and LB were grouped in the same category.

Main results and the role of chance:

Categories including transference scores associated to similar PR are the same categories found when the likelihood of singleton delivery (SDR) is studied. DET of 4-cells grade one embryos were associated to the highest PR (>54%) and SDR (>36%). The second category, including at least one top-quality embryo, is associated to similar PR (40,85% to 53,33%, p>0.05) and SDR (30,23% to 35,34%, p>0.05). It is followed by the third category in which at least one of the embryos has 4 cells but exhibits mild to moderate fragmentation (PR:32,60%-36,60% p>0.05; SDR:23,35%-26% p>0.05). In DET included in the fourth category (PR:22,27%-27%, p>0.05; SDR:16,81%-20%, p>0.05) were transferred both embryos transferred showed impaired number of blastomeres and/or fragmentation degree. Interestingly, when the number of blastomeres of one of the embryo is different of 4 and the other embryo has more than 5 or less than 4 cells with severe fragmentation are associated to limited reproductive success (PR:14,29%-17,86%, p>0.05; SDR:8,2%-10,71%, p>0.05), only followed why DET in which both embryos transferred exhibit more than 5 or less than 4 blastomeres, regardless the fragmentation degree (PR:11%, p>0.05), that in all cases lead to miscarriage. It can be affirmed that TSS is not affected by female age.

Limitations, reasons for caution:

The current study is limited to day-2 DET since in our laboratories is the most common sort of embryo transference, although the methodology can be translated to day-3 DET.

Wider implications of the findings:

Experience-based transference-scoring demonstrates the necessity of developing this sort of tools to improve the final reproductive outcome. Moreover, the methodology used to construct TSS is worthy itself since it can be used for DET and SET of cleavage embryos of different days of development.

Trial registration number:

This is not a clinical trial

O-298 Progesterone supplementation in natural cycles improves live birth rates after frozen embryo transfers through higher implantation rate and reduced pregnancy loss

K. Wånggren[1]

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Study question:

Does supplementation with progesterone in natural cycle after frozen embryo transfer (FET) improve live birth rate, pregnancy rate and miscarriage rate?

Summary answer:

Supplementation with vaginal tablets of 100 mg progesterone two times daily in a natural FET cycle significantly improved live birth rate.

What is known already:

Progesterone is needed to support endometrial receptivity for embryo implantation as well as for continuous pregnancy until the eighth week of pregnancy. Implantation rates after FET in natural cycles are still relatively low. Women with regular menstrual cycles may have insufficient progesterone production in the luteal phase. Supplementation with vaginal progesterone during the luteal phase and early pregnancy could therefore improve the outcome of FET. There are so far only a few published trials on if FET and progesterone supplemented natural cycles increase pregnancy rate.

Study design, size, duration:

During a period of six years, 2013-2018, 500 women scheduled for FET in a natural cycle were enrolled in a randomized trial. Randomization was achieved using sealed envelopes. Primary endpoint was live birth rate. Secondary endpoints were pregnancy rate and miscarriage rate. The study was approved by the Regional ethical approval board, Stockholm Dnr: 2012/1845-32.

Participants/materials, setting, methods:

Infertile women with regular menstruation cycles, scheduled for FET were enrolled for the study at two university based reproductive medicine centers. After giving informed consent, the women were randomized to either supplementation with vaginal tablets of micronized progesterone 100 mg twice daily, starting at FET until eight weeks of pregnancy or no treatment. Pregnancies were followed up until the end of pregnancy. Comparison between the two groups was analyzed by use of Fisher´s exact test.

Main results and the role of chance:

The study group included 500 patients. Half the group received progesterone supplementation according to the study protocol. Primary endpoint was live birth. In the progesterone supplemented group, 82 patients (32.8%) and in the control group, 56 patients (23,6%) had a live birth, p=0.0286. Three patients in the progesterone treatment group had twin births while one patient in the control group had twins, all after single embryo transfers. Secondary endpoints, number of pregnancies were 100 (40%) and 84 (33.6%) p=0.1158, respectively. Pregnancy losses 9 (9.9%) and 15 (21.7%) p=0.2957, respectively. Biochemical pregnancies 12 (12%) and 13 (13.1%) p=0.8342, respectively. The mean ages were similar between the two groups 33.2 years in the progesterone group and 33 years in the control group, at embryo freeze and 34.6 versus 34.0 years at FET. There were no significant differences between day of freezing of the embryos between the two groups.

Limitations, reasons for caution:

The study was not blinded. The start of progesterone treatment was different depending on which day the embryos were frozen (day 2,3 or 5,6). This could affect the results negative for the patients having blastocysts transferred since they had a shorter period of supplementation with progesterone before implantation.

Wider implications of the findings:

The study confirms the result from a previous randomized controlled trial that progesterone supplementation in the luteal phase improves the live birth rate after FET in natural cycle. Therefore, patients undergoing FET in natural cycles should be offered luteal phase support with progesterone.

Trial registration number:

NTR4305

O-299 Low serum bHCG concentration on outcome day (trigger day +17) identifies a subgroup of women at risk of poor perinatal outcome

M. Gaudoin[1], **R. Payne**[2], **T. Hamilton**[3], **C. Fairbairn**[4], **J. Lyon**[4], **N. Gibson**[5], **P. Ambrose**[6], **R. Fleming**[4]

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Study question:

To determine the immediate and subsequent perinatal outcomes of patients whose βHCG concentration was less than 70 IU/L on trigger day +17.

Summary answer:

A βHCG concentration < 70 IU/L was associated with an increased risk of miscarriage, preterm delivery and small for gestational age and higher stillbirth rate.

What is known already:

Algorithms combining maternal age and various hormone concentrations, originally in the second trimester but progressively into the first trimester, have been used to predict adverse perinatal outcome (these include AFP, HCG, placental growth factor and PAPP-A, amongst others). HCG is the first known hormonal signal by the embryo, promoting embryo/endometrial molecular cross-talk, cytotrophoblast proliferation and subsequent invasion. HCG contributes to the maternal tolerance of the embryo via T-cell modulation and influences the degree of macrophage migration by up-regulation of macrophage migration inhibitory factor of the endometrial stromal cells. Failure of these mechanisms may result in miscarriage or poor placentation.

Study design, size, duration:

Retrospective analysis of outcomes from fresh ART cycles from 2008-2016 inclusive, in a single UK centre where the pregnancy outcome day was exactly 17 days after the pre-oocyte retrieval trigger injection. Subsequent perinatal outcomes (delivery after 24 weeks' gestation) were limited to singletons.

260 women had a βHCG < 70 IU/L, 534 had βHCG ≥ 70 IU/L.

Comparisons between groups of continuous data performed by Mann-Whitney test; comparisons of categorical data performed by Chi-squared analysis.

Participants/materials, setting, methods:

Serum bHCG measured using a Beckman Coulter Access II analyser.

Definitions. Positive pregnancy test: βHCG >10 IU/L. Biochemical pregnancy: non-viable pregnancy up to first pregnancy scan at 8 weeks' gestation. Miscarriage: fetal heartbeat identified but pregnancy loss up to 24 weeks' gestation. Preterm delivery: delivery < 37 weeks' gestation. Small for gestational age, SGA: birthweight $<10^{th}$ centile for expected gestation (UK birthweight charts). Stillbirth: fetal demise after 23 completed weeks' gestation.

Main results and the role of chance:

When βHCG was < 30 IU/L (N = 82), the biochemical pregnancy rate was 93% and only 2 pregnancies progressed to a positive heartbeat (2%).

When βHCG was 30-50 IU/L (N = 68), the biochemical pregnancy rate was 60% and the ongoing pregnancy rate was 24%.

When βHCG was 50-70 IU/L (N = 110), the biochemical pregnancy rate was 36% and the ongoing pregnancy rate was 53%.

For all women with βHCG < 70 IU/L (N = 260), the ongoing pregnancy rate was 29%.

When βHCG was ≥70 IU/L (N = 534), the biochemical pregnancy rate was 8%, and the ongoing pregnancy rate was 86%.

Comparing women with βHCG < 70 IU/L vs. ≥ 70 IU/L, there was no difference in age, AMH or BMI. However, the former group delivered singletons with a lower birthweight (P = 0.006), shorter gestational age at delivery (P = 0.005) and lower birth centile for gestational age (39 ± 28 vs. 52 ± 28 centile, P = 0.006). They were more likely to be born preterm (28% vs. 13%, P = 0.014) and be SGA (22% vs. 8%, P = 0.003). There were 3 stillbirths in each group (8% vs. 0.6%, P = 0.005).

Limitations, reasons for caution:

The analysis was limited to women with βHCG concentration on trigger day +17. Very few pregnancies with βHCG < 30 IU/L progressed to viability so the overall proportion of viable pregnancies when βHCG was < 70 IU/L was low, resulting in a relatively small dataset for analysis of perinatal outcomes.

Wider implications of the findings:

These data allow us to counsel women with greater certainty about the likelihood of a viable pregnancy. The low βHCG concentration also appears to be a marker of poor perinatal outcome, identifying an especially high-risk group and provides a model for future potential research into implantation and placentation.

Trial registration number:

Not applicable

O-300 Soluble urokinase plasminogen activator receptor (suPAR) in plasma as a biomarker of early first trimester pregnancy viability and location compared with hCG, progesterone and estradiol.

A. García-Alix Grynnerup[1], J. Friis Petersen[2], N.H. Mitchell[3], K. Løssl[4], S. Sørensen[5], A. Lindhard[6], L.J. Friis-Hansen[7], A. Pinborg[4], A. Nyboe Andersen[4], E.C.L. Løkkegaard[2]

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[7]*North Zealand Hospital- Copenhagen University Hospital, Department of Clinical Biochemistry, Copenhagen, Denmark*

Study question:

Can suPAR plasma levels improve location and viability diagnostics of early pregnancies compared with known and regularly used biomarkers?

Summary answer:

suPAR had a modest predictive value of early pregnancy location and viability inferior to progesterone especially before 6 weeks' gestation.

What is known already:

Early pregnancy loss is the most common complication of pregnancy, as roughly 25% of recognized pregnancies end in a miscarriage and 1-2% will be ectopic. Currently, serial hCG measurements and ultrasound examinations are used to diagnose early pregnancy location and viability.

suPAR, a part of the plasminogen activator system, is a novel biomarker increasingly used to monitor systemic inflammation. In healthy pregnancies circulating suPAR levels have been reported higher in first trimester compared with non-pregnancy levels, and thus plasma suPAR may have a role as a biomarker for early pregnancy location and viability, which has not previously been investigated.

Study design, size, duration:

A cross-sectional case control study merged two cohorts in total comprising 313 women and analysed plasma samples from biobanks. The first cohort included 201 women with a naturally conceived pregnancy between 4 and 8 weeks' gestation recruited from the public of the Copenhagen Capitol Region (2016–2017). The second cohort included 112 women diagnosed with a tubal pregnancy at three university hospitals in Denmark (2014–2016), who had blood samples biobanked before treatment.

Participants/materials, setting, methods:

The patients were divided into three groups: A) 164 women with a viable intrauterine pregnancy (IUP) resulting in a live birth; B) 37 women with an IUP resulting in early miscarriage; C) 112 women admitted and treated for a tubal pregnancy. Prediction of pregnancy location (C vs. A+B) and viability (A vs. C+B) was assessed using area under the receiver-operating characteristic curve (AUC) in pregnancies before and after full 6 weeks' gestation.

Main results and the role of chance:

The three groups were similar regarding age (p=0.06), BMI (p=0.57) and smoking status (p=0.38), but group C had higher parity (p<0.001) and gestational age (GA) with a median of 6.9 weeks (IQR 6.0-7.4) compared with 5.9 (IQR 5.1-6.6) and 5.9 (IQR 5.0-6.4) in group A and B, respectively (p=0.001). suPAR levels (median (IQR) in µg/L) were significantly lower in group C (1.9 (1.7-2.32)) compared with group A (2.4 (2.1-2.7)) and B (2.4 (2.0-2.7)), p<0.001. Likewise, hCG, estradiol and progesterone levels were all significantly lower (p<0.001) in group C compared to group A and B.

In pregnancies with GA<6 weeks (n=125), suPAR poorly predicted pregnancy location (AUC=0.69; 95% CI 0.54-0.83) and pregnancy viability (AUC=0.58; 95% CI 0.48-0.69), whereas progesterone was the best predictor of pregnancy location (AUC=0.95; 95% CI 0.87-1.00) and viability (AUC=0.84; 95% CI 0.75-0.92).

In pregnancies with GA>6 weeks (n=188) suPAR was a modest predictor of location (AUC=0.71; 95% CI 0.63-0.78) and viability (AUC=0.70; 95% CI 0.63-0.78) compared with hCG (AUC=0.96; 95% CI 0.93-0.99 and AUC=0.96; 95% CI 0.93-0.98), estradiol (AUC=0.92; 95% CI 0.88-0.97 and AUC=0.92; 95% CI 0.88-0.96) and progesterone (AUC=0.92; 95% CI 0.87-0.96 and AUC=0.88, 95% CI 0.83-0.93).

Limitations, reasons for caution:

Merging cohorts from different studies introduces an important methodological risk of selection bias. Our findings should be transferred with caution to the general population where the prevalence of ectopic pregnancies is several folds lower.

Wider implications of the findings:

Our findings demonstrate that suPAR levels are significantly lower in tubal compared with intrauterine pregnancies suggesting a relation to the formation of the placenta. However, in the present population progesterone and hCG outperformed suPAR as a predictor of very early pregnancy location and viability.

Trial registration number:

not applicable

O-301 Adverse/ reproductive outcomes of reduced pregnancy and live birth rates after In Vitro Fertilization in women with previous caesarean section: a retrospective cohort study

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[2]Amsterdam UMC- Vrije Universiteit Amsterdam, Epidemiology and Biostatistics, Amsterdam, The Netherlands

Study question:

Does a previous caesarean section effect the reproductive outcomes including live births in women after In Vitro Fertilization or Intracytoplasmic Sperma Injection?

Summary answer:

A previous caesarean section does have a negative effect on pregnancy and live birth rates after In Vitro Fertilization or Intracytoplasmic Sperma Injection.

What is known already:

Rates of elective caesarean sections (CS) are rising worldwide. Late sequaelae of CSs related to a niche (CSdefect) include gynaecological symptoms and obstetric complications. A systematic review of Gurol-Urganci (2013) reported a lower pregnancy rate after a previous CS (RR 0.91 CI 0.87-0.95) compared to a previous vaginal delivery (VD). However the underlying cause is unclear, included studies do not differentiate between problems of fertilisation, embryo transportation, or implantation. Using an IVF population it is possible to study the effect of a previous CS on the implantation of embryo's in relation to a previous vaginal delivery.

Study design, size, duration:

In this retrospective cohort study we included all women who underwent an IVF or ICSI treatment at the IVF centre, Amsterdam UMC, location VU university, Amsterdam, the Netherlands between 2006 & 2016 with one previous delivery. In total 1317 women were included, 334 women with a previous caesarean section and 983 women with a previous vaginal delivery

Participants/materials, setting, methods:

All secondary infertile women, with only one previous delivery either by CS or VD were included. If applicable, only the first fresh embryo transfer was taken into account in the analyses. Patients without an intention for embryo transfer were excluded. The primary outcomes was live birth and clinical pregnancy. Multivariate logistic regression analyses were used with adjustment for possible confounders (age, BMI, smoking, indication for IVF/ICSI, uterine pathology, endometrioses, duration of infertility) if applicable.

Main results and the role of chance:

Baseline characteristics of both groups were comparable. Live births rates were significantly lower in women with a previous CS 15.9% (51/320) versus 23.3% (219/941) for women with a previous VD (OR 0.63 95% CI 0.45-0.87) in the ITT analyses. The rates were also lower for ongoing pregnancy (20.1% vs. 28.1% (OR 0.64 95% CI 0.48-0.87)) clinical pregnancy (25.7% vs. 33.8% (OR 0.68 95% CI 0.52 -0.90)) and biochemical pregnancy (36.2% vs. 45.5% (OR 0.68 95% CI 0.53-0.88)) in women with a previous CS. The quality of embryos did not differ between the two groups. 77% received a single embryo transfer. Rated difficulty concerning the embryo transfer was significantly higher in the caesarean section group (9.3% versus 1.0% (OR 10.0 95% CI 4.61-21.54)). The results of the per protocol analyses, including only patients who actually had an ET, were comparable.

Limitations, reasons for caution:

Despite its large size, this study was limited by its retrospective design. Furthermore, 55 (4,3%) cases missed data regarding delivery outcomes, but these were equally distributed between the two groups.

Wider implications of the findings:

The lower clinical pregnancy rates in combination with equal embryo quality, indicates that in particular implantation may be hampered after a CS. It's relation with a niche (CS defect) in the uterine CS scar needs to be studied. Our results should be discussed with women considering an elective CS.

Trial registration number:

This study is submitted to the Dutch Trial Register. http://www.trialregister.nl TRIAL REGISTRATION DATE: 11-01-2019.

SELECTED ORAL COMMUNICATIONS

SESSION 78: INTRINSIC AND LABORATORY DETERMINANTS OF IVF SUCCESS

Wednesday 26 June 2019 Haydn 4 14:00 - 15:30

O-302 Should we be performing blastocyst transfers at 120±12hrs post-ovulation? An analysis of 1,079 single frozen-thawed euploid blastocyst transfers

B. An[1], M. Chapman[1], L. Tilia[2], C. Venetis[3]

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Study question:

Does performing single frozen-thawed euploid blastocyst transfers at 120±12hrs post-ovulation optimise live birth rates (LBR)?

Summary answer:

Performing a single frozen-thawed euploid blastocyst transfer at 120±12hrs post-ovulation is independently associated with higher LBR as compared to transfers outside this window.

What is known already:

There is compelling evidence for maintaining embryo-endometrial synchrony to optimise clinical outcomes following frozen embryo transfers (FETs). Although it seems physiologically intuitive to replace the blastocyst on the exact day of the embryo's developmental age after ovulation (i.e. ~120hrs post-ovulation for blastocysts), it has been suggested that the implantation window is actually significantly wider and earlier or later transfers can lead to similar or in some cases higher probability of live birth. Whether restricting embryo transfers to 24 hrs around this 120hrs post-ovulation period is beneficial has not yet been properly investigated.

Study design, size, duration:

This is a multi-centred retrospective cohort study analysing 753 patients who underwent 1,079 single frozen-thawed euploid blastocyst transfers following trophectoderm biopsy and PGT-A between January 2015 and May 2018. Limiting the analysis to single euploid embryo transfers allowed for a more accurate estimation of the endometrial synchrony factor by controlling for the developmental stage of the embryo (≥full blastocyst) and its genetic composition. Live birth rate (LBR) per FET was the primary outcome measure.

Participants/materials, setting, methods:

Patients underwent natural or ovulation-induction preparation of the endometrium and serial serum LH and progesterone measurements were obtained during the late follicular phase in order to estimate ovulation time. For triggered cycles (n=218), ovulation was assumed to occur 36hrs post-trigger. Optimally timed transfers were defined as those conducted 120±12hrs post-ovulation.

Statistical analysis was performed using the generalised estimating equations (GEE) framework to control for the clustered nature of the data while adjusting for potential confounders.

Main results and the role of chance:

The mean age of patients was 36.2 years and the main indications for PGT-A was patient/doctor request (n=378; 34.9%) and advanced reproductive age (n=244; 22.5%). The mean post-thaw survival rate (proportion of cells surviving the thaw) for the thawed embryos was 95.7% (Standard Deviation-SD: 7.9). The mean duration between estimated ovulation and transfer was 108.2hrs (SD: 21.2). Four hundred twenty-two embryo transfers (39.1%) were performed at 120±12hrs post-ovulation.

A multivariate regression GEE model including the pre-freezing embryo quality (top vs. good quality), the post-thaw survival rate and whether ovulation during the FET cycle was triggered or not, as covariates revealed that optimal timing was positively associated with LBR (OR 1.34, CI 1.03-1.73) per embryo transfer.

A sensitivity analysis in which all embryo transfers performed >24hrs before or after the optimal timing (120±12hrs post-ovulation) were excluded, yielded similar results (OR 1.35, CI 1.04-1.77).

Limitations, reasons for caution:

This study is retrospective and the presence of residual unknown bias cannot be excluded. Furthermore, the embryos included in this study had reached full blastocyst stage and had undergone trophectoderm biopsy for PGT-A prior to their freezing.

Wider implications of the findings:

The results of this study underline the significance of embryo-endometrial synchrony for the optimization of embryo transfer outcome. This study shows that although implantation and live birth is possible outside the optimal window, performing embryo transfers 120±12hrs post-ovulation can improve the probability of live birth.

Trial registration number:

Not applicable

O-303 Laser assisted hatching and continued culture of embryos within the same culture dish does not impact embryo development potential in PGT cycles

S. Dalati[1], N. Hesketh[1], S. McArthur[1], M. Bowman[1]

[1] *Genea, Embryology, Sydney- NSW, Australia*

Study question:

To determine whether laser assisted hatching and continued culture of embryos within the same culture dish is possible without compromising embryo development potential in PGT cycles?

Summary answer:

Hatching and culturing embryos within the same culture dish is possible, reduces embryo disturbance and has no impact on embryo development and quality.

What is known already:

The standard protocol for hatching embryos destined for PGT involves their movement to a separate dish for the laser hatching process performed on Day 3/4, followed by a move back to the culture dish. With the advent of time-lapse incubator systems there is a greater need for optimizing uninterrupted embryo culture, raising the possibility of performing the hatching process in-the same-dish. There is scant data assessing the impact of laser assisted hatching in-same-dish (HISD) on embryo development and quality. Initial in-house mouse studies indicated that HISD minimized environmental impact on embryos without any deleterious effects on embryo development potential.

Study design, size, duration:

A retrospective cohort study comparing human embryo development following laser assisted hatching in a separate dish from August-December 2017 to hatching in-same-dish August-December 2018 at Genea Sydney. A total of 6187 embryos were analyzed and development was compared. Embryos had to have reached a minimum of a blastocyst stage or above to be included in the study.

Participants/materials, setting, methods:

Embryos from IVF cycles were cultured in a well-in-well dish in a time-lapse system (Geri) in continuous medium (Genea-Biomedx). A total of 3136 2PN embryos were moved out on D4, hatched in a separate dish and returned to continuous culture. A total of 3051 2PN embryos were hatched on D4 within the culture dish. Laser assisted hatching was performed using a Hamilton Thorne near infra-red laser.

Main results and the role of chance:

Overall results showed that blastocyst development rates and utilization of embryos hatched in-same-dish (HISD) were comparable to those embryos hatched outside of the dish (HOD), and that embryos HISD did not impact embryo development and quality. There were no differences between D5 blastocyst development (HISD:48.8% and HOD:46.8%), D5 blastocyst development with grade1/2 embryos (HISD:31.3% and HOD:31.3%), D5/6 utilization rates (HISD:55.2% and HOD:52.9%) and D5/6 grade1/2 utilization rates (HISD:38.6% and HOD:37%) p>0.05. Whilst there were no statistical differences between the groups for developmental outcomes that were measured, the trends were in favor of HISD.

Limitations, reasons for caution:

Clinical outcomes should be closely monitored following the implementation of this hatching method into clinical practice. Implementation of a randomized control trial may be performed in the future to know the complete impact of laser assisted hatching within the same dish has on embryo development.

Wider implications of the findings:

This study has demonstrated that hatching and culturing embryos within the same environment is possible with no impact on embryo development potential. By limiting the exposure of embryos to environmental disturbances we can ensure that embryos are developing to their maximum potential in an IVF setting.

Trial registration number:

Not applicable

O-304 Paternal age over 50 years reduces the success of IVF/ICSI - ET

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[2] *Centre for Reproductive and Genetic Health, Embryology, London, United Kingdom*

Study question:

Does increasing paternal age affect clinical pregnancy rates (CPR) and miscarriage rates (MR) following in vitro fertilisation (IVF)/ intracytoplasmic sperm injection (ICSI) treatment?

Summary answer:

Paternal age over 51 years significantly decreases the clinical pregnancy rate (CPR) following IVF/ICSI when maternal age is controlled. Miscarriage rate (MR) is not affected.

What is known already:

Semen quality deteriorates with increasing paternal age however there is conflicting evidence for any impact paternal age may have on the outcome of in vitro fertilisation (IVF)/ intracytoplasmic sperm injection (ICSI). Several retrospective and prospective cohort studies have shown that paternal age increases the MR and reduces the CPR. Some studies have shown no effect of paternal age on CPR or MR. Studies involving donor oocytes have shown no impact of paternal age on outcomes. The age at which there is a significant change in outcome is not known and there is no limit to paternal age in IVF/ICSI treatment.

Study design, size, duration:

This was a retrospective cohort study conducted at the Centre for Reproductive and Genetic Health clinic, London UK. A total of 4271 men and 4833 cycles of IVF and ICSI, including all causes of subfertility, from 01/12/2009 to 01/08/2018 were analysed in the study.

Participants/materials, setting, methods:

All cycles of IVF/ICSI performed using freshly ejaculated sperm. Cycles using donor oocytes/sperm and surgically retrieved sperm were excluded.

Data for each ART cycle was held on IDEAS version 6 Mellowood Medical and analysed using SPSS version 24.0 and STATA version 15.

Male partners were grouped into age ranges ≤35; 36 – 40; 41-44; 45-50; ≥51 years for analysis. Male age and female age <35 years was used as the reference groups for comparison.

Main results and the role of chance:

Median maternal and paternal ages were 36 years (IQR 33 – 39) and 38 years (IQR 35 – 42) respectively.

Significantly fewer men over 51 years met WHO semen analysis criteria (56/133, [42.1%, 95% CI 34.1 – 50.6]) compared to men under 51 years (2530/4138 [61.1%, 95% CI 60.0 – 62.6]) (p = 0.001).

2019/4833 (41.8%, 95% CI 40.4 – 43.2) of cycles resulted in clinical pregnancy. CPR declined with increasing maternal age ≤35 (1074/2102, 51.1% 95% CI 49.0 – 53.2), ≥40 (202/929, 21.7% 95% CI 19.2 – 24.5) (p = 0.001). CPR also declined with increasing paternal age; ≤35 (715/1433, 49.9% 95% CI 47.3 – 52.5), 36 – 40 (735/1731, 42.5% 95% CI 40.2 – 44.8), 41 – 45 (379/1076, 35.2% 95% CI 32.4 – 38.1), 46 – 50 (129/393, 32.8% 95% CI 28.4 – 37.6), ≥51years (61/200, 30.5% 95% CI 24.5 – 37.2). We performed multivariate logistic regression analysis with clinical pregnancy as dependent variable and maternal and paternal age class as independent variables. Maternal and paternal age were retained in the model. For all maternal age subgroups the probability of pregnancy decreased with paternal age over 51years (OR 0.655, 95% CI 0.477 – 0.927) (p = 0.001).

Limitations, reasons for caution:

The main limitation of this study is that it is retrospective and therefore vulnerable to confounding and bias. 80% of men ≥51years received ICSI treatment even though 42% had normal semen parameters. This may have confounded the results and reduced the perceived effect of increased paternal age.

Wider implications of the findings:

Paternal age over 50 significantly affects the chance of success of ART and there should be a public health message for men to not delay fatherhood. Alternative options for procuring semen for men over the age of 50 years may be discussed at the time of consultation.

Trial registration number:

not applicable

O-305 Factors influencing live birth outcomes of day 7 blastocysts following autologous single vitrified-warmed blastocyst transfer: A single-centre large cohort study

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Study question:

What factors influence live birth outcomes of day 7 blastocysts following single vitrified-warmed transfer (SVBT)?

Summary answer:

Live birth outcomes of day 7 blastocysts following SVBT are influenced by maternal age, time from insemination to blastulation, expansion time, and blastocyst diameter.

What is known already:

Preimplantation genetic testing has been promoted to prevent miscarriage after embryo transfer. Therefore, blastocyst culture has become standard for in vitro fertilisation (IVF), but it is unclear whether day 7 (d7) blastocysts should be cryopreserved and transferred. Clinical outcomes with d7-frozen blastocyst transfer (FBT) have been studied. However, as the usefulness of d7-blastocysts remains controversial, clinical outcomes of d7-FBT require further evaluation with a large cohort study. Investigation of factors influencing live birth (LB) after d7-FBT is also necessary to prioritize selection of blastocysts.

Study design, size, duration:

A retrospective cohort study of 49,044 autologous SVBTs (23,715 patients, mean age: 37.6±4.1) was conducted in a single centre between 2006 and 2015. Study 1: Factors influencing LB using d7-blastocysts following SVBT were investigated. Study 2: Cut-off values of factors influencing LB and criteria of d7-blastocysts were established and live birth rates (LBRs) for, d4-blastocysts (d4-SVBT), d5-blastocysts (d5-SVBT), d6-blastocysts (d6-SVBT) and d7-blastocysts (d7-SVBT) following SVBT were compared.

Participants/materials, setting, methods:

Blastocysts were vitrified using the Cryotop method according to criteria based on blastocyst diameter (Ueno et al., 2014). SVBT was performed in natural ovulatory cycles. Multivariable logistic regression (mLR) analysis was used to analyse the factors influencing LB. Multivariate receiver operating characteristic (ROC) curve analysis was used to establish cut-off values and criteria for d7-blastocysts. Chi-square tests were performed to compare LBR at >22 weeks of pregnancy among the groups.

Main results and the role of chance:

Study 1: mLR analysis to calculate adjusted odds ratios (aORs) included the following independent factors: age of females and males, number of previous IVF cycles, time from insemination to starting blastulation (tSB), expansion time (tExp: time from tSB to expanded blastocyst), and blastocyst diameter. mLR analysis revealed that age of females (aOR: 0.83, $P < 0.05$), tSB (aOR: 0.95, $P < 0.05$), tExp (aOR: 0.92, $P < 0.05$), and blastocyst diameter (aOR: 1.02, $P < 0.05$) were significantly correlated with LB in d7-SVBT. Study 2: ROC curve analysis determined cut-off values as follows: age of females < 39 years, tSB > 143 h post insemination, tExp: within 19 h, and blastocyst diameter > 210 μm (area under the curve: 0.76). The LBRs of d4-, d5-, d6- and d7-SVBT groups were 52.6% (n = 154), 40.1% (n = 23,484), 27.0% (n = 22,928), and 14.2% (n = 2,478), respectively. There were significant differences between the groups ($P < 0.05$). LBRs of d7-blastocysts that met the criteria (Adjusted-d7-SVBT group) were comparable to those of the d6-SVBT group (28.2% vs. 27.0%), but this rate was significantly lower compared to that for d4- and d5-SVBT groups (28.2% vs. 52.6% and 40.1%, $P < 0.05$).

Limitations, reasons for caution:

The present study did not include blastocyst morphology as an independent factor in the mLR analysis, because all d7-blastocysts analysed in this study had low-grade morphology. In addition, d7-blastocyst criteria may be influenced by clinical settings such as culture environment.

Wider implications of the findings:

Our results demonstrate that LBRs following d7-SVBT are comparable to those after d6-SVBT if d7-blastocysts fulfil strict criteria. Therefore, d7-blastocysts may have potential clinical usefulness for FBT.

Trial registration number:

None

O-306 The use of pronuclear transfer to overcome infertility disorders in mice

B. Heindryckx[1], M. Tang[1], A.K. Boel[1], P. Stamatiadis[1], M. Van der Jeught[1], R. Guggilla[1], D. Deforce[2], R. Van Coster[3], P. De Sutter[1]

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[3]Ghent University Hospital, Department of Pediatric Neurology and Metabolism, Gent, Belgium

Study question:

Can pronuclear transfer (PNT) overcome inferior embryonic development or reproductive ageing in a mouse model?

Summary answer:

The PNT technology is very efficient to restore embryonic developmental potential in infertility disorders such as reproductive ageing or embryo arrest.

What is known already:

Nuclear transfer such as PNT is being used to avoid the transmission of mitochondrial disorders. There is increasing interest to also use PNT for infertility disorders, however, scientific evidence in both animal and human models to support the use of PNT for infertility is currently lacking. It is important to reveal which infertility indications could benefit from this novel technology.

Study design, size, duration:

We used a reproductively-aged model (B6D2F1 mice) with age ranging from 6-8-weeks (control), 13-months (aged) to 16-months (very-aged); corresponding to women of <30, ~36 and ~45-year-old respectively. Secondly, another mouse strain (NZB mice, 6-8w) showing two-cell block was used. Finally, cytoplasmic transfer (CT) was performed by injection of a limited amount of

young B6D2 cytoplasm in both reproductively-aged and embryo-arrest models. We evaluated embryonic development in reconstituted PNT (n = 572) and CT (n=365) embryos.

Participants/materials, setting, methods:

Ovarian reserve was assessed by histological analysis in reproductively-aged mice. The Mitochondrial membrane potential (△Ψm) was measured by JC-1 staining in MII oocytes. The spindle-chromosomal morphology was examined by confocal analysis. PNT was performed by transferring pronuclei from fertilized oocytes (after ICSI) to enucleated counterpart zygotes between aged/very-aged and young mice, or between NZB/OlaHsd (embryo-arrest) and B6D2F1 (non-arrest control) mice.

Main results and the role of chance:

In comparison to the young mice, the ovarian reserve in aged/very-aged females was severely diminished, reflected by a lower number of ovarian follicles and lower ovulation rate ($P<0.001$). The average △Ψm in aged/very-aged mouse oocytes was significantly reduced ($P<0.001$) compared to young mice. Moreover, the rate of abnormal spindle-chromosome configuration in MII oocytes of aged/very-aged group was significantly higher ($P<0.05$) than young mice. Following ICSI, oocytes from aged/very-aged mice showed significantly lower fertilization (60.7% and 45.3%) and blastocyst formation rates (51.4% and 38.5%) than the ICSI control with young mouse oocytes (FR = 89.7%, blastocyst 87.3%) ($P<0.001$). After PNT from aged/very-aged to young mice, the blastocyst formation rates were significantly improved (74.6% and 69.2%, respectively).

Similarly, as model of embryo arrest, most (61.8%) of *in vivo* zygotes of NZB/OlaHsd strain displayed two-cell block during *in vitro* culture, with a significantly decreased blastocyst rate compared to B6D2F1 strain (13.5% vs. 90.7%, respectively) ($P<0.001$). When transferring PN from embryo-arrested (NZB/OlaHsd) to non-arrested (B6D2F1) zygotes, most of reconstructed zygotes developed beyond two-cell stage, with significantly increased blastocyst rates (89.7%) ($P<0.001$). The application of CT did not overcome inferior embryonic development in both aged/very-aged and embryo-arrested mice ($P>0.05$).

Limitations, reasons for caution:

Considering the differences between animal models and women regarding various biological processes, validation of PNT in infertility patients to explore the reliability of this technique is still required.

Wider implications of the findings:

This is the first study in mice to determine whether PNT could overcome certain female infertility indications such as advanced maternal age or embryonic developmental arrest. Our data support that PNT, refreshing the oocyte or zygote cytoplasm, may represent a novel reproductive strategy to increase embryonic developmental potential.

Trial registration number:

NA

O-307 Impact of trophectoderm grade on infant physiological characteristics and gender after single frozen blastocyst transfers: an analysis of 1109 singletons after 3822 frozen blastocyst transfers

M. Kataoka[1], Y. Nakajo[1], H. Hattori[1], Y. Nakamura[1], M. Takahashi[1], N. Okuyama[2], N. Aono[1,2], T. Tai[1,2], M. Toya[1], T. Hashimoto[2], H. Igarashi[1], K. Kyono[1,2]

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Study question:

Does trophectoderm (TE) morphology grade affect infants' physiological characteristics and gender ratio after frozen blastocyst transfer?

Summary answer:

TE morphology grade was uncorrelated with birth weight, height or congenital abnormality in infants. High TE grade was significantly associated with gender ratio towards male.

What is known already:

Gardner's scoring criteria is often used to evaluate blastocyst quality. A strong correlation between blastocyst morphology grade and clinical outcome has been reported by many studies. However, the correlations between TE grade and physiological characteristics and gender ratio of infants have not been fully understood.

Study design, size, duration:

This retrospective study was conducted at Kyono ART Clinic in Japan from 2012 to 2017. This study includes a total of 1109 singletons born after 3822 single frozen blastocyst transfer in 2166 patients.

Participants/materials, setting, methods:

TE morphology was graded according to Gardner's scoring system and divided into the three grades (Grades A, B, and C). Grade A includes 638 cycles, 447 patients, and 254 children; Grade B, 1731 cycles, 944 patients, and 526 children; and Grade C, 1453 cycles, 775 patients and 329 children. The correlations between TE grade and clinical outcomes (pregnancy rate, miscarriage rate and ongoing pregnancy rate), physiological characteristics, and gender ratio were evaluated.

Main results and the role of chance:

Average maternal age in Grade C was significantly higher than in Grades A and B (35.5±3.1 vs. 34.8±3.2 and 35.1±3.2, $p<0.01$). Pregnancy rates in Grades A, B, and C were 60.7%, 50.5%, and 36.9%, respectively; Grade A was significantly higher than Grade C ($p<0.01$). Ongoing pregnancy rates in Grades A, B, and C were 54.1%, 45.5%, and 30.6%, respectively; Grade A was significantly higher than Grade C ($p<0.01$). Miscarriage rates in Grades A, B, and C were 12.1%, 11.5%, and 10.7%, respectively (N.S: not significant). Average gestational age (weeks) in Grades A, B, and C were 38.8±1.8, 38.7±2.3, and 38.4±2.4, respectively (N.S). Average height (cm) and weight (g) in Grades A, B, and C were 49.3±2.4, 49.0±3.7, and 49.0±3.2 (N.S), and 3,066±0.4, 3,047±0.5, and 3,040±0.5, respectively (N.S). Premature birth rates and low birth weight rates in Grades A, B, and C were 5.5% (n=14), 8.4% (n=44) and 7.9% (n=26) (N.S), and 6.3% (n=16), 9.3% (n=49), and 8.5% (n=28), respectively (N.S). Congenital abnormality rates in Grades A, B, and C were 2.8%, 2.9%, and 1.5% (N.S). Gender ratios (male:female) in Grades A, B, and C were 1.92:1, 1.26:1, and 0.94:1, respectively; Grade A was significantly higher than Grade C ($p<0.01$).

Limitations, reasons for caution:

In this study, we did not assess the correlation between inner cell mass and infants' physiological characteristics and gender ratio. Since male embryos grow faster than female embryos, high TE grade male embryos might be selected as transferred blastocysts in our study.

Wider implications of the findings:

It was particularly noteworthy that high grade TE clearly skews gender ratio towards male. Blastocyst TE grade is responsible for pregnancy rate. However, miscarriage rate, infants' physiological characteristics and congenital abnormality are not affected by TE grade.

Trial registration number:

None.

SELECTED ORAL COMMUNICATIONS
SESSION 79: NOVEL INSIGHTS IN PCOS
Wednesday 26 June 2019 Strauss 1+2 14:00 - 15:30

O-308 Association of childhood adiposity with irregular menstruation and polycystic ovary syndrome in adulthood: the Childhood Determinants of Adult Health Study and the Bogalusa Heart Study

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Study question:

Is abdominal obesity in childhood associated with menstrual irregularity and polycystic ovary syndrome (PCOS) in later life?

Summary answer:

Abdominal obesity measured as waist-to-height ratio (WHtR) in childhood was positively associated with a significantly increased risk of self-reported menstrual irregularity and PCOS in adulthood.

What is known already:

While adult obesity, particularly abdominal obesity, is associated with irregular menstruation and PCOS in women, few studies have explored the relationships with childhood adiposity. Increased childhood body mass index (BMI) has been associated with irregular menstrual cycles and PCOS symptoms in adulthood in two longitudinal studies but no study has reported on associations with childhood abdominal obesity. Few studies have investigated whether there are racial differences in the associations of adiposity with PCOS though there has been some suggestion that associations with high BMI may be stronger in white girls than in black girls.

Study design, size, duration:

The study included 3,152 participants aged 7-15 years at baseline from two cohorts (1,551 from the Childhood Determinants of Adult Health Study (CDAH) in Australia and 1,601 from the Babies sub-study of the Bogalusa Heart Study (BBS) in the United States). At follow-up, questions about menstruation (current for CDAH or before age 40 for BBS) and PCOS were asked in two follow-ups in CDAH (between ages 26-41) and once in the BBS (ages 27-57).

Participants/materials, setting, methods:

Childhood anthropometrics including measured height, weight and waist circumferences. Race was available in BBS (59% white; 41% black). In CDAH, a single childhood visit was conducted in 1985. In BBS, multiple childhood visits occurred from 1973-2000. Obesity was defined by international age-sex-specific standards for BMI. Waist/height ratio (WHtR) was considered as an indicator of abdominal obesity. Multilevel mixed-effects Poisson regression estimated relative risks (RRs) adjusting for education and age at menarche.

Main results and the role of chance:

The prevalence of childhood obesity was 1.1% in CDAH and 7.1% in BBS (white:5.0%; black:10.1%). At follow-up, the prevalence of menstrual irregularity was 13.9% in CDAH and 17.4% in BBS (white:18.9%; black:15.2%). PCOS was reported by 7.5% in CDAH and 6.5% in BBS (white:8.7%; black:3.4%). In CDAH, compared with normal weight girls, those who were obese in childhood were more likely to report menstrual irregularity (RR=2.94, 95% confidence interval [CI]:1.24-6.99) and PCOS (RR=4.35, 95% CI:1.17-16.19). Each 0.01unit increase in childhood WHtR led to a 5% (95% CI:1%-9%) greater likelihood of menstrual irregularity and 5% (95% CI:0%-10%) greater likelihood of PCOS. Overall, in BBS, childhood obesity was associated with an increased risk of menstrual irregularity (RR=2.67, 95% CI:1.77-3.12) and PCOS (RR=1.70, 95% CI:1.03-2.79). A 0.01unit increase in childhood WHtR was associated with an 8% (95% CI:2%-14%) increase likelihood of PCOS. However, when stratifying by race in BBS, the association of childhood adiposity measures with PCOS was not present in black girls. In white girls in BBS, childhood obesity increased the risk of self-reported PCOS when compared with normal weight girls (RR=2.46, 95% CI:1.41-4.30) and an average 0.01unit higher childhood WHtR was associated with a 9% (95% CI:3%-15%) greater risk of PCOS.

Limitations, reasons for caution:

The classification of menstrual irregularity and PCOS was based on self-report by questionnaire, which may have led to misclassification of these outcomes. While the study samples were population-based, loss to follow-up means the generalizability of the findings is uncertain.

Wider implications of the findings:

Greater childhood adiposity indicates a higher risk of menstrual irregularity and PCOS in adulthood. Whether this is causal or an early indicator of underlying hormonal or metabolic disorders needs clarification. The stronger associations of adiposity with PCOS in white girls suggest that other environmental or genetic factors are also important.

Trial registration number:

Not applicable

O-309 Quantitative differences in TGF-β superfamily growth factors measured in small antral follicle fluids from women with PCOS

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Study question:

Are there measurable differences in intrafollicular levels of TGF-β superfamily growth factors in small antral follicles from women with and without PCOS?

Summary answer:

Altered levels of Inhibins and Growth-and-Differentiation-Factor-9 (GDF9), but not Anti-Müllerian-Hormone (AMH), were found in the follicle fluids (FF) of small antral follicles from polycystic ovaries.

What is known already:

PCOS is the most common endocrine disorder in women. Anovulation in PCOS is characterized by growth-arrest of small antral follicles (hSAF) (2–9 mm in diameter) in the ovary due to the abnormal endocrine environment. Members of the TGF-β superfamily have been implicated in aberrant follicle development in PCOS, but there is no data on FF from hSAF during the natural cycle due to the scarcity of such material and assay limitations. Previous studies have shown that AMH levels in both serum and large antral follicles are significantly higher in women with PCOS compared to controls.

Study design, size, duration:

Donated FF were aspirated and collected from small antral follicles found in the ovaries of 55 women undergoing ovarian tissue cryopreservation for fertility preservation at the University Hospital of Copenhagen, Denmark, from 2011-2018.

Participants/materials, setting, methods:

Follicle fluids (N=192) were aspirated from whole ovaries laparoscopically removed from patients during their natural cycle and before initiation of gonadotoxic treatment. Women with PCOS were identified in the cohort by ovarian volume, number of antral follicles, and hormone profiles. A new line of hypersensitive immunoassays from Ansh Labs in Texas, USA were used to detect levels of GDF9, AMH, Inhibin-A and -B, Activin-A, -B and AB, and Follistatin.

Main results and the role of chance:

A total of 90 FF were collected from 15 women with PCOS (median age: 25 years) and 102 FF were collected from 40 control women without PCOS (median age: 25 years). The diameters of small antral follicles ranged from 4.6-10.7 mm in both groups. Intrafollicular levels of both Inhibin-A and -B were significantly lower in FF from women with PCOS compared to controls, and the low levels were especially pronounced in follicles just before selection of the dominant follicle (hSAF $<$ 8 mm). Moreover, intrafollicular levels of GDF9 were found to be significantly higher in PCOS FF in hSAF exceeding 6 mm in diameter compared to controls. Interestingly, intrafollicular levels of AMH did not differ between PCOS and controls, only in the hSAF larger than 8 mm AMH levels were significantly higher in PCOS FF compared to control FF. Thus, intrafollicular AMH levels remain high after follicle selection in polycystic ovaries instead of decreasing to low levels like in the control ovaries with normal follicle growth. Intrafollicular levels of Activin-B, Activin-AB and Follistatin did not differ between PCOS and controls, and levels of Activin-A were under the assay detection limit.

Limitations, reasons for caution:

Only a small number of hSAF larger than 8 mm in diameter were available for analysis. Further studies are needed to elucidate on the molecular mechanisms and functional effects of the altered levels of Inhibins, GDF9 and AMH at specific stages of small antral follicle development in polycystic ovaries.

Wider implications of the findings:

This is the first time oocyte-specific GDF9 has been measured in small antral human FF using a hypersensitive immunoassay. Altered levels of TGF-β superfamily growth factors at certain follicular stages of small antral follicle development may reflect molecular defects of folliculogenesis, especially around follicle selection, in women with PCOS.

Trial registration number:
not apllicable

O-310 Favorable changes in characteristics, phenotype and androgens as result of weight loss in a randomised controlled three-component lifestyle intervention in women with PCOS

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Study question:
What is the effect of weight loss on PCOS characteristics ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology during a three-component lifestyle intervention.

Summary answer:
Weight loss had a significant impact on ovulatory dysfunction and hyperandrogenism, resulting in a milder phenotype in the majority of overweight/obese women with PCOS.

What is known already:
The first line treatment for women with PCOS is a multicomponent lifestyle intervention to improve weight. Overweight and obesity seem to worsen PCOS characteristics and phenotypic expression. Although insights in the dynamic character of PCOS characteristics have been described due to female aging or anthropomorphic appearance, little is known about the effect of weight loss due to a lifestyle intervention on PCOS characteristics, phenotypic expression and androgens.

Study design, size, duration:
An RCT was performed consisting of a three-component (cognitive behavioral therapy, healthy diet and physical therapy) one-year lifestyle intervention (LS) compared to care as usual (CAU). Overall, 209 women were included between May 2009 and August 2016. The lifestyle intervention group received 20 group sessions during one year addressing the three components. The control group received CAU which consisted of advices to lose weight autonomously.

Participants/materials, setting, methods:
Women diagnosed with PCOS, aged 18-38 years, having a wish to conceive and a BMI above 25 kg/m^2 were included at the Erasmus MC, The Netherlands. Outcome variables were evaluated every three months and included anthropomorphic measurements, ultrasound and an endocrine assessment. Multilevel linear and logistic regression was applied for longitudinal analyses.

Main results and the role of chance:
No differences were found between the LS and the CAU group concerning changes in PCOS characteristics, phenotype expression and androgens after 12 months except for a significant decrease of testosterone ($p<0.05$) and androstenedione ($p<0.05$) serum levels in the LS group. However, when we evaluated weight loss in general we observed a significant decrease in the prevalence of ovulatory dysfunction (OD) ($p<0.001$) and hyperandrogenism (HA) ($p<0.001$). When a 5% and 10% weight loss was achieved, 10.2% and 26.4% improvements in ovulatory function and 6.3% and 15.1% improvements in HA were found respectively. Changes in polycystic ovarian morphology were not observed. Furthermore, the chance to be classified as phenotype A (full blown phenotype) was significantly lower due to weight loss ($p<0.001$). In case of achieving 5% or 10% weight loss the chance of having phenotype A diminished with 12.7% and 27.9% respectively.

Limitations, reasons for caution:
Improvement in ovulatory function was defined as regaining cycle regularity, however progesterone serum levels were not used to confirm ovulation, a limitation of the study.

Wider implications of the findings:
These findings confirm the dynamic character of PCOS which is apparently modulated by BMI. Additionally, they support the necessity of multicomponent lifestyle interventions in overweight and obese women with PCOS to achieve weight loss.

Trial registration number:
This study was registered at the Dutch Trial Register, number NTR2450.

O-311 Cardiometabolic health in offspring of women with polycystic ovary syndrome (PCOS) compared to healthy controls: A systematic review and Individual Participant Data Meta-analysis.

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Study question:
Are cardiometabolic features in PCOS offspring, both females and males, between the age of 1 to 18 years less favorable in comparison with controls?

Summary answer:
We observed subtle signs of compromised cardiometabolic health in children born to women diagnosed with PCOS, predominantly in female offspring.

What is known already:
The Barker hypothesis implies that children's health and development is distinctly affected by the intrauterine environment. An association between maternal health and offspring health has been convincingly demonstrated in the general population. Women with PCOS often present with unfavorable cardiovascular risk factors such as obesity and insulin resistance. Currently confirmation of this association within women with PCOS and their offspring is limited. The sample size of these studies is too small to draw definitive conclusions, and the majority does not report on sex differences. Moreover, existing literature concerning cardiometabolic outcomes in PCOS offspring is inconsistent.

Study design, size, duration:
We conducted a systematic review and individual participant data meta-analysis. For each included study approval of the local medical ethics committees, and written informed consent from all parents was obtained before measurement of offspring outcomes.

Participants/materials, setting, methods:
PubMed, Embase and grey literature were searched (updated: February 1th, 2018). Relevant key terms such as "offspring" and "PCOS" were combined. Internal validity was assessed using the Newcastle Ottawa Quality Assessment Scale. Outcomes were reported as age-specific standardized deviation scores of cardiometabolic parameters: body mass index, blood pressure, insulin resistance, lipid profile and metabolic sum score. Linear mixed models were used for analyses with age-standardized estimated mean differences as outcome, corrected for multiple testing.

Main results and the role of chance:
Nine relevant observational studies could be identified which jointly included 1,367 children originating from the Netherlands, Chile and the United States. After removal of neonates, duplicate records and follow-up screenings 885 subjects remained, of which 298 children were born to mothers with PCOS and 587 controls.

PCOS offspring exhibited increased plasma levels of fasting insulin (β=0.21(SE:0.10), p=0.046), insulin–resistance (β=0.21(Standard Error

(SE:0.10)), p=0.042), triglycerides (β=0.19(SE:0.09), p=0.030) and HDL-cholesterol concentrations (β=0.31(SE:0.12), p=0.008), but reduced birth weight (β=-116(SE:40), p=0.004) compared to controls.

Interaction tests for sex revealed differences between males and females comparing PCOS offspring versus controls. Higher 2-hour fasting insulin was observed within females (estimate for females (β_f)=0.45(Standard Error(SE:0.20)) compared to males (estimate for males (β_m)=-0.20(SE:0.20)), interaction-test: p=0.030). LDL-cholesterol differences were lower within females (β_f=-0.39(SE:0.12), β_m=0.27(SE:0.15), interaction-test: p<0.001). Total cholesterol was also lower in female PCOS offspring versus female controls (β_f=-0.31(SE:0.13)) in comparison to the difference within males (β_m=0.28(SE:0.15), interaction-test: p=0.012). HDL-cholesterol was higher in female PCOS offspring versus controls (β_f=0.53(SE:0.17)), compared to the difference within males (β_m=0.13(SE:0.09)), interaction-test: p=0.007). The overall metabolic sum score appeared lower in females (β_f=-0.23(SE:0.46)), than within males (β_m=0.84(SE:0.48)), interaction-test: p=0.004).

After multiple testing correction (significance level: p=0.05/3=0.017) however, differences in insulin and triglycerides lost their statistical significance.

Limitations, reasons for caution:

We used international external reference sources to calculate standardized deviation scores for BMI and systolic blood pressure which already takes sex of the child into account. The additional correction for sex by using interaction terms and stratification methods, may have resulted in an underestimation of differences between males and females.

Wider implications of the findings:

We observed subtle signs of a compromised cardiometabolic health predominantly in female offspring between 1 and 18 years of age of women with PCOS. Further high validity studies should elucidate the influence of PCOS diagnosis on males and females during the fetal development and consequently during childhood.

Trial registration number:

Not applicable

O-312 Effects of a three-component lifestyle intervention on emotional well-being in women with PCOS

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Study question:

Is a multidisciplinary one-year cognitive-behavioural lifestyle treatment more effective to decrease emotional well-being than usual care?

Summary answer:

Depression scores only decreased significantly more in the lifestyle group compared to the usual care group during the first 5.38 months, thereafter the difference decreased.

What is known already:

Women with PCOS experience more depressive and anxiety complaints, have a lower self-esteem and have a more negative body-image compared to women without PCOS. Especially obese women with PCOS have higher depression rates compared to non-obese women with PCOS. Lifestyle interventions based on two-components have demonstrated short-term effects on well-being in women with polycystic ovary syndrome (PCOS). In general, three-component interventions including diet, exercise, and cognitive behavioral therapy (CBT) have shown to be effective at the long-term to improve emotional well-being. This has not yet been studied in women with PCOS.

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 emotional well-being in overweight/obese women with PCOS. A total of 164 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 164 women diagnosed with PCOS according to the Rotterdam criteria, a Body Mass Index above 25 kg/m^2 were included. We measured depression with the Beck Depression Inventory, self-esteem with the Rosenberg Self-Esteem Scale and body image with the Fear of Negative Appearance Evaluation scale, all measured at start and at three, six, nine and twelve months.

Main results and the role of chance:

Depression scores only decreased significantly (26.7%, P=0.04) during the first 5.38 months of lifestyle compared to care as usual. After 5.38 months the difference decreased while weight loss continued. In the long-term, depression scores in lifestyle were not significantly lowered compared to care as usual (P=0.376). Self-esteem scores improved 11% comparing lifestyle to care as usual at 12 months (P=0.053). Body image improved 17.7% comparing the lifestyle group to the care as usual at 12 months (P=0.038). Also, a lower depression score at baseline was significantly associated with 5% weight loss (OR 0.96; CI 95% 0.93-1.00; P=0.039) but not with 10% weight loss. A more positive body image at baseline was significantly associated with 10% weight loss (OR 0.93; CI 95% 0.87-0.99; P=0.020).

Limitations, reasons for caution:

A limitation of our study is that we did not use a structured clinical interview such as the SCID-I to diagnose clinical mood disorder to verify clinically relevant depression scores.

Wider implications of the findings:

A three-component lifestyle intervention program for obese women with PCOS resulted in temporal lower depression scores and long term improvements for self-esteem and body image if we compared lifestyle to control. Women with PCOS need long-term three-component lifestyle interventions for weight loss and additional psychological treatment to improve mood.

Trial registration number:

Registered at the Netherlands National Trial Register with number NTR2450 on August 2nd, 2010.

O-313 Decrease in serum AMH levels during prepregnancy metformin therapy associates with improved pregnancy and live-birth rates in women with PCOS: a multicentre, double-blind, placebo-controlled RCT

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Study question:

What are the clinical, hormonal or metabolic parameters that associate with pregnancy and live-birth (LB) during metformin in anovulatory women with polycystic ovary syndrome (PCOS)?

Summary answer:

Decrease in serum anti-Müllerian hormone(AMH) levels during metformin treatment was the only parameter that associated significantly with improved pregnancy and LB rates in PCOS women.

What is known already:

Metformin treatment has been shown to improve pregnancy and LB rates in women with PCOS and anovulatory infertility. However, one third of PCOS women do not respond to metformin treatment. Whether the responsiveness to metformin treatment in PCOS is related to the severity of menstrual disorders, metabolic disturbances, obesity, degree of insulin resistance or androgen

excess remains controversial. Previously, metformin treatment has been shown to decrease serum AMH levels in women with PCOS, however, the possible association with improvement in the pregnancy and LB rates has not been investigated.

Study design, size, duration:

Multicenter, randomized (1:1), double-blind, placebo-controlled study investigating the efficacy of metformin in anovulatory infertility. Three-hundred-twenty women with PCOS women were randomized receiving metformin or placebo until the 12th week of pregnancy or maximally nine months. Women with BMI≥27kg/m^2 received 1000 mg×2/day and women with BMI<27kg/m^2 500mg+1000mg/day, or identical placebo. Serum samples were obtained at 0 and 3 months. After 3 months of treatment with metformin/-placebo alone, another appropriate infertility treatment was combined, if necessary.

Participants/materials, setting, methods:

Clinical (weight, waist/hip circumferences, BMI, hirsutism), metabolic (fasting insulin and glucose, area under curve for insulin and glucose during OGTT, insulin resistance indexes), and hormonal (testosterone, sex hormone binding globulin [SHBG], free androgen index, androstenedione, AMH) parameters were assessed at baseline and at 3 months. Women getting pregnant before 3 months or cases with missing sample at 3 months were excluded. Comparisons were done between the women achieving and not achieving pregnancy/LB after 3 months'treatment.

Main results and the role of chance:

Metformin group (N=126): Women achieving pregnancy (N=56) or LB (N=49) had shorter duration of infertility (p=0.031 and p=0.004, respectively). During 3 months of treatment, serum AMH levels decreased significantly more among women later achieving pregnancy (decrease: 10.13±6.93ng/mL to 8.98±5.90ng/mL vs. 9.20±5.16ng/mL to 9.22±5.06mg/mL, change:-1.15±3.37ng/mL vs. 0.02±2.74ng/ml,p=0.039) or LB (decrease: from 10.49±7.29ng/mL to 9.24±6.21ng/mL vs. 9.05±5.00ng/mL to 9.02± 6.21ng/mL, change: -1.25±3.59ng/mL vs. -0.03±2.61ng/mL,p=0.034). In multivariate regression analysis, AMH decrease during 3 months of treatment was the only predictive factor for pregnancy and LB when previous pregnancies, infertility duration, age and BMI change were taken into account (pregnancy:OR 1.149[95%CI 1.004–1.315]p=0.044, LB:OR 1.149[95%CI 1.003–1.315]p=0.044).

Placebo group (N=125): Women achieving pregnancy (N=43) or LB (N=36) after 3 months of treatment were less hirsute (p=0.031 and p=0.047 respectively) and had higher serum SHBG levels (p=0.018 and p=0.029 respectively) at baseline. BMI (p=0.008 and p=0.003, respectively) and waist circumference (p=0.007 and p=0.012, respectively) decreased more during 3 months of treatment in women who later achieved pregnancy or LB. In multivariate regression analysis, BMI decrease was the only predictive factor for pregnancy and LB when previous pregnancies, infertility duration and age were taken into account (pregnancy: OR 1.841[95%CI 1.145–2.961]p=0.012, LB: OR 2.019[95%CI 1.233–3.308]p=0.005).

Limitations, reasons for caution:

All women did not attend the control visit at 3 months of treatment and the women who had achieved pregnancy before that point were excluded. Non-obese women used a lower metformin dose which may have biased the results in the metformin group.

Wider implications of the findings:

Among several clinical, metabolic and hormonal parameters, the decrease of serum AMH levels was the best indicator for patients' responsiveness to metformin treatment. These results open interesting new insights into the mechanisms of metformin action and possible use of AMH levels in predicting pregnancy outcomes in anovulatory women with PCOS.

Trial registration number:

NCT00994812

POSTER VIEWING
ANDROLOGY

P-001 Relationship between Vascular Endothelial Growth Factor (VEGF) gene polymorphism (rs1570360) and sperm DNA damage

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Study question: Is there an association between Vascular Endothelial Growth Factor VEGF -1154 guanine(G)>adenine(A) (rs1570360) polymorphism and sperm DNA integrity?

Summary answer: The VEGF G>A polymorphism seems to influence sperm quality. Men carrying the A allele (GA or AA genotypes) exhibited increased levels of sperm apoptosis.

What is known already: VEGF is a major factor in angiogenesis and a prime regulator of endothelial cell proliferation. It is involved in oocyte maturation, trophoblast proliferation, embryo implantation and development, angiogenesis of the placenta, and the growth of maternal/foetal blood vessels. Reports indicate that several VEGF gene polymorphisms affect VEGF protein activity and expression. The VEGF -1154G>A polymorphism characterised in the promoter region of the gene has been correlated with variations in protein production and recurrent implantation failure. Unfortunately, the association between VEGF gene polymorphisms and male fertility is not commonly analysed.

Study design, size, duration: The study enrolled 363 men submitted to infertility assessment. Semen analyses were performed according to WHO guidelines/morphology motile sperm organelle morphology examination (MSOME). For DNA integrity analysis, the proportions of DNA fragmentation (TUNEL), abnormal chromatin packaging/underprotamination (chromomycin A3/CMA3), and apoptosis (annexin-V) were recorded. At least 200 spermatozoa were examined in each evaluation. Potential confounders (age, abstinence, smoking, drinking alcohol, and varicocele) were also observed.

Participants/materials, setting, methods:

DNA was extracted from peripheral blood samples taken from each participant and the VEGF single nucleotide polymorphism (SNP) -1154G>A was genotyped by real-time PCR. For purposes of comparison, the subjects were divided into three genotype groups according to their results: GG; GA; or AA. The level of significance was set at $P<0.05$. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.

Main results and the role of chance: No correlations was observed between VEGF-1154G>A and age, abstinence, smoking, drinking alcohol, or varicocele. Genotypes carrying the A allele (GA or AA genotypes) were associated with higher proportions of spermatozoa apoptosis (Table 1). No association was observed between genotypes VEGF-1154G>A and other semen parameters (Table 2).

Table 1 VEGF-1154G>A vs. DNA integrity analysis

VEGF Genotypes	DNA fragmentation (%)	Apoptosis (%)	CMA3 positivity (%)
GG	13.3±6.9	**18.2±7.2[a,b]**	56.4±17.4
GA	13.4±7.9	**21.1±9.0[a]**	55.2±16.8
AA	14.8±9.6	**21.0±7.7[b]**	54.5±15.9
P	0.77	**[a]0.01;[b]0.04**	0.76
GG+GA	13.4±7.4	19.4±8.1	55.9±17.1
AA	14.8±9.6	21.0±7.7	54.5±15.9
P	0.37	0.29	0.64
GG	13.3±6.9	**18.2±7.2**	56.4±17.4
GA+AA	13.8±8.4	**21.0±8.6**	55.0±0.3
P	0.61	**0.005**	0.49

Table 2 VEGF-1154G>A vs. general and semen parameters

Parameters	VEGF Genotypes			
	GG	GA	AA	*P*
n	179	132	52	
Age (years)	38.1±5.8	38.1±6.1	37.7±6.3	0.87
BMI (Kg/m^2)	28.6±4.4	28.3±4.2	28.4±4.4	0.76
Abstinence (days)	3.2±1.9	3.2±2.1	3.7±2.7	0.62
pH	8.1±0.2	8.1±0.3	8.1±0.2	0.32
Volume (ml)	2.9±1.4	2.7±1.3	3.0±1.8	0.37
Concentration (mlx10^6)	64.3±52.9	59.8±53.6	67.4±42.7	0.30
Progressive motility (%)	52.5±17.2	53.8±15.8	54.5±15.6	0.84
Total motility (%)	59.6±16.7	61.0±16.4	61.0±14.7	0.89
Normal sperm structure (%)	0.6±0.8	0.7±0.78	0.7±0.6	0.40
Leukocytes (x10^6/ml)	0.5±0.8	0.4±0.6	0.3±0.3	0.77
Vitality (%)	61.8±16.2	62.5±14.8	62.8±15.6	0.97

Limitations, reasons for caution: Differences in the genetic backgrounds of various ethnic populations should be considered. The sperm were obtained from men submitted to fertility assessment. Generalization of these results to the general population should be performed with caution

Wider implications of the findings: The results indicated that VEGF -1154G>A (rs1570360) gene polymorphisms were related to sperm apoptosis. Further investigations with larger populations should be conducted to confirm these findings and to determine their clinical implications.

Trial registration number: Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

P-002 Is there an association between the TP53 codon 72 polymorphism and sperm DNA damage?

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Study question: Is there an association between the TP53 codon 72 polymorphism (which encodes arginine [Arg] or proline [Pro]) (rs1042522) and sperm DNA damage?

Summary answer: The TP53 codon 72 polymorphism seems to be linked to sperm DNA damage.Sperm DNA fragmentation was more pronounced in the presence of the Arg allele.

What is known already: The TP53 protein plays a critical role in maintaining genomic stability and mediates pregnancy by regulating the activation of steroid hormones and functionally related genes essential for foetal-maternal interactions during implantation. The literature provides evidence that maternal genotype for the TP53 codon 72 polymorphism (rs1042522) is associated with repeated implantation failure after IVF/ICSI or recurrent miscarriage. However, the relevance of male genotypes has not been determined yet.

Study design, size, duration: This cross-sectional study included the semen samples of 364 men (38.1±6.0 years) from couples seeking care at a fertility clinic. The semen analyses derived from one semen sample of each enrolled subject were recorded. For DNA integrity analysis, the following proportions were determined:

- Spermatozoa with DNA fragmentation using the TUNEL assay;
- Spermatozoa with abnormal chromatin packaging/underprotamination using chromomycin A3 (CMA3);
- Spermatozoa with abnormal mitochondrial membrane potential (MMP) using MitoTracker Green;
- Spermatozoa apoptosis using annexin-V.

Participants/materials, setting, methods: DNA was extracted from peripheral blood, and the TP53 gene codon 72 single nucleotide polymorphism(SNP) Arg>Pro was genotyped using real-time PCR with the Taqman Universal PCR Master Mix and Taqman SNP genotyping assays. The subjects were categorised based on their genotypes into three groups: Arg/Arg(n:187); Arg/Pro(n:151); or Pro/Pro(n:26). Potential confounders(age, abstinence time, smoking, drinking alcohol, and varicocele) were also observed. Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies.

Main results and the role of chance: No correlation was observed between TP53 gene codon 72 SNP Arg>Pro and age, abstinence, smoking, drinking alcohol, or varicocele. Although there was no observed association between TP53 genotype or alleles and underprotamination, apoptosis or mitochondrial damage, the proportions of sperm DNA fragmentation were higher in men carrying the Arg allele (Arg/Arg and Arg/Pro genotypes). Table 1 shows the results.

Table 1 TP53 gene codon 72 single nucleotide polymorphism vs. DNA damage parameters: group comparisons.

TP53 Genotypes	DNA fragmentation (%)	Apoptosis (%)	CMA3 positivity (%)	Abnormal MMP (%)
Arg/Arg	**14.6±7.8^{a,b}**	20.1±9.3	56.4±17.1	26.5±17.7
Arg/Pro	**12.4±7.4^{a}**	19.0±6.2	55.5±17.0	26.0±16.2
Pro/Pro	**9.7±3.1^{b}**	19.6±6.8	53.3±16.3	22.4±17.0
P	**^{a}0.02, ^{b}0.004**	0.85	0.70	0.73
Arg/Arg+ Arg/Pro	**13.7±7.9**	19.6±8.1	56.0±17.0	26.3±17
Pro/Pro	**9.7±3.1**	19.6±6.8	53.3±16.3	22.4±17.0
P	**0.03**	0.98	0.50	0.39
Arg/Arg	**14.6±7.8**	20.1±9.3	56.4±17.1	26.5±17.7
Arg/Pro+ Pro/Pro	**12.0±6.9**	19.1±6.3	55.1±16.8	25.3±16.3
P	**0.002**	0.64	0.55	0.65

Values within each column with the same superscript letter were significantly different.

Limitations, reasons for caution: The data are cross-sectional and specifically focus on the association between a specific TP53 polymorphism and DNA damage. Only males from infertile couples were sampled. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings: The TP53 gene codon 72 SNP Arg>Pro (rs1042522) might impair sperm DNA integrity and probably affects male reproductive capacity. Additional validation of the analysed SNP with a greater number of cases is required to confirm these findings and provide more robust evidence of possible clinical implications

Trial registration number: Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

P-003 Evaluation of the Herpes Virus-Associated Ubiquitin-Specific Protease (HAUSP) gene polymorphism (rs1529916) on semen quality and sperm DNA damage

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Study question: Are there correlations between the herpes virus-associated ubiquitin-specific protease (HAUSP) gene guanine (G)>adenine (A) (rs1529916) polymorphism and semen quality or sperm DNA damage?
Summary answer: There seems to be no association between HAUSP G>A polymorphism and semen or DNA damage parameters.
What is known already: Single nucleotide polymorphisms (SNPs) in the p53 pathway have been linked to fertility in humans. An important regulator of this pathway is HAUSP (or USP 7), a specific deubiquitinase that stabilises the p53 protein. The literature provides evidence that the HAUSP gene G>A (rs1529916) polymorphism in women is associated with fertility. However, the impact of the HAUSP gene polymorphism on male fertility is often disregarded.
Study design, size, duration: This prospective cohort study enrolled 359 males seeking fertility care. DNA was extracted from peripheral blood, and the HAUSP gene G>A (rs1529916) was genotyped using real-time PCR with the Taqman Universal PCR Master Mix and Taqman SNP genotyping assays. Patients were genotyped for HAUSP polymorphisms GG (n=186), GA (n=145) and AA (n = 28). The different genotype groups were compared for their semen analysis results
Participants/materials, setting, methods: A portion of each semen samples was used for analysis according to the WHO guidelines/morphological analysis by motile sperm organelle morphology examination(MSOME). The remainder of the semen samples were tested for sperm DNA fragmentation using TUNEL assays; sperm apoptosis was analysed using the annexin V assay; sperm chromatin packing/protamination was assessed using chromomycin A3(CMA3) staining; and sperm mitochondrial membrane potential(MMP) was analysed using MitoTracker Green. At least 200 spermatozoa were examined in each evaluation
Main results and the role of chance: Hardy–Weinberg genotype distributions indicated concordance between the observed/expected frequencies. No correlation was observed between HAUSP genotype and potential confounders (age, abstinence time, smoking, drinking alcohol, or varicocele). No association was observed between HAUSP genotype and general semen parameters or sperm DNA damage. Table 1 shows the data.
Limitations, reasons for caution: Additional validation of the analysed SNP (using a greater number of cases) will be important to provide more information. Although all available eligible males were enrolled in the study, the sample size was limited. Differences in the genetic backgrounds of various ethnic populations should also be considered
Wider implications of the findings: Despite previous reports of an association between the HAUSP G>A (rs1529916) polymorphism and human fertility, there appeared to be no relation between genotypes and semen quality or sperm DNA damage. This polymorphism is probably not useful in the evaluation of male infertility
Trial registration number: Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

Table 1 HAUSP gene G>A (rs1529916) genotypes vs. population and semen parameters

Parameters	HAUSP gene G/A genotypes			
	GG	GA	AA	*P*
Age (years)	37.9±5.8	37.7±6.0	39.9±6.1	0.17
BMI (Kg/m^2)	28.8±4.3	28.2±4.5	28.1±3.7	0.48
Smoking (%)	9.7%	11.0%	3.6%	0.55
Drinking alcohol (%)	57.5%	63.4%	60.7%	0.56
Varicocele (%)	16.7%	15.9%	17.9%	0.97
Vitamin supplementation (%)	17.2%	15.2%	17.9%	0.86
Abstinence (days)	3.4±2.5	3.0±1.4	3.4±1.9	0.24
pH	8.1±0.3	8.1±0.3	8.1±0.3	0.53
Volume (ml)	2.9±1.6	2.7±1.2	2.8±1.0	0.41
Concentration (mlx10^6)	62.5±54.3	59.9±49.2	71.7±45.4	0.37
Progressive motility (%)	53.4±17.3	52.0±16.0	57.0±11.1	0.19
Total motility (%)	60.4±16.8	59.6±16.6	62.9±11.4	0.49
Leukocytes (x10^6/ml)	0.4±0.6	0.4±0.7	0.4±1.1	0.16
Vitality (%)	62.3±15.5	61.3±16.7	65.2±9.7	0.58
Normal sperm structure (%)	0.7±0.8	0.6±0.7	0.8±1.3	0.60
DNA fragmentation (%)	13.3±7.4	14.2±8.3	12.4±7.0	0.56
Apoptosis (%)	19.3±7.6	19.8±9.1	20.0±5.5	0.81
CMA3 positivity (%)	56.0±16.2	57.1±17.6	49.5±18.4	0.16
Abnormal MMP (%)	25.6±16.8	25.5±17.0	29.9±18.3	0.67

P-004 The methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism (rs1801133) might increase the risk of sperm DNA fragmentation

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Study question: Is it possible to determine association between methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism (rs1801133) and impaired structure of sperm DNA and/or general sperm parameters?
Summary answer: Genotypes with the T allele appear to be an additional molecular genetic risk for increased in DNA sperm fragmentation and for worse seminal quality.
What is known already: The 5-methylenetetrahydrofolate reductase is a key regulatory enzyme involved in folate metabolism, critical factor in DNA methylation and spermatogenesis. The change from C to T at the nucleotide position 677 of the MTHFR gene causes the substitution of alanine for valine in the MTHFR enzyme with consequent reduction in its activity. Meta-analyses have associated MTHFR C677T polymorphism and the risk of male infertility. However, the results are still inconsistent, frequently presenting significant heterogeneity between the studies analysed. On the other hand, association between MTHFR C677T polymorphism and damage in sperm DNA structure (fragmentation) has been paucity evaluated.
Study design, size, duration: 348 men from couples who underwent infertility evaluation/treatment was recruited. Pathological conditions of the genital tract, varicocele, azoospermia and vitamine suplemente use were exclusion criteria.

For DNA integrity analysis, the following percentages were determined:

- DNA fragmentation(by TUNEL assay);
- Abnormal chromatin packaging/underprotamination(by chromomycin A3/CMA3);
- Abnormal mitochondrial membrane potential(MMP/by MitoTracker Green);
- Apoptosis(by annexin-V).

The remainder portion of each sample was used for analysis according to the WHO guidelines/morphological analysis by motile sperm organelle morphology examination(MSOME).
Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from each participant and the MTHFR C677T single nucleotide polymorphism (SNP) (rs1801133) was genotyped by real-time PCR TaqMan SNP genotyping assay**.** For comparisons the men were divided into three genotype groups according to their result: CC (n:155), CT (n:141) and TT (n:52). The level of significance was set at $P<0.05$.
Main results and the role of chance: No correlation was observed between MTHFRC677T and age, BMI, abstinence, smoking, or drinking alcohol.

Table 1 MTHFRC677T vs. semen parameters parameters

Parameters	MTHFRC677T Genotypes						
	CC	CT	TT	*P*	CC	CT+TT	*P*
pH	8.0±0.2	8.1±0.3	8.1±0.2	0.37	8.0±0.2	8.1±0.3	0.29
Volume(ml)	2.7±1.2	2.9±1.6	2.8±1.7	0.75	2.7±1.2	2.9±1.6	0.23
Concentration(mlx10^6)	**87.7±64.2[a]**	76.6±51.1	**67.3±51.2[a]**	**[a]0.03**	**87.7±64.2**	**74.1±51.2**	**0.03**
Progressive motility(%)	**57.4±13.4[a]**	54.8±14.7	**51.5±14.3[a]**	**[a]0.007**	**57.4±13.4**	**53.9±14.6**	**0.02**
Total motility(%)	**63.8±12.8[a]**	61.8±15.1	**58.9±14.0[a]**	**[a]0.01**	63.8±12.8	61.0±14.9	0.05
Leukocytes(x10^6/ml)	0.4±0.9	0.5±1.1	0.3±0.3	0.19	0.4±0.9	0.5±1.0	0.28
Vitality(%)	**66.5±10.7[a]**	**62.5±14.2[a]**	**62.4±12.3[b]**	**[a]0.02;[b]0.03**	**66.5±10.7**	**62.5±13.7**	**0.01**
Normal sperm(%)	**0.8±0.9[a,b]**	**0.6±0.7[a]**	**0.5±0.7[b]**	**[a]0.02;[b]0.04**	**0.8±0.9**	**0.5±0.7**	**0.008**
LNV(%)	**32.1±19.0[a,b]**	**37.0±18.1[a]**	**38.5±18.6[b]**	**[a]0.02;[b]0.03**	**32.1±19.0**	**37.7±18.2**	**0.002**
DNA fragmentation(%)	**13.7±7.5[a]**	**16.2±8.2[a]**	**16.3±8.6[a]**	**[a]0.007;[b]0.03**	**13.7±7.5**	**16.2±8.3**	**0.003**
Apoptosis(%)	18.8±7.1	20.2±7.0	20.2±8.9	0.46	18.8±7.1	20.2±8.2	0.10
CMA3 positivity(%)	55.9±16.2	54.9±16.1	56.0±17.7	0.88	55.9±16.2	55.2±16.5	0.82
Abnormal MMP(%)	25.8±17.5	25.3±16.5	24.9±18.0	0.92	25.8±17.5	25.2±16.8	0.82

Despite the lack of an association with sperm apoptosis, protamination and mitochondria damage, males carrying the T allele (genotypes C/T and T/T) presented higher proportions of sperm DNA fragmentation. In addition, T allele was associated with decreased sperm concentration, motility, vitality, and normal forms, and increased proportions of sperm with large nuclear vacuole(LNV:vacuoles occupy >50% of the nuclear area)(Table 1).

Limitations, reasons for caution: This study was conducted in couples seeking fertility treatment and might, therefore, be biased toward infertility. Folate and homocysteine measurements might help in data analysis. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings: Infertile men with high DNA fragmentation and/or poor semen quality should be screened for MTHFR C677T. A folate-rich diet or folate supplementation (e.g.: 5-methyl tetrahydrofolate) should be recommended as part of therapy. Other mutations of the MTHFR gene should also be considered.

Trial registration number: Not applicable. The local ethics committee authorised the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

P-005 The obstructive interval predicts pregnancy chance in post-vasectomy patients undergoing intracytoplasmic sperm injection with surgical sperm retrieval

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Study question: What is the influence of the obstructive interval (OI) on surgical sperm retrieval (SSR) and intracytoplasmic sperm injection (ICSI) in couples with previous vasectomy?

Summary answer: Higher OI reduces the chance of finding epididymal sperm, increasing the necessity of testicular sperm retrieval. Moreover, it reduces blastocyst development, implantation and pregnancy rates.

What is known already: Nearly 6% of the couples with obstructive azoospermia due to prior vasectomy often seek medical care for vasectomy reversal. Although satisfactory pregnancy rates have been demonstrated after reversal, some patients still struggle to achieve pregnancy after reversal or even opt to undergo ICSI with SSR, namely, percutaneous epidydimal sperm aspiration (PESA) and testicular sperm aspiration (TESA). The OI is an important factor to consider, since higher incidence of anti-sperm antibodies and sperm clumping with decreased motility have been reported to occur over time in vasectomized men. The literature addressing the influence of OI on ICSI outcomes, with SSR, is scarce.

Study design, size, duration: This historical cohort study analyzed the medical records of 148 patients (194 cycles) with azoospermia due to vasectomy, who presented for PESA and ICSI, between Jan/2012 and Feb/2017. The sample size calculation revealed that a sample of at least 132 treatment cycles had 95% power to detect a 10% effect (α of 5%). The OI was recorded for each couple. Couples were included only in the presence of isolated secondary azoospermia due to vasectomy.

Participants/materials, setting, methods: This study was performed in a private university–affiliated in vitro fertilization center. General Mixed Models were used to investigate the associations between OI and the outcomes of SSR and ICSI. Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive value of OI on pregnancy achievement. The best cutoff value was defined by Youden's index (J), according to the maximized sensitivity and specificity.

Main results and the role of chance: The obstructive interval was negatively correlated with the presence of sperm (β: -0.032, p: 0.009) and motile sperm (β: -0.031, p: 0.010) during PESA. The need to convert to TESA was significantly influenced by the obstructive interval (β: 0.012, p: 0.003). The obstructive interval did not influence the fertilization rate (β: -0.098, p: 0.747) and the high-quality embryos rates on days 2 (β: -0.001, p: 0.777) and 3 (β: 0.001, p: 0.472). The blastocyst development rate on day 5 was inversely correlated with the obstructive interval (β: -0.011, p: 0.014). Implantation and pregnancy rates were negatively influenced by the obstructive interval (β: -1.107, p: 0.039 and β: -0.016, p: 0.031, respectively). The miscarriage rate was not significantly associated with the obstructive interval (β: 0.006, p: 0.483). The ROC curve analysis demonstrated that the obstructive interval has a predictive value on the achievement of pregnancy (AUC: 0.667, CI: 0.573 - 0.752, p: 0.001). The cutoff value defined by Youden's index demonstrated a negative predictive value on the chance of pregnancy with > 17 years of vasectomy (J: 0.3385, sensitivity: 90.32, specificity: 43.53).

Limitations, reasons for caution: The main limitation of this study is its retrospective design. Although being the largest cohort series, our study is still limited in size when compared with the large cohort of couples undertaking ICSI.

Wider implications of the findings: For the patients planning on being vasectomized, we recommend sperm banking. For those who are already vasectomized, we suggest that they seek for reproductive medical help as soon as the fatherhood desire is felt. The use of ICSI with TESA should be considered when pregnancy has failed with PESA.

Trial registration number: None.

P-006 Male infertility treatment for cancer survivors: Do anticancer agents affect infertility treatment?

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Study question: We investigated whether anticancer agents affected infertility treatment in cancer survivors who consulted our male infertility division.
Summary answer: Anticancer agents did not affect the sperm retrieval rate.
What is known already: The cancer survivors of the reproductive age increase according to the recent advanced cancer treatment. It is well known that cancer treatments are harmful for spermatogenesis.
Study design, size, duration: Cancer survivors who desired to have their own genetic offspring between 2008 and 2018, were enrolled in this study.
Participants/materials, setting, methods: Of 1,525 male infertility patients who consulted our division between 2008 and 2018, 56 (3.7%) were cancer survivors who desired to have their own genetic offspring. Of these, 32 had received anticancer treatment (group A) and 24 were treated with surgery alone or were seen before anticancer treatment (group B).
Main results and the role of chance: The pathology in group A included hematologic cancer (11 cases), testicular cancer (8), colorectal cancer (4), osteosarcoma (3), extragonadal cancer (2), parotid cancer (1), adrenal glioblastoma (1), rhabdomyosarcoma (1), and prostate cancer (1). Group B included testicular cancer (11), colorectal cancer (4), kidney cancer (3), extragonadal cancer (2), lung cancer (1), gallbladder cancer (1), thymus cancer (1), and leukemia (1). Sperm cryopreservation before anticancer treatment was performed in testicular, colorectal, lung, and thymus cancer cases. Semen analysis in the leukemia case in group B revealed azoospermia due to Klinefelter's syndrome. Sperm retrieval surgery was performed in 13 cases in group A and 10 cases in group B. Motile sperm were recovered in 7 cases in group A and in 8 cases in group B. Four healthy deliveries in group A and 2 in group B were achieved and two ongoing pregnancy were observed in group B with intracytoplasmic sperm injection.
Limitations, reasons for caution: The size of our sample was limited in a single-institute setting. A larger sample size is needed.
Wider implications of the findings: Anticancer treatment did not affect successful sperm retrieval in this study. However, some patients abandoned infertility treatment due to the cost of testing and sperm retrieval surgery. Support for the cost of infertility treatment in cancer survivors is necessary.
Trial registration number: not applicable

P-007 Effect of antioxidant supplementation on the sperm proteome in idiopathic infertile men

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Study question: What changes are observed in the sperm proteome after antioxidant therapy in idiopathic infertile men?
Summary answer: The protein profile of spermatozoa before and after-antioxidant treatment are different. There is an upregulation of proteins that are beneficial to spermatozoa after antioxidant treatment.
What is known already: Antioxidant supplementation is one of the treatment option for idiopathic male infertility. Several studies have reported the beneficial effect of antioxidants on semen parameters. However, its role in modulating spermatozoa at molecular level has not been reported. The objective of this study was to evaluate the sperm protein profile of idiopathic infertile men before and after antioxidant supplementation.
Study design, size, duration: A prospective case controlled study was conducted in men with idiopathic infertility. Patients were provided with antioxidant capsules (FH Pro for Men) for a period of 6 months. Semen samples were collected before and after antioxidant supplementation.
Participants/materials, setting, methods: Proteomic profiling of pooled sperm samples from before (n=5) and after antioxidant (n=5) groups were performed using liquid chromatography-tandem mass spectrometry. Ingenuity pathway analysis software was used to select the key differentially expressed proteins (DEPs) associated with normal sperm function. DEPs associated with oxidative phosphorylation (NDUFS1), sperm binding (CCT3) and sperm motility (PRKARA1) were validated by western blot.
Main results and the role of chance: Out of 379 DEPs identified 274 DEPS were overexpressed in men after antioxidant supplementation. Bioinformatic analysis revealed the activation of canonical pathway such as oxidative phosphorylation, and involvement of key upregulated DEPs in spermatogenesis, maturation of sperm, binding of sperm, fertilization and normal reproductive function. Transcriptional factors associated with sperm motility and capacitation (PPARGC1A), and free radical scavenging system (NFE2L2 & HSF2) were functionally activated after antioxidant supplementation. Western blot validation revealed the overexpression of NDUFS1 (6.62 fold change, P=0.0163), CCT3 (5.91 fold change, P=0.0452) and PRKARA1 (4.24 fold change, P=0.0451) after antioxidant supplementation.
Limitations, reasons for caution: Sample size (n=5) is one of the limitation of the study.
Wider implications of the findings: Our proteomic findings suggest antioxidant supplementation of 'FH Pro for Men' in idiopathic infertile men has beneficial effect on sperm function proteins associated with fertility at molecular level.
Trial registration number: None

P-008 Identification of fertility associated proteomics biomarkers in normozoospermic infertile men

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Study question: What are the molecular changes in the spermatozoa affecting the fertility as potential of normozoospermic infertile men?
Summary answer: Differential expression of sperm proteins in normozoospermic infertile men alters the homeostasis of development and function of reproductive system.
What is known already: Up to 30% of men with normal semen parameters are diagnosed infertile and the reason for their infertility is unknown. Change in the expression of the sperm proteins may be a major cause of fertility issues in these men.
Study design, size, duration: Proteomic profiling was performed on pooled semen samples from 20 normozoospermic fertile men and 9 infertile patients with normal semen parameters. Four proteins identified by global proteomic analysis as differentially expressed (SPA17, SERPINA5, ANXA2 and PRDX2) were selected for validation by western blot in individual samples from normozoospermic fertile men (n=10) and infertile men (n=10).
Participants/materials, setting, methods: Global proteomic analysis was performed using liquid chromatography-tandem mass spectrometry. The abundance of each protein was categorized as very low, low, medium or high based on the spectral count. The normalized spectral abundance factor ratio was calculated to categorize the expression profile of differentially expressed proteins (DEPs) as underexpressed, overexpressed or unique to one of the groups. Functional pathway analysis of the DEPs was done using the Ingenuity Pathway Analysis (IPA) software.
Main results and the role of chance: LC-MS/MS detected a total of 1139 and 1095 proteins in normozoospermic fertile and infertile groups, respectively. 162 proteins were identified as DEPs. Canonical pathway related to free radical scavenging was enriched with upregulated DEPs in fertile healthy men. Thus, suggesting the presence of poor antioxidant defense system in normozoospermic infertile men. The proteins associated with reproductive system development and function were overexpressed in fertile men. In addition the proteins related to ubiquitination pathway were underexpressed in normozoospermic infertile men. Western blot analysis revealed the overexpression of SPA17 (3.25-fold change), SERPINA5 (2.39-fold change), and underexpression of ANXA2 (0.49-fold change) in normozoospermic fertile group. Validation of proteins associated with acrosome reaction and fertilization process (SPA17), spermiation and sperm maturation process (ANXA2) and morphology of sperm (SERPINA5) showed that the sperm functions such as hyperactivation,

capacitation and acrosome reaction are compromised in normozoospermic infertile men.
Limitations, reasons for caution: Validation of additional proteins by large sample size is a limitation of the current study.
Wider implications of the findings: We have demonstrated by proteomic analysis defective/ altered reproductive pathways in spermatozoa of normozoospermic infertile men. Our data suggests that SPA17, ANXA2 and SERPINA5 may serve as potential non-invasive protein biomarkers to assess the fertilizing ability of the spermatozoa in unexplained male infertility.
Trial registration number: None

P-009 Comparative sperm proteomics of cancer patients and fertile healthy men using LC-MS/MS

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Study question: Does cancer have an adverse effect on the sperm proteins associated with fertility potential?
Summary answer: Sperm proteome of the men with cancer is different from that of fertile healthy men.
What is known already: Cancer affects the reproductive health of males. Fertility in these men are compromised after cancer treatment. Sperm banking before cancer treatment is the recommended procedure to preserve their fertility. However the effect of cancer on fertility status in these men cannot be explained using conventional semen analysis. Proteomics can identify the subcellular changes and molecular factors related to the fertilization potential of spermatozoa. Therefore, the main objective of the study was to compare the sperm proteome of cancer patients before initiating cancer therapy with that of healthy fertile men.
Study design, size, duration: Cryopreserved semen samples from cancer patients before starting cancer therapy were used in the current study. Type of cancer patients included were: Testicular cancer (n=28), Hodgkin's disease (n=20), Lymphoma (n=8) and Leukemia (n=5). Pooled samples from the cancer patients were used for proteomic analysis. Proteome of cancer group was compared with fertile men (n=7).
Participants/materials, setting, methods: Pooled samples from cancer group and fertile healthy men were run in triplicates on 1D-SDS PAGE. Each lane was cut into six gel pieces. The proteins and peptides eluted from the gel were subjected to quantitative global proteomic profiling using liquid chromatography-tandem mass spectrometry (LC-MS). Proteins and peptides were identified using search programs MASCOT and SEQUEST. The functional bioinformatic analysis of the differentially expressed proteins (DEPs) was done by Ingenuity Pathway Analysis (IPA) software.
Main results and the role of chance: A total of 1138 proteins were quantifed in the cancer and fertile groups. 460 DEPs were identified between the two groups. 208 DEPs were underexpressed and 62 overexpressed in cancer group. In addition, 182 proteins were uniquely expressed in fertile group, whereas only 8 were unique to cancer group. Bioinformatic analysis revealed mitochondrial dysfunction ($P=7.41E^{-24}$),oxidative phosphorylation ($P= 1.31E^{-15}$) and protein ubiquitination pathway ($P=1.51E^{-09}$) as the top most canonical pathways. These findings suggest that sperm function in cancer patients is compromised as a result of defective mitochondrion and ubiquitination system. In addition, upstream regulation analysis predicted that rapamycin-insensitive companion of mTOR was activated (P= 2.54E-29), suggesting that the progression of cancer affects the spermatogenic process in cancer patients.
Limitations, reasons for caution: The study lacks validation of DEPs due to fewer samples with adequate sperm concentration for western blot analysis.
Wider implications of the findings: Our proteomic findings indicate pathways associated with normal physiological sperm function are compromised in cancer patients. Fertility of these patients is at risk due to altered expression of critical sperm proteins. Screening of sperm proteome from cancer patients before treatment helps in the identification of molecular factors dysregulated in spermatozoa.
Trial registration number: None

P-010 Association of sleep quality, bedtime, and sleep duration with semen quality in males seeking fertility treatment

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Study question: Are sleep quality, bedtime, and self-reported sleep duration associated with semen quality in males seeking fertility treatment?
Summary answer: Early bedtimes (<22:30) and sleep durations of 7.5-7.99 hours were associated with higher semen quality. No association found between global sleep quality and semen quality.
What is known already: Relationships between sleep characteristics and semen quality remain poorly understood. One study found an inverse u-shaped association between sleep quality and semen quality. Another study found that the association between subjective sleep quality and semen quality became non-significant when adjusting for sleep duration. Late bedtimes have also been linked to impaired semen quality, and studies on sleep duration and semen quality suggest that an optimal amount of sleep may exist. Of the limited number of studies regarding sleep and male fertility, only one study included males referred to a fertility clinic and did not rigorously investigate sleep quality nor duration.
Study design, size, duration: As a part of a randomized controlled trial, 104 men and their partners receiving treatment in cooperating fertility clinics in Denmark between 2010-2012, completed an online-version of the Pittsburgh Sleep Quality Index (PSQI). The result of the semen analysis (normal/reduced) was self-reported by the participants based on the information given at the fertility clinics, where the semen analysis was measured in according to WHO specifications.
Participants/materials, setting, methods: Only when both the man and his partner agreed that the male had either reduced (56 men) or non-reduced semen quality (48 men) was the male included. Data analyses included non-parametric statistical analyses including bivariate logistic regressions to investigate potential associations between semen quality and global sleep quality, bedtime, and sleep duration. Results are presented as odds ratios (ORs).
Main results and the role of chance: Early bedtimes (<22:30) were associated with better semen quality than both regular (22:30-23:29) or late (≥23:30) bedtimes (OR: 2.75, 95% CI: 1.1-7.1, p = 0.04 and OR: 3.97, 95% CI: 1.2-13.5, p = 0.03 respectively). Conventional sleep durations (7.5-7.99 hours) were associated with better semen quality than very short (<7.0 hours) or short (7.0-7.49 hours) sleep durations (OR: 6.18, 95% CI: 1.6-24.2, p = 0.01 and OR: 1.36, 95% CI: 1.2-12.9, p = 0.03 respectively). No association was found between long sleep durations (≥8 hours) and semen quality (OR: 0.75, 95% CI: 0.6-7.5, p = 0.25). Furthermore, no association was found between optimal (PSQI ≤6) vs. borderline (PSQI 7-8) or poor (PSQI ≥9) sleep quality in relation to semen quality (OR: 1.19, 95% CI: 0.4-3.4, p = 0.75 and OR: 2.43, 95% CI: 0.8-7.1, p = 0.11 respectively).
Limitations, reasons for caution: The relatively small number of respondents may reduce the statistical power, increase the risk of type-2 error, and limit generalizability of the results. The limited response rate (23%) may increase the risk of biased responses. Lastly, sleep parameters were self-reported rather than measured objectively.
Wider implications of the findings: The present study offers further insight into links between sleep quality, bedtime, and sleep duration and semen quality, suggesting potential areas of optimization in relation to treatment of male infertility.
Trial registration number: The original study: Clinicaltrials.gov, trial no. NCT01187095.

P-011 Effect of a high-fat diet on male fertility and sperm physiology in high fertility performance mice

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Study question: Does high-fat diet (HFD) induced metabolic syndrome (MetS) affect male fertility and sperm physiology in mice with high reproductive performance?
Summary answer: Although HFD altered some sperm functional parameters, it did not impair male fertility in high reproductive performance mice.
What is known already: The prevalence of the MetS has increased in alarming proportions, being currently around 20-25% in the global population. The MetS has been defined as an increase in at least 3 of the following factors: abdominal obesity, blood pressure, triglycerides, cholesterol and fasting glucose. Although MetS had been originally associated with advanced age, changes in lifestyle have advanced the appearance of symptoms, coinciding now with the reproductive age. Therefore, the study of the effect of MetS on fertility emerges as a novel area of research.
Study design, size, duration: This basic research study was an *in vitro* and *in vivo* experimental approach involving the use of mouse males from a hybrid strain presenting high reproductive performance. Nine animals were included in both control and experimental groups.
Participants/materials, setting, methods: Three-week-old hybrid (C57BL/6xBALB/c) F1 male mice received *ad libitum* control (CD, 6% fat) or high-fat diets (HFD, 30% fat) for 19 weeks. Food intake and animal weight were determined weekly. Triglycerides, cholesterol, fasting glucose and glucose intolerance were measured at the end of the treatment. The impact of MetS on fertility was evaluated through *in vivo* and *in vitro* fertilization experiments. Sperm count, viability, motility, and acrosome reaction were determined through standard determinations.
Main results and the role of chance: HFD mice ingested a higher amount of fat ($p<0.01$) but less total food ($p<0.01$) and only 12% more calories than CD animals ($p<0.05$), indicating that HFD animals received a poor-quality diet. Since week 13 of treatment, HFD mice gained more weight compared to CD mice ($p<0.001$). At the end of the treatment, serum triglycerides levels were similar in both groups but there was a significant increase in cholesterol ($p<0.001$), fasting glucose ($p<0.05$) and glucose intolerance ($p<0.05$) in HFD mice, compatible with MetS. When fertility was evaluated, there was no significant difference between groups in the *in vivo* fertilization rate or in the percentage of embryos that developed to blastocysts *in vitro*. The male reproductive tracts from both groups were similar under macroscopic observation including testicular weight, although there was a higher amount of gonadal fat in HFD mice compared to controls ($p<0.01$). *In vitro* studies were performed as a more restricted condition to unveil sperm defects. Whereas there was no difference in sperm viability, motility or acrosome reaction between groups, sperm count was lower in HFD compared to controls ($p<0.05$). Finally, *in vitro* fertilization assays showed no differences in either fertilization or embryo development rates between groups.
Limitations, reasons for caution: The use of a mouse model to explore human aspects of male fertility which cannot be performed in human subjects and gametes because of ethical and technical limitations.
Wider implications of the findings: The lack of severe effects of HFD on male fertility in animals with high reproductive performance may have clinical relevance for young men with MetS seeking for pregnancy. A point of caution could be the lower sperm counts in combination with other own individual defects or the partner´s fertility status.
Trial registration number: This study was supported by CONICET (PUE 2017) and Roemmers´ Grant.

P-012 Noninvasive evaluation of testicular function by measurement of oxygen saturation of scrotum-testicular tissue using near-infrared spectroscopy

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Study question: To study whether the scrotum-testicular tissue oxygen saturation (S-T StO_2) measured by finger-mounted near-infrared spectroscopy (NIRS) is useful for the evaluation of testicular function.
Summary answer: S-T StO2 was lower than in almost other measurable tissues and had a significant negative correlation with testicular size and positive correlation with LH.
What is known already: It has been reported that partial pressure of oxygen of testes in mammals is lower than that of other tissues, and that Hypoxia Inducible Factor-1 (HIF-1), the expression of which is induced by hypoxic environments is important for normal testicular function. We used NIRS as a noninvasive method of evaluating oxygen saturation (arterial-venous oxygen content) of testis.
Study design, size, duration: Between Jan 2017 and May 2018, 73 men making their first visit to the Tawara IVF clinic (Shizuoka, Japan) for possible causes of male infertility were recruited for this cross-sectional study.
Participants/materials, setting, methods: Seventy-three patients (average age: 35.2 ± 5.6) with measured S-T StO2 were recruited. The causes of infertility were: 14 patients with non-obstructive azoospermia (NOA), two with obstructive azoospermia (OA), 34 with varicocele and 29 with other causes, including poor semen parameters and sexual problems. Among non-NOA patients, 52.6% (n = 30) had low sperm concentration (one patient had no semen analysis data).
Main results and the role of chance: We found that S-T StO2 was low compared to the oxygen saturation of other measurable tissues. The relationship between S-T StO_2 and the following factors was evaluated by simple linear regression: age ($r = 0.04$), scrotum temperature ($r = 0.04$), and testis size ($r = 0.49$). We found a negative correlation between S-TStO_2 and testis size ($p < 0.05$). Consistent with this, S-T StO_2 was significantly higher in NOA patients who have small testis size than in non-NOA patients (NOA patients: 41.4 ± 6.3%, non-NOA patients: 34.5 ± 4.2%, $p < 0.05$). Furthermore, we found that there was a significant positive correlation between S-T StO2 and LH even in non-NOA patients ($r = 0.34$, $p < 0.05$). On the other hand, S-T StO2 did not differ between patients with low sperm concentration and patients with normal sperm concentration (34.4 ± 4.3 vs. 34.4 ± 4.4), suggesting that S-T StO2 correlated with testicular function rather than sperm production. Finally, we found that S-T StO2 tended to be lower in the left-sided testis than right-sided testis, and varicocele affected the S-T StO2.
Limitations, reasons for caution: This study has some limitations. One is that we could not obtain the cutoff value of S-T StO2 for evaluating decreased testicular function. Another is that the S-T StO2 value could not be compared with the oxygen partial pressure of the testes at the tissue level.
Wider implications of the findings: Male infertility contributes to half of infertility cases. In this study, we found that S-T StO2 may be useful for the evaluation of testicular function and the presence or absence of varicocele. Our noninvasive approach provides a new perspective on the novel male infertility assessment method.
Trial registration number: not applicable

P-013 clinical usefulness of groin color doppler ultrasound (CDU) for artery preservation in microsurgical varicocelectomy (MV)

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Study question: In MV, is it possible to predict the number of arteries to be preserved by groin CDU?
Summary answer: CDU is capable of imaging blood flow in arteries measuring more than 1.5mm in diameter, and 50% in arteries measuring 1 to 1.5mm in diameter.
What is known already: In MV, ligating the spermatic vein and securing blood flow by preserving arteries are key to better outcomes. To preserve arteries, an ultrasonic blood flowmeter (UBF) is useful. Since the number of arteries varied, it is unclear how many arteries can be preserved before surgery. Although there are reports of a technique to preserve only the deferential artery, it

cannot be ignored that testicular arteries also have blood flow in the testis. Several testicular arteries are found in the spermatic cord, and these are difficult to identify, because the spermatic veins surround the testicular artery as the pampiniform plexus.

Study design, size, duration: Patients (19-50y.o. 35.0+/-6.84) with grade 2 and 3 varicoceles (n=85) were candidates for surgery (Lt=65, Rt=1, Bil=19), from April 2018 to December 2018. y.o. Before CDU was performed in an operating room, the number of arteries with a flow was counted. We compared semen parameters and serum hormone before and 3 months after the surgery. Clinical outcome was evaluated in cases where post-surgery progress follow-up was conducted.

Participants/materials, setting, methods: CDU was performed with a 12MHz probe on the external inguinal ring before incision. MV was performed with local anesthesia. UBF was used to preserve arteries. Then, the arteries were counted and their diameters were measured in surgery. The arteries were separated into three groups depending on their diameter. (group A: >1.5mm, group B: 1mm to 1.4mm, group C: <1mm).

Main results and the role of chance: By CDU, the average number of preserved arteries was 2.49 (1-4) in one side. In surgery, the average number of arteries was 4.36 (2-7). The average artery count was 2.17 (1-4) in group A, 1.24 (0-4) in group B, and 1.05 (0-3) in group C.

The number of arteries in group A was smaller than that of total count of arteries by CDU, and the detected rate in group B was 45.6% by CDU.

There is a significant middle correlation between the number of A+B and CDU ($P<0.05$, $r=0.55$).

Sperm concentration (SC) ($19.42x10^6$/ml vs $32.03x10^6$/ml), sperm motility (SM) (41.66% vs 50.07%), progressive sperm motility (PSM) (33.73% vs 42.67%), and total motile sperm count (TMSC) ($23.22x10^6$/ml vs $42.67x10^6$/ml) were significantly improved after 3 months ($p<0.01$). Semen volume (SV) (2.70cc vs 2.73cc) showed no significant change ($p<0.05$).

In semen parameters, testosterone (5.24ng/ml vs 6.25 ng/ml) had changed significantly after 3 months ($p<0.01$). FSH (6.70 mlU/mL vs 6.39 mlU/mL), LH (6.51 mlU/mL vs 5.55mlU/mL), and E2 (19.73pg/mL vs 23.73 pg/mL) had not changed significantly ($p>0.05$).

Improvement rate of TMSC was 84.5%. No recurrence, testicular atrophy or other adverse event was observed after 3 months.

Limitations, reasons for caution: Only the results of semen examination were evaluated. In the future, it is necessary to evaluate clinical outcomes of ART.

Wider implications of the findings: By CDU, it is possible to predict the number of high-flow arteries, and lead to the improvement of semen parameters. Before surgery, predicting the number of arteries can increase the accuracy of surgery, and it can be expected to reduce the stress of the surgeon.

Trial registration number: none

P-014 Evaluation of seminal plasma proteomic profile in patients with secondary infertility

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Study question: What are the differentially expressed proteins in the seminal plasma proteome of men with secondary infertility?

Summary answer: Differential expression of seminal plasma proteins in infertile men with secondary infertility affects the normal physiological functions of spermatozoa.

What is known already: Male factor is estimated to contribute to approximately 50% of all infertility cases. Basic semen analysis is the first step in the assessment of the male fertility potential. However, men with normal semen parameters can be infertile. The seminal plasma protein profile is a reflection of spermatozoa homeostasis. Alterations in the seminal plasma proteome can have adverse effects on the fertilization potential of spermatozoa. The aim of this study was to analyze the seminal plasma proteomic profile of patients with secondary infertility in comparison with that of proven fertile donors.

Study design, size, duration: The study included 10 seminal plasma samples, divided into two groups: 1) proven fertile donors, and 2) patients with secondary infertility (n=5/group). Following basic semen analysis, the seminal plasma was separated from the spermatozoa by high-speed centrifugation.

Participants/materials, setting, methods: Seminal plasma proteins were extracted and quantified. For the comparative proteomic analysis, a pooled sample from each group was analyzed using LC-MS/MS and differentially expressed proteins (DEPs) were identified. To analyze the seminal plasma proteomic profile of each group, a quantitative proteomic analysis by liquid chromatography tandem mass-spectrometry (LC-MS/MS) was performed. Mascot and SEQUEST software were used to search the Human Reference Sequence database. Biological relevance of the identified DEPs was evaluated by bioinformatics analysis.

Main results and the role of chance: Proteomic analysis identified a total of 523 proteins, of which 418 were common to both groups, 4 unique to proven fertile donors, and 101 unique to patients with secondary infertility. Among the 53 DEPs identified, 2 were underexpressed (SEMG1, SEMG2), 38 overexpressed (e.g. ANXA2, C3, C4) and 13 unique to patients with secondary infertility (e.g. HSPA8, APP). Semenogelins help form a gel matrix surrounding ejaculated spermatozoa while ANXA2 is critical for blood-testis barrier integrity and timely release of spermatids. As proteins of the immune system, C3 is involved in membrane fusion during fertilization while C4 is essential to the classical complement pathway. HSPA8 enhances sperm membrane fluidity and increases sperm-oviduct binding ability. APP is involved in cell adhesion, cell motility, cell signaling, and apoptosis. Bioinformatics analysis revealed cellular movement, cell-to-cell signaling, interactions and metabolic diseases, carbohydrate metabolism, and inflammatory response as the processes affected in the top protein interaction networks of DEPs. Additionally, inflammation (complement system), immune response (phagocytosis), and phagosome antigen presentation were the other processes enriched with many DEPs. The main transcriptional regulators were androgen receptor and zinc finger protein 143. Dysregulation of the androgen receptor and the pathways essential for the sperm function affected the fertilization process.

Limitations, reasons for caution: The sample size is a major limitation in this study. The use of only 5 different samples for each group is not enough to overcome the biological variability seen in fertile men and that of infertile males with secondary infertility.

Wider implications of the findings: We have demonstrated that men with secondary infertility have altered cellular pathways. Differences in seminal plasma proteome between proven fertile men and males with secondary infertility suggest that these proteins play an important role in fertility impairment. Seminal plasma proteins are a promising source for identification of secondary infertility-related biomarkers.

Trial registration number: Not applicable

P-015 Clinical evaluation of a fully automated semen analysis system (LensHooke X1 PRO) based on the new AIOM (Artificial Intelligence Optical Microscopic) technology

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Study question: The performance and efficiency of the LensHooke X1 PRO (X1 PRO) semen analysis device was evaluated by the clinical application of precision, accuracy and time.

Summary answer: LensHooke X1 PRO offers excellent accuracy and efficiency in analyzing the bulk semen parameters.

What is known already: The major shortcomings of standardizing manual semen analysis (MSA) are due to the subjective nature of this test and its dependence on the operator (laboratory technologist). The development of computer-assisted semen analyzers (CASA) over three decades ago has allowed partial automation of routine semen analysis but with limited success due to a complicated operation, lack of accuracy at low and high sperm count range and a high cost of virtually all CASA devices.

Study design, size, duration: 1) Compare speed of analysis (volume, pH, concentration, motility and morphology) between MSA and X1 PRO by a single operator on twenty samples, 2) intra observer correlation (48 readings of sperm count and motility from 16 samples; each sample repeated 3 times) and last 3) inter device correlation (100 readings of sperm count and motility from 20 samples and each sample tested on 5 different X1 PRO devices). The study was conducted during August 2018.
Participants/materials, setting, methods: Semen samples from seven volunteers were tested for sperm count and motility by 1) MSA using Leja slides and by 2) a X1 PRO using semen test cassette. The operator of this study was skilled in routine microscopy but has no experience in using X1 PRO. This pilot study approved by the Ethics Committee was conducted at the Cleveland Clinic's Andrology Center.
Main results and the role of chance: A complete semen analysis by X1 PRO device as compared to MSA method took 90% less time (52.5 + 4.8 vs. 5.2 + 0.5 minutes, P< 0.001). Intra observer's precision between the MSA method and X1 PRO was 4.3% (median 3.8% [2.7 - 4.7%]) and 6.1% (median 5.5% [1.5 - 8.7%]) for sperm concentration, 6.1% (median 4.2% [2.8 - 9.0%]) and 7.4% (median 2.9% [1.9 - 12.9%]) for sperm motility, and 6.0% (median 5.1% [2.7 - 7.9%]) and 9.0% (median 2.3% [0.6 - 17.6%]) for progressive motility. The regression correlation (r) of the X1 PRO with manual method was > 0.95 for sperm concentration and motile sperm concentration (MSC). In addition, sperm concentration and motile sperm concentration were both greater than 85% in sensitivity and specificity as assessed by diagnostic reference values (World Health Organization, 2010 Fifth edition). Inter device precision results showed that the precision performance of X1 PRO was 9.2% (median 7.4% [5.3 - 12.4%]) for sperm concentration, 5.8% (median 5.9% [4.1 - 6.5%]) for sperm total motility, 7.1% (median 6.4% [3.7 - 8.7%]) for progressive motility and 13.7% (median 12.4% [10.5 - 16.1%]) for sperm morphology.
Limitations, reasons for caution: The sample size of seven specimens was a limitation.
Wider implications of the findings: The LensHooke X1 PRO is a simple device which offers fast, reliable and reproducible results of semen analysis.
Trial registration number: Not applicable

P-016 Reversal of oxidative stress by antioxidant therapy improves routine and advanced sperm functions

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Study question: Can supplementation with antioxidants reverse a seminal oxidative stress (OS) state?
Summary answer: Antioxidants can be used to treat seminal OS with subsequent significant improvements in conventional semen parameters and sperm DNA fragmentation (SDF) levels.
What is known already: OS has been recognized as an important cause of male factor infertility. It can trigger sperm lipid peroxidation, DNA fragmentation and abortive apoptosis. Oxidation reduction potential (ORP) measures the balance between oxidants and reductants in a given medium. While various measures of OS have been used to examine the effect of antioxidant treatment, ORP has not been particularly investigated. An ORP reference value of 1.38 mvolts/10^6 sperm has been reported previously to discriminate between abnormal and normal semen quality.
Study design, size, duration: This was a prospective study conducted in a tertiary medical centre between January to June 2018 including 148patients presenting with male infertility of >1year duration, at least one abnormal semen parameter and documented normal female partner fertility. Patients with varicocele, leukocytospermia, genitourinary infections, any febrile illness in the past 6 months and exposure to chemo-radiation were excluded from this study. Patients with seminal OS detected by a high ORP level were included in this analysis.
Participants/materials, setting, methods: All participants were treated with the antioxidant supplement FH-PRO (Fairhaven Health, Bellingham, WA) for a period of 3 months. Semen analysis, SDF (sperm chromatin dispersion test) and ORP (MiOXSYS, Aytu BioScience, Englewood, CO) tests were performed initially and immediately following treatment. Results were compared using Kruskal Wallis Test and a p value of <0.05 was considered statistically significant. The SPSS version 20 (IBM, Armonk, NY) was used to conduct the statistical analysis.
Main results and the role of chance: A total of 116 participants with high ORP value of 12.84 ± 1.6 mVolts/10^6sperm were included in this analysis. The study participants' mean age and body mass index were 36.3 ± 0.6 years and 29.8 ± 0.43 Kg/m^2 respectively. Following treatment, the ORP level decreased significantly in 99/116 (85.3%) but were normalized in only 41/116 (35%) patients. Overall, significant improvements were noted in sperm concentration (+9.02 ± 1.41 x106/ml, p<0.001), total motility (+3.97 ± 1.59%, p=0.013), progressive motility (+3.31 ± 0.79%, p<0.001), normal morphology (+1.09 ± 1.99 %, p<0.001) and SDF (-6.03 ± 1.99%, p=0.004). Patients with normalized ORP levels following treatment had better improvements in conventional semen parameters and SDF values than patients in whom the ORP level improved but remained abnormal (Table 1). These differences were statistically significant only for conventional semen parameters.

Variable	Normal ORP posttreatment (n=41)	Abnormal ORP posttreatment (n=58)	P value*
Semen Volume (ml)	-0.04±0.17	-0.27±0.18	0.572
Sperm Count (10^6/ml)	+21.52±2.51	+3.49±1.2	<0.001
Total Motility (%)	+12.54±2.56	+1.2±2.09	0.001
Progressive Motility (%)	+7.90±1.42	+1.72±0.95	<0.001
Normal Morphology (%)	+2.19±0.48	+ 0.82±0.26	0.017
SDF (%)	-8.19±2.5	-5.03±3.33	0.508

SDF: Sperm DN fragmentation **Kruskal Wallis Test*

Limitations, reasons for caution: The study lacked a placebo group which could be considered as one limitation.
Wider implications of the findings: Antioxidant supplements can reverse the seminal OS state and in turn improve semen parameters and SDF levels. There improvements are expected to hold a favorable effect on the male's fertility potential.
Trial registration number: 16351/16

P-017 Can oxidative stress testing in semen predict sperm DNA fragmentation?

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Study question: Can seminal oxidation reduction (ORP) potential accurately predict Sperm DNA Fragmentation (SDF) and thereby serve as a surrogate marker for male infertility?
Summary answer: ORP and SDF are interrelated but they measure independent sperm functions. One test cannot predict or replace the other in the evaluation of sperm function.
What is known already: Oxidative stress (OS) is the underlying cause of sperm dysfunction in 25 to 40% of men with idiopathic male infertility and is known to correlate highly with sperm DNA fragmentation (SDF).
Study design, size, duration: This retrospective study included 1,147 patients presenting with male factor infertility to a tertiary medical center over a period of 18 months. The inclusion criteria were infertility duration of > 1year and normal female partner.

Participants/materials, setting, methods: SDF and seminal OS were recorded for all patients. SDF testing was done using the Halosperm G2 test kit (Halotech DNA SL, Madrid, Spain) with a cut-off of 30%, while ORP was determined using the MiOXSYS system (Aytu BioScience, Englewood, CO) with a cut-off of 1.36mV/10^6sperm. Statistical analysis was performed using MedCalc Statistical Software version 18.10 (MedCalc Software bvba, Ostend, Belgium) using non-parametric tests (Spearman Rank correlation, Mann-Whitney test, Fisher's Exact Test, ROC curve analysis).

Main results and the role of chance: Percentage SDF and seminal ORP correlated significantly positively (r=0.225; P<0.0001) and ORP values differed significantly (P<0.0001; Median: 1.57mV/10^6sperm vs. 2.33mV/10^6sperm) between patients with low and high SDF, respectively. Although the correlations between SDF and ORP were still significant in the individual groups (low/high SDF, low/high ORP), the association between the parameters was markedly lower (r=0.0876 to r=0.209). Fisher's Exact test indicates a significant (P<0.0001) relationship between the two classification factors ((low/high SDF, low/high ORP). However, ROC curve analysis showed that although high ORP can significantly (P<0.0001) predict high SDF with a specificity of 57.9% and a sensitivity of 62.4%, this prediction is only moderate.

Limitations, reasons for caution: The main limitation is the retrospective design of the study however this should not affect the study findings.

Wider implications of the findings: Multiple sperm function tests are still needed in order to evaluate the fertilizing ability of the human spermatozoa.

Trial registration number: n/a

P-018 The sperm chip imitating the cervical environment for high performance sperm isolation from raw semen

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Study question: We designed sperm selection chip which imitating the condition of female reproductive organ and cervical mucus. Can this sperm chip isolate high performance sperm from raw semen?

Summary answer: Similar structure for female reproductive track and viscous media imitating cervical mucus show high quality sperm isolation from semen without any mechanical or chemical treatment.

What is known already: Female reproductive track is known to have a naturally filtering function for selection of high quality sperm from raw semen. This process is not only safer for sperm than artificial sperm retrieval procedures and it is also more efficient for isolate high quality sperm. However, artificial sperm retrieval methods like centrifugation can damage sperm because they cause physical damage.

Study design, size, duration: The sperm chip platform which imitates the structure of the female reproductive organ, was manufactured by laser cutting the polyethylene board(WLH : 3cm X 3cm X 0.25cm). The sperm chip consists of three parts. Startion point(10ul), filtering tract(35ul), end point(10ul). And then, we designed the viscous filtering media for imitating cervical mucus using Ham's F10 and PVP(1%~3%). Normal criteria semen donated by healthy men and approved by IRB from CHA University were used for experiment.

Participants/materials, setting, methods: Sperm chip filled with PVP viscous media has been set as the treatment group. Then we set up the control group which filled with non-viscous media, Ham's F10. Liquefied semen was 10ul dropped in starting point of the platform. After 5min, 10min, 20min, and 30min time point, we collected the sperm from the end point for sperm DNA fragment and kinetics analysis. Each sample was analyzed by cell tracking plug-in ImageJ software for kinetics analysis.

Main results and the role of chance: In this study, we designed a similar female reproductive track condition as sperm chip filled PVP mucus media. It showed the isolation of highly progressive motile sperm from the debris and WBC contaminated semen. PVP filtering sperm chip has a very simple and highly cost-effective method for the motile sperm filtering. Most advanced point of the PVP chip is that there is no need for any mechanical support like the motor system. PVP sperm chip's sperm directly took action without sperm washing and there wasn't any time that was spent on sperm purification. Therefore, sperm that underwent PVP filtering shows a significantly lower number of vacuole sperms than the controlled sorting sperm.

Limitations, reasons for caution: Sperm volume loaded to starting point was very low, so retrieved sperm using the sperm chip was also low. In order for this device to be used in clinical field, the retrieved sperm concentration must be increased and stability verification for fertilization and pregnancy must precede use of this device.

Wider implications of the findings: Sperm chip sperm selection is a more natural and efficient method than the conventional sperm retrieval methods. And sperm chip can retrieve sperm at low cost and easily. In view of this, sperm chip could be used efficiently for intracellular cytoplasmic sperm injection.

Trial registration number: Not applicable.

P-019 The Mu- and Delta- opioid receptors modulate the human sperm acrosome reaction by blocking the calcium channels.

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Study question: Are the Mu- and –Delta- opioid receptors involved in the regulation of human sperm capacitation and acrosome reaction?

Summary answer: Mu- and Delta- opioid receptor agonist do not regulate the human sperm capacitation but inhibit the acrosome reaction by the blocking of the calcium channels.

What is known already: Opioids exert their effect by binding to mu-, delta- and kappa- opioid receptors in human spermatozoa. As it is known, the three opioid receptors are involved in the regulation of sperm motility. Recently we have described the involvement of the kappa-opioid receptor in the inhibition of the sperm motility and acrosome reaction by blocking the calcium channels and producing phosphorylation changes in sperm-specific proteins. However, the role of mu- and delta- in the human sperm capacitation and acrosome reaction, as well the signaling pathways underlying them are largely understood.

Study design, size, duration: We used 100 human seminal samples obtained from the Cruces University Hospital. The isolated sperm cells were used for inhibitory studies of calcium signaling pathways using: Morphine: selective mu-opioid receptor agonist, DPDPE: selective delta-opioid receptor agonist, U73122: Phospolipase C inhibitor, Mibefradil high doses: a calcium channel activator, NNC55-0395: CatSper specific inhibitor. The samples were treated with the different substances for an hour, and the untreated samples (Control) were compared with the treated ones (p<0.05).

Participants/materials, setting, methods: After the treatmens, immunoblotting studies were conducted to evaluate capacitation using the anti-phosphotyrosine antibody and the results were analyzed by densidometry. Acrosome reaction was measured by Flow cytometry using the FITC-anti-CD46 antibody. The statistical analysis was performed using the SPSS Statistics 22 program.

Main results and the role of chance: Morphine and DPDPE did not change the phosphotyrosine levels in human spermatozoa following 1 hour stimulation. However, both agonists inhibited the human sperm acrosome reaction blocking the calcium channels. Morphine and DPDPE were able to blunt the acrosome reaction induced by Mibefradil (p<0.05). The inhibition of the CatSper calcium channel and the Phospholipase C by NNC55-0395 and U73122, respectively had effect on human sperm acrosome reaction, and the agonists had no statistically significant synergic effects.

Limitations, reasons for caution: Further studies are needed to describe the whole signaling pathways underlying each opioid receptor.

Wider implications of the findings: The mu- and delta- opioid receptors participate in the regulation of the acquisition of the fertile capacity in human sperm. This supports the idea that opioid system could be used as a therapeutic target in the finding of new male contraceptives.
Trial registration number: CEISH-UPV/EHU (M10/2016/254).

P-020 A pilot study to identify the level of plagiarism in the most cited andrology related articles

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Study question: What is the level of plagiarism in the most cited articles published in andrology journals?
Summary answer: Evaluation of similarity index using iThenticate revealed moderate to high level of plagiarism in the most cited andrology articles.
What is known already: The act of seizing words, data, processes or even ideas from another person without acknowledging or providing appropriate credit is referred as plagiarism. It is a serious problem that could extensively jeopardize the quality of scientific publication. Though several journals have published reviews on plagiarism in scientific writing, there is no transparency on the permissible/non-permissible level of plagiarism in peer reviewed journals. In fact, there is no report on the extent of plagiarism in andrology related articles. Investigating the level of plagiarism in the published articles would provide a broader picture on the current status of plagiarism in andrology-related articles.
Study design, size, duration: Most cited articles (n=34) listed on 7 andrology journals website were analyzed for similarity index using iThenticate and Turnitin software. The articles were categorized based on year (before & after 2012) and type of publication (review vs. research article). The similarity index was compared using both softwares. Articles analyzed using iThenticate were categorized based on the level of plagiarism (low, moderate, high and very high) and incidence rate determined using arbitrary range for similarity index.
Participants/materials, setting, methods: All articles were analyzed using following inclusion and exclusion criteria:

1. Articles/cross references published before the year of analyzed article were included
2. Original source article being analyzed was excluded
3. Bibliography and all match instances below word count of 10 were excluded
4. Comparison with material available from internet sources were excluded
5. Articles/cross references published on the same year or later of the analyzed article were excluded

Main results and the role of chance: The level of plagiarism detected by iThenticate (23.56% ± 12.46) and Turnitin (19.38% ± 11.83) were comparable with a positive correlation of 0.684. Analysis based on the year of publication revealed no significant difference in plagiarism content in articles published before and after 2012 using iThenticate (26.54% ± 12.95 vs 21.72% ± 12.08, P=0.3476) as well as Turnitin (26.54 ± 11.13 vs 18.62 ± 12.45, 0.5350). Similar results were observed upon analysis based on the type of publications (reviews vs research articles) using iThenticate (22.69 ± 10.97 vs. 25.36 ± 15.55, P=0.8486) and Turnitin (18.87 ±11.53 vs. 20.45 ±12.94, P= 0.7025). The incidence rate of low, moderate, high and very high levels of plagiarism was determined to be 9%, 50%, 35%, and 6%, respectively. The above results were obtained based on an arbitrary similarity index (%) range (≤10, 11-20, 21-50, >50) using iThenticate software. Our results indicate that the level of plagiarism based on similarity index in the most cited andrology articles is moderate to high.
Limitations, reasons for caution: Analysis was performed on limited number of articles. Sample size limits the determination of clear cut-off value for the permissible/non-permissible level of plagiarism in andrology-related publications. Internet sources with similarity index (<1%) were excluded from the report.
Wider implications of the findings: Our research sheds light on the level of plagiarism in andrology related articles. Furthermore, it provides a transparency to researchers about the criteria considered in plagiarism check and helps them to be more cautious while penning their findings or ideas and improve the overall standard of scientific writing.
Trial registration number: Not applicable

P-021 Microfluidic sperm selection by the ZyMōt sperm separation device concentrates sperm with significantly less DNA damage for subsequent ART procedures.

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Study question: Can the ZyMōt™ sperm separation device isolate fractions of sperm with less DNA denaturation from ejaculates of men with normal to abnormal thresholds of sperm chromatin fragmentation?
Summary answer: The DNA fragmentation index (DFI) of sperm retrieved from the ZyMōt™ sperm separation device post-processing was significantly lower than that in the fresh ejaculate.
What is known already: With activation of the paternal genome during embryogenesis, subsequent development of a normal embryo is dependent upon sperm DNA integrity. Studies correlate an elevated DFI with male sub-fertility and reduced probability of a successfully pregnancy. Although DFI analysis of sperm is becoming more routine for male fertility assessment, there have been minimal advancements regarding how to isolate and concentrate sperm from the ejaculate which are within the normal DFI range.
Study design, size, duration: This study was conducted between July 2018 and January 2019 at California Fertility Partners. Fresh semen specimens were voluntarily provided by patients scheduled for DNA fragmentation testing by their referring physician. Sperm from fresh semen and sperm harvested from the ZyMōt™ sperm separation device were flash frozen respectively and sent for DNA fragmentation index determination off-site. At that site sperm were then stained with acridine orange and subsequently sorted by flow cytometry.
Participants/materials, setting, methods: A total of 30 semen specimens were analyzed and a portion of the fresh ejaculate was aliquoted to cryogenic storage vials, frozen rapidly, and shipped to ReproSource for the Advanced Semen Report (ASR 2.0) ™. The remaining semen was added to the ZyMōt™ microfluidic device, incubated for 30 minutes and the harvested sperm were also aliquoted to cryogenic storage vials, frozen rapidly and submitted for DFI analysis.
Main results and the role of chance: In our controlled comparison, the study showed that sperm DNA fragmentation index was significantly different between sperm from fresh semen specimens and post processed sperm via the ZyMōt™ device ($p < 0.0001$). The sperm harvested from the ZyMōt™ sperm separation device demonstrated an average sperm DFI decrease of 20%; 95% confidence interval for a decrease between 15% to 25%.
Limitations, reasons for caution: Further validation with larger patient populations is required to definitively establish the significant difference in sperm DFI, however, our preliminary results establish a trend towards the benefit of using the ZyMōt™ microfluidic device. Additional investigation is also needed to confirm that this difference improves embryonic development and clinical outcomes.
Wider implications of the findings: The present study has indicated a compelling decrease in sperm DNA fragmentation; it is likely that the ZyMōt™ microfluidic device may be beneficial across a broad range of male infertility etiologies.
Trial registration number: Not applicable.

P-022 A pilot study to investigate the sperm zona-adhesion score (SZAS) as a predictor of successful implantation

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Study question: Is it possible to predict the implantation success by evaluating the sperm zona-adhesion ability?
Summary answer: Evaluation of the sperm zona-adhesion ability is predictive for successful implantation.
What is known already: Disordered sperm-zona recognition and adhesion is one of the main causes for male infertility, but cannot be detected by the routine semen analysis. Hence, additional sperm-binding tests have been developed using whole zona pellucida or hemizona. It has been demonstrated that the hemizona binding assay (HZA) could predict the outcomes of ART. However, the existing methods have some drawbacks, such as using different zonae in each test that results in irrelevant sample comparison. In this study the predictive value of an optimized zona-adhesion assay and sperm zona-adhesion score (SZAS) for the implantation outcome was assessed.
Study design, size, duration: This cross-sectional study was carried out at Nadezhda Woman's Health Hospital (Sofia, Bulgaria) between June 2018 and August 2018. Data on implantation success and spermatozoa zona-adhesion ability were collected for 42 couples who fulfilled the inclusion criteria of male infertility factor and good quality oocytes.

The sperm zona-adhesion assessment was carried using acid solubilized zonae pellucidae that were obtained from healthy donors. All participants have signed an informed consent.
Participants/materials, setting, methods: Semen samples were assessed for routine parameters using the WHO-2010 guidelines and were processed for standard ICSI procedure.

Sperm zona-adhesion score (SZAS) was evaluated in samples of 250 000 motile spermatozoa by calculating the number of adhered spermatozoa per mm^2 of surface that was previously coated with acid-solubilised zonae-solution.

Implantation was determined by peripheral blood HCG concentration on day 14 after embryo transfer.

Statistics: Pearson correlation, t-test, receiver operating characteristic (ROC) curves, logistic regression analysis.
Main results and the role of chance: Patients were classified according to the implantation outcome as successful (n=21) and unsuccessful implantation (n=21). There was no statistical difference in the common semen parameters (sperm count, concentration, motility or morphology) between the two groups. Correlation analysis revealed no relation of the evaluated SZAS to the sperm concentration ($p>0.05$), sperm count ($p>0.05$), motility ($p>0.05$) nor morphology ($p>0.05$) among the studied patients. However, the mean SZAS in the successful implantation group was found to be significantly higher (149 ± 67) compared to the unsuccessful implantation group (84 ± 37) ($p=0.002$).

According to the ROC curve, SZAS above the evaluated cut off of 65 predicted successful implantation with sensitivity 95.2% and specificity 52.4% (AUC, 80.2%; 95% CI 0.66-0.94). In addition, SZAS was found to be a significant parameter for the prediction of implantation outcome by logistic regression analysis. The accuracy, specificity and sensitivity of the developed predictive model were 73.8%, 76.2% and 71.7%, respectively.
Limitations, reasons for caution: In this study, results are limited to the implantation stage. Data regarding live birth rates will be available before June 2019. Also increased number of participants are needed to further confirm our finding.
Wider implications of the findings: This study demonstrates the predictive value of the sperm zona-adhesion ability for successful implantation after ICSI in patients with good quality oocytes. The evaluation of SZA score could be useful tool in directing the couples with male infertility to appropriate assisted reproduction therapy.
Trial registration number: Not applicable.

P-023 CD147 deficiency in testes regulates germ cell development but not Sertoli cell-germ cell interaction during spermatogenesis

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Study question: Global depletion of CD147 led to male infertility due to germ cell degeneration. However, whether CD147 has a role in other cell types during testis development and spermatogenesis remains elusive.
Summary answer: Through conditional knockout of CD147, we revealed that CD147 was only essential for germ cell development but not for Sertoli cells and Sertoli-germ cell interactions.
What is known already: Previous studies have demonstrated the germ cells degenerated in CD147 null mutant testis and the ectoplasmic specialization was not intactness. Our previous studies also suggested that CD147 regulated spermatocytes apoptosis but not spermatogonia.
Study design, size, duration: Laboratory-based investigation
Participants/materials, setting, methods: Floxed CD147 mice were crossbred with Amh-Cre and DDx4-Cre mice to produce offspring with the genotype of CD147 $^{-/-}$;AMH-Cre and CD147 $^{-/-}$;DDx4-Cre. Testis at various time-point were isolated for histological and western blot analyses. Primary Sertoli cells isolation from testes and western blot were performed to confirm the knockout effect of CD147 in the CD147$^{-/-}$;AMH-Cre mice.
Main results and the role of chance: In the testes of CD147 $^{-/-}$; AMH-Cre adult mice, all of the different stages of germ cells which including spermatogonia, spermatocytes, spermatids and spermatozoa were observed in the seminiferous tubules, suggesting the normal spermatogenesis occurred. The sperm concentration and sperm motility were no significant differences in all genotypes. Consistent with the results of CD147 null mutant mice and our previous studies, significant decrease of the testes weight and morphology in CD147$^{-/-}$; DDx4-Cre mice were observed compared to the testes of WT mice. Furthermore, developing germ cells were arrested at the pre-meiosis phase and apoptotic cells were observed in the CD147$^{-/-}$; DDx4-Cre testes. These results suggested CD147 deletion in Sertoli cells did not affect testis development and Sertoli cell-germ cell interactions.
Limitations, reasons for caution: The mechanism underlying the CD147 depletion induced germ cell arrest still needs further investigation.
Wider implications of the findings: Our results not only validate those from global CD147 KO studies, but also indicate that CD147 might be involved in the progression of azoospermia.
Trial registration number: No clinical trials.

P-024 Outcome of sperm selected by microfluidic technique in high DNA fragmentation index sperm samples.

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Study question: Does sperm selected by microfluidic sorting technique helps in improving reproductive outcome in 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 and reduced miscarriage rate.
What is known already: DNA damage is not recognisable 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 like swim up and density gradient are associated with centrifugation, which may induce reactive oxygen species and DNA damage in sperm. Microfluidic based sperm sorting allows for the selection of highly motile, morphologically normal sperm from an unprocessed specimen. Using human semen samples, it has been demonstrated that microfluidic sperm sorting technique could provide sperm with significantly reduced percentage of sperm with DNA damage.
Study design, size, duration: A prospective randomised control study was conducted from 1st August 2017 to 31st December 2018. One hundred and ninety eight 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 while in group B (n=98) sperm were processed by density gradient technique and morphologically normal motile sperm were injected by Intracytoplasmic sperm injection (ICSI) technique in all mature oocytes.

Participants/materials, setting, methods: During the study period all normozoospermic patients with high DNA fragmentation index (>25%) were included in the study, while oligospermic, asthenozoospermic samples, patients with poor ovarian reserve and advanced maternal 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. 38%, p-0.003), clinical pregnancy rate (62% vs. 41%, p-0.004) while a significant decrease in miscarriage rate (12% vs. 25%, p-0.028). On the other hand there was no statistical difference observed in fertilisation rate (82% vs. 78%, p-0.48).

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 is not only correlated with better DNA integrity but also with the reproductive outcome. Using it in routine practice can help in reducing the extraneous effect of sperm processing techniques and achieving higher pregnancy rate and sustaining it.

Trial registration number: MCDH/2017/35

P-025 Cap-Score™ accurately predicts the probability of generating pregnancy across maternal age stratifications

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Study question: Do predicted probabilities of generating pregnancy based on the Cap-Score male fertility assay differ from observed outcomes even when stratified by maternal age?

Summary answer: In women eligible for intrauterine insemination (IUI), Cap-Score remains predictive across maternal age stratifications, with predicted and observed clinical pregnancy outcomes matching closely.

What is known already: Sperm must capacitate to fertilize. Cap-Score, which quantifies capacitation status to functionally assess male fertility, was prospectively shown to predict pregnancy. Based on clinical pregnancy outcomes from IUI patients at five fertility clinics and across a wide age range, the relationship between Cap-Score and the probability of generating pregnancy (PGP) was previously defined. However, maternal age is linked with reduced fertility. The ability of Cap-Score to predict PGP across stratified maternal ages is unknown and tested here. IUI was chosen as an experimental model since the number and timing of inseminations relative to ovulation could be documented and controlled.

Study design, size, duration: Data were collected (11/2016-09/2018) from 175 couples who generated a pregnancy within, or completed, 3 rounds of IUI, and 18 couples who became pregnant through natural conception (NC). Relationships between maternal age and PGP were tested using analysis of variance (ANOVA). Differences between predicted and observed pregnancy rates, and age and outcome, were examined using Chi-Square analysis. The potential relationship between Cap-Score and delivery or miscarriage was also evaluated on a preliminary basis (t-test).

Participants/materials, setting, methods: Semen was collected as part of a standard fertility evaluation at 5 different centers. Samples having fewer than $10x10^6$ motile sperm were excluded. Fixed specimens were shipped overnight to Androvia, where the Cap-Score assay was performed. Only female fertility that would preclude attempts at IUI led to exclusion, resulting in a representative test population of patients pursuing IUI across age ranges.

Main results and the role of chance: Observations were separated into the following age groups: ≤29, 30-34, 35-39, and ≥40. There was no relationship between outcome and age group (p=0.5). The average PGP derived from the Cap-Scores (predicted, PRED) and the observed pregnancies (OP) in each group were, respectively: ≤29 (35% PRED, 26% OP, n=27); 30-34 (36% PRED, 38% OP, n=87); 35-39 (34% PRED, 38% OP, n=53); and ≥40 (39% PRED, 50% OP, n=8). There were no differences between observed and predicted pregnancy rates in any maternal age group (p=0.431, 0.626, 0.472, and 0.317, respectively). Cap-Scores and resultant PGPs reflect male fertility and did not differ across maternal age stratifications (ANOVA p=0.677).

Preliminary data from one center from 20 couples pregnant by IUI (65% live births; 35% miscarried) and 18 by NC (61% live births; 39% miscarried) were also evaluated to determine if live births were similar between high and low Cap-Scores. There was no difference in Cap-Score between miscarriages and live births in either the IUI (p=0.226) or NC groups (p=0.982). The role of chance is increased when evaluating data from a single center versus multicentric studies, and when evaluating smaller versus larger datasets.

Limitations, reasons for caution: Caution is needed when evaluating smaller datasets and those from a single center. Women over 40 had the smallest sample size and thus the greatest risk of stochastic impact. Preliminary data suggest no relationship between Cap-Score and miscarriage. More data from multiple clinics are needed to address this issue definitively.

Wider implications of the findings: Female age and fertility are indisputably linked; however, if eligible for IUI, then PGP based on Cap-Score accurately predicted outcomes even when stratified by maternal age. This likely reflects that PGPs were originally quantified based on actual clinical pregnancy outcomes collected across a representative age range.

Trial registration number: not applicable

P-026 Longitudinal analysis of patients who eventually achieved live birth after micro dissection testicular sperm extraction (micro TESE) and ICSI at a single fertility center

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Study question: How many attempts including embryo transfers(ET) are required to give birth to a first child in micro TESE-ICSI couples, and a there more congenital anomalies?

Summary answer: The maximum and median number of oocyte retrieval and ET cycles were 9 and 1, 10 and 2.5, respectively, and congenital defect rate was 4.0%.

What is known already: Micro TESE-ICSI provides a high therapeutic effect for non-obstructive azoospermic (NOA) couples, however the condition of retrieved testicular sperm often affects treatment efficacy. Because the background of NOA patients is various and, in many cases abnormal and/or immotile sperm are utilized, many couples who were subjected to micro TESE-ICSI feel that children from testicular sperm may have a higher birth defect rate than ejaculatory sperm.

Study design, size, duration: This retrospective study was conducted with infertile couples who underwent micro TESE, including unexplained NOA, Klinefelter's syndrome, after orchidopexy, azoospermia factor (AZF) c microdeletions, cryptozoospermia, severe oligozoospermia with successful acquisition of sperm by micro TESE, consequently obtained at least one live-born between September 2013 to December 2017. Patients were followed longitudinally during consecutive ICSI and embryo transfer cycles with testicular sperm.

Participants/materials, setting, methods: The maternal age at the time of micro TESE-ICSI was 32.8±3.1 years and their average AMH levels was 4.9±3.8 ng/ml. We assessed the maximum and median numbers for micro TESE, total oocytes used for ICSI, transplanted embryos, oocyte retrieval cycles, embryo transfer (ET) cycles, respectively. Time spent for ICSI per oocyte, total time required to find sperm were also evaluated. Finally, we examined sex, average birth weight, and congenital anomaly rate in 177 newborns.

Main results and the role of chance: The maximum and median numbers for micro TESE were 3 and 1, total oocytes for ICSI were 85 and 14, transplanted embryos were 15 and 3, oocyte retrieval cycles were 9 and 1, and ET cycles were 10 and 2.5, respectively. The maximum and median minutes of time spent for ICSI per oocyte were 76 and 8, total time to find sperm for ICSI was 1482 and 136, respectively. A case where only immotile testicular sperm were available for ICSI even after pentoxifylline treatment obtained a healthy baby. Of the 177 children including 10 cases of twins, the sex of the children was 97 males (54.8%) and 80 females (45.2%). The average weight was 2691±626g, and congenital defects were confirmed in 7 children (4.0%). These include atrial septal defect, ventricular septal defect and hydronephrosis. The incidence of the birth defects was not different from that of ejaculated sperm ICSI (2.7%). The congenital defects were not observed in cases with AZFc microdeletions. In this group of patients, the sperm searching time for ICSI was the longest among NOA cases.
Limitations, reasons for caution: Since this study is a single center retrospective observational study, large cohort study is necessary. Female factors that may be attributable for clinical outcome were not fully evaluated.
Wider implications of the findings: A very severe male factor infertility implementing such as micro TESE-ICSI can be devastating for couples who are uncertain about their ultimate prognosis. This study will help us to predict outcomes and provide information for NOA couples.
Trial registration number: N/A

P-027 Evaluate effect on sperm DNA fragmentation, semen parameters of empirical supplement in single-arm cohort study on sub-fertile male partner with primary or secondary infertility

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Study question: Study whether sperm parameters and sperm chromatin integrity is affected post 3 month usage of empirical supplement in sub-fertile males and with any adverse outcomes.
Summary answer: DNA Fragmentation Index reduction by 57% observed in supplement cohort and no significant differences seen in sperm motility, morphology or concentration with no adverse outcomes
What is known already: Semen impairment and DNA damage in ejaculated spermatozoa has its source in testicular micro-environment, epididymal transit and seminal tract antioxidant capacity, affecting paternal genetic content adversely. Once the required reactive ion threshold is crossed in either of these checkpoints, single and double strand nicks in DNA cause irreparable damage. Current adjunct therapy of male sub-fertile patients is with empirical antioxidants with little data on its effects on fragmented sperm DNA combined with semen parameters and whether any adverse outcomes result.
Study design, size, duration: Prospective single arm cohort study was designed as single group continuous endpoint with calculated sample size (n=48) between Feb 2017- Sep 2018; over 19 months for patients opting for over the counter supplement. Considering loss to follow up, n = 58 patients were recruited who partook bi-diurnal dose of one tablet and repeat SCD and SFA was done post 3 months. Eight patients were lost to follow up and (n=50) cohort study data was analysed.
Participants/materials, setting, methods: Men aged 23- 44 years with primary or secondary infertility were analysed for sperm DNA fragmentation index (DFI) by sperm chromatin dispersion assay (SCD) and for concentration, motility & morphology by semen analysis testing (SFA) by W.H.O laboratory manual (fifth edition). Patients with >15% DFI and normal or impaired semen parameters were included. Subjects with endocrine disorders, autoimmune disease, secondary antioxidants, azoospermia, testicular cancer were excluded. Analysis of ejaculate was done according to W.H.O guideline.
Main results and the role of chance: The statistical power of the study is 80% with alpha = 0.01, beta = 0.2. DFI by SCD assay in sample before beginning regimen , termed period T_0 and post 3 months as period T_1 are statistically very significant ($p < 0.001$),by Mann-Whitney U test with Bonferri -Holm correction. DFI was lowered from median 21% +/- 8.63 (T_0) to median 9 % +/- 2.94 (T_1) post treatment showing absolute 57% reduction in DFI. There was no statistical difference in three other predetermined semen parameters, evaluated for multiple comparisons by Mann-Whitney U Test with Bonferri-Holm correction. The difference was non-significant in progressive sperm motility (p=1), sperm concentration (p=1) or morphology p=1), conducted in triplicate by two experienced andrologists in those two periods. The response rate of oral supplement was, in this cohort study was 100% with every treated patient showing reduction in DFI but we did not observe any effect on semen analysis parameters. All tests are done counting 400 spermatozoa, conducted in triplicate and with two experienced technicians.
Limitations, reasons for caution: Limitations is in being a Single Arm study, using a standard DFI threshold which is varying in different publications and use of SCD assay which suffers with non-standardization .Use of Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labelling or the TUNEL assay. recently acquired. shall be added in follow up studies.
Wider implications of the findings: Results show lower DFI at T_1 with no reported adverse outcomes or cases of unintentional harm to patient andrological parameters. The findings agree with the overall review of meta-analysis on effect of antioxidants on sperm DNA Fragmentation as seen in a Cochrane review of Antioxidants in Male Subfertility (2015).
Trial registration number: N/A

P-028 Tail swelling pattern in hypo-osmotic solution as a predictor for human spermatozoa with DNA fragmentation

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Study question: Does the hypo-osmotic swelling test (HOST) have a significant correlation with sperm DNA fragmentation (SDF) level after sperm preparation?
Summary answer: SDF level was significantly lower in HOST type 'd' followed by 'g' type and 'f' type.
What is known already: HOST is a non-invasive technique to select a live single spermatozoon without DNA damage. When semen samples are incubated in a hypo-osmotic solution, the specific swelling pattern of the sperm tail can be further classified as six types. The presence of any swelling ('b' - 'g') represents healthy cell membrane function, thus considered to be viable. Sperm vitality measured by the HOST in raw semen (HOST score) has been reported to be correlated with sperm motility, percentage of normal form, and SDF level.
Study design, size, duration: A prospective study was performed from 2016 to 2017. Semen samples were obtained from 21 healthy donors. Tail swelling pattern and head halo pattern were assessed in 200 spermatozoa per slide. In each person, 10 slides were prepared thus the final counted spermatozoa was 2,000 per person.
Participants/materials, setting, methods: The semen samples were thawed and centrifuged, then processed by using the swim-up method. HOST was performed and for sperm chromatin dispersion (SCD) test, a Halo sperm kit was used. Each sperm head was counted as big halo, medium halo, small halo, no halo, or degraded. Spermatozoa with 'small halo', 'no halo' or 'degraded' were considered as spermatozoa having fragmented DNA.
Main results and the role of chance: The median HOST score was 16.7% (interquartile range: 10.4% - 20.7%) and the median SDF level was 1.40% (interquartile range: 0.65% - 2.83%). The HOST score was not related with age of male, sperm concentration, total motility, or the percentage of normal form. And the SDF level was not related with age of male, sperm concentration, or motility, but negatively correlated with the percentage of normal form (Spearman's coefficient of rank correlation r = -0.473, p=0.034).

The HOST score was not related with the SDF level, but the probability of choosing sperm without DNA fragmentation is highest in 'd' type spermatozoa (100%) followed by 'g' type (98.67%) and 'f' type (98.17%).
Limitations, reasons for caution: At the time of semen collection, Marriage or fertility status of sperm donors, their medical or surgical history, and prescribed medication were unknown. Another limitation is that we have a relatively small number of 'd' type spermatozoa.
Wider implications of the findings: The application of HOST may be a valuable tool in the routine identification and selection of viable,

DNA-intact individual spermatozoa for ICSI after further research to demonstrate its efficacy and safety.
Trial registration number: Not applicable

P-029 sperm motility, seminal l-carnitine and sperm fatty acids

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Study question: Do seminal L-carnitine and sperm fatty acids correlate with sperm motility?
Summary answer: Sperm fatty acids (FA) and seminal L-carnitine (LC) could be predictors for sperm motility.
What is known already: LC is a small water-soluble molecule derived from lysine. The main function of carnitine is to transfer long-chain fatty acids (FAs) to mitochondria for β-oxidation. Although a considerable amount of data is currently available on lipid metabolism and L-carnitine in somatic cells, there are few studies on LC and FAs in spermatozoa. It has been suggested that endogenous FAs of spermatozoa may represent an energy source for the cell. However, little is known about endogenous sperm FAs as a source of ATP.
Study design, size, duration: The samples had previously been obtained from men who participated in a study of semen quality related to BMI (Andersen et al., 2016). Men (n=128) aged between 22 and 61 years were included. Semen samples were collected in the period 2008-2013. Semen analysis was performed according WHO guidelines (2010). Seminal LC was analyzed in samples stored at -80 °C.
Participants/materials, setting, methods: Only semen samples produced after sexual abstinence of 2–7 days prior to sample collection were included. FA content of spermatozoa was assessed by gas chromatography while seminal LC analysis was performed on high performance liquid chromatography. FA levels are expressed as weight percentage (wt%) of the total FA, seminal LC as µg/ml and sperm progressive motility (PR) as percentage (%).
Main results and the role of chance: Associations between PR, spermatozoa FAs and seminal LC adjusted for age and sexual abstinence time were tested using Pearson correlation analysis. PR correlated positively with seminal LC ($r=0.364$; $p<0.001$), total polyunsaturated FA in spermatozoa ($r=0.448$; $p<0.001$), n-3-polyunsaturated FA (n-3-PUFA) ($r=0.463$; $p<0.001$), docosahexaenoic acid (DHA) ($r=0.462$; $p<0.001$) and palmitic acid (PA) ($r=0.251$; $p<0.05$). A stepwise multiple regression analysis was performed to predict PR based on age, days of sexual abstinence, BMI, seminal LC, DHA and PA. The total variance explained by the model for sperm motility was 40%, $F(6,90)=10.018$, $p<0.001$, thus $R^2=0.4$. Three independent variables were statistically significant: LC (unadjusted B 17.16; SE 5.9; $p<0.01$), DHA (unadjusted B 0.032; SE 5.9; $p<0.001$) and age (unadjusted B -0.643; SE 0.202; $p<0.01$).
Limitations, reasons for caution: The sample size is relatively small.
Wider implications of the findings: FAs and seminal LC could be predictors for sperm motility. The positive associations between sperm FAs, sperm motility and seminal LC indicate that FAs might be metabolic substrates for the spermatozoon, although it is not known if sperm membrane FAs, the major part of sperm FAs, contribute to energy metabolism.
Trial registration number: Not applicable.

P-030 Novel homozygous pathogenic variants in CCDC103 identified in patients with situs-inversus-totalis and absence of axoneme dynein arms: further insights on reproductive function

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Study question: To study CCDC103 expression profiles in reproductive and other tissues, and genotype-phenotype correlations in *situs-inversus-totalis* patients carrying novel homozygous *CCDC103* variant
Summary answer: CCDC103 expression showed tissue-specific features, suggesting distinct tissue regulation. *CCDC103* variants led to absence of axoneme dynein arms (DA) and affected gene and protein expression.
What is known already: Primary ciliary dyskinesia (PCD) is due to genetic anomalies causing axoneme defects, being characterized by chronic respiratory infections, infertility and organ laterality defects. We previously described an infertile male patient with *situs-inversus-totalis,* absence of sperm DA and presence of a novel missense homozygous *CCDC103* variant. This gene was previously associated with the genetic aetiology of PCD and its role in DA assembly, being required for microtubule structure stabilization.
Study design, size, duration: To study CCDC103 expression profiles we collected sperm (SZ), testicular germ cells (GC), Sertoli cells (SC), oocytes and somatic cells (nasal and white blood cells), from human healthy individuals. To understand patient genotype-phenotype correlations, two patients were included in the present study, an infertile PCD male patient (Patient-1) and a female PCD patient (Patient-2), both with situs-inversus-totalis. Two different homozygous CCDC103 gene were identified, a missense change in Patient-1 and a frameshift in Patient-2.
Participants/materials, setting, methods: Quantitative PCR (qPCR), western-blot (WB) and immunofluorescence (IF) were performed in SZ, GC, SC and somatic cells. For oocytes, only qPCR was performed. Axonemes of nasal cilia of both patients were analysed by transmission electron microscopy. As previously performed for Patient 1, we also used whole-exome sequencing (WES) analysis in the Patient 2. qPCR, WB and IF were performed in both patients and compared to healthy controls. Biological samples were obtained after patient informed consent.
Main results and the role of chance: Here we firstly report that *CCDC103* is expressed at different levels in reproductive tissues and somatic cells. In controls, different mRNA expression levels were found, with the highest values detected in SZ. WB showed the presence of dimers and higher-order oligomers whose sizes are tissue-specific, suggests that *CCDC103* possibly express different mRNA transcripts in each cell type or have post-translational modifications that are tissue specific. Regarding immunolocalization, CCDC103 was restricted to the mid-piece of SZ and present in the cytoplasm of the other cells. Besides the former described missense variant in Patient-1, a *CCDC103* novel homozygous frameshift variant was found in Patient 2. Both patients evidenced absence of DA and nexin links in the axonemes, which suggests that regardless of the variant location and type, *CCDC103* pathogenic variants lead to *situs-inversus-totalis* and absence of DA. Moreover, both patients presented reduced *CCDC103* mRNA expression and reduced labelling, although more prominent in cells of Patient 2. Overall, our data provides additional evidences for the *CCDC103* involvement in PCD aetiology. Further, it suggests that CCDC103 may have different assemblies and roles in cilia and sperm flagella biology that are still unexplored.
Limitations, reasons for caution: CCDC103 possesses specific biochemical characteristics making WB experiences especially difficult. Although cases with *situs-inversus-totalis* are rare, the main limitation of our study was the reduced number of patients. Further studies on animal models are needed to better understand these genotype-phenotype correlations, namely the role of CCDC103 in human fertility.

Wider implications of the findings: Our work increases the knowledge regarding the expression and sub-cellular location of the CCDC103 protein. Such data constitutes an additional piece to the complex puzzle that constitutes the process of axonemal dyneins assembly and ultimately to understand concerning PCD pathophysiology

Trial registration number: 'not applicable'

P-031 Impact of severe male factor on cumulative live birth rates during ICSI-cycles with preimplantation genetic testing for aneuploidies.

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Study question: Does severe male factor (SMF) impact the cumulative live birth rate (CLBR) during ICSI-cycles with preimplantation genetic testing for aneuploidies (PGT-A)?

Summary answer: CLBR is mainly dependent on maternal age and number of inseminated oocytes, however non-obstructive azoospermia (NOA) seems to impact this outcome.

What is known already: Severe oligoasthenotheratozoospermia (OAT), obstructive azoospermia (OA) and NOA have been previously reported to significantly affect fertilization rate and oocyte developmental potential to blastocyst. However, no hard evidence of a similar impact on blastocyst euploidy rate and/or LB after euploid single embryo transfer (SET) exist. Similarly, little is known about CLBR according to SMF. In this study, we attempted to quantify the putative impact of SMF, if any, on CLBR during PGT-A cycles conducted at the blastocyst stage through comprehensive chromosome testing.

Study design, size, duration: Observational cohort study involving 2396 ICSI-cycles with qPCR-based PGT-A (April-2013 to September-2017). Vitrified-warmed euploid SETs up to April-2018 were included. The primary outcome was the CLBR per oocyte retrieval among ended cycles. A cycle was considered ended when the patient delivered or did not deliver after transferring all euploid blastocysts (if any) obtained. Main confounders were age, kind, duration and cause of infertility, PGT-A indication, sperm factor, number of oocytes, kind of incubator and media.

Participants/materials, setting, methods: Ovarian stimulation (recombinant-gonadotrophins in an antagonist protocol), blastocyst culture and biopsy, and vitrification were conducted. In case of OA or NOA, sperm were retrieved by fine-needle-aspiration (FNA) or testicular-sperm-extraction (TESE), respectively. PGT-A cycles were sub-clustered according to maternal age at retrieval (<36yr, 36-38yr, 39-41yr, and ≥42yr) and sperm factor (normozoospermia-N, moderate male factor-MMF, OAT, OA and NOA). Logistic regressions were conducted. Lastly, a decision tree was built to predict CLBR based on significant confounders.

Main results and the role of chance: Mean maternal and paternal age were 39.5±3.3yr (range:23-45) and 41.8±5.6yr (range:26-65), respectively. Maternal age in OA- and NOA-group was significantly lower than N-group (37.8±3.2, 36.5±4.6 and 39.8±3.2, respectively; p<0.01). Indeed, it biased the overall CLBR, which resulted similar among the 5 groups (27.4% (n=291/1063), 29.6% (n=222/751), 33.4% (n=120/359), 37.2% (n=16/43) and 25.0% (12/48) in N, MMF, OAT-s, OA and NOA; p=NS). If CLBR was sub-clustered according to maternal age at retrieval, it resulted significantly reduced in NOA- compared to N-group for women <36yr (n=6/19, 31.6% versus n=59/104, 56.7%; p=0.05). This was probably due to the greater effect of woman aging in women ≥36yr, which might have hindered the negative impact of NOA itself. Logistic regressions indeed outlined that the CLBR is highly dependent on maternal age (OR=0.79, 95%CI:0.77-0.82; p<0.01) and number of inseminated oocytes (OR=1.15, 95%CI:1.12-1.18; p<0.01). Among the sperm factor groups, only NOA corrected for these confounders showed a negative association with the primary outcome when compared to the N-group (OR=0.24, 95%CI:0.11-0.52; p<0.01). A decision-tree to predict CLBR in this dataset was then built based on maternal age at retrieval, number of inseminated oocytes and sperm factor group. However, to date, this last variable was disregarded from the model.

Limitations, reasons for caution: CLBRs could be higher since in 5.5%(132/2396) of the cycles a pregnancy has not been achieved yet but there is still ≥1 euploid blastocyst left. Moreover, only the first LB is accounted in the analysis. Lastly, the sample size in the groups of ICSI-cycles with surgically-retrieved sperms should be increased.

Wider implications of the findings: A multicenter study with a larger NOA population is desirable to confirm the general value of these data. NOA might represent the group of patients achieving the highest benefit in terms of CLBR per cycle due to the maximization of oocyte yield after ovarian stimulation.

Trial registration number: None

P-032 Home sperm freezing kit achieves same sperm quality after thawing.

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Study question: Are there any differences in terms of quality of the seminal sample depending on whether it has been cryopreserved by patients or by experienced biologists?

Summary answer: Semen quality after thawing does not decrease if the sample has been frozen by the patient.

What is known already: Sperm freezing with reproductive purposes is indicated in several causes: in order to preserve fertility in oncology patients, before a vasectomy, for patients with sexual dysfunction and problems to obtain a sperm sample, and for those patients who cannot be present on the day of the In vitro fertilisation (IVF). The male is compelled to deliver the sample at an exact moment and, often, also in a determined setting, usually in a clinic. Many experience this as a stressful situation that, furthermore, obliges them to travel if they happen to live far from the IVF clinic.

Study design, size, duration: Prospective study. From November 2016 to March 2017 41 volunteers delivered a sperm sample in rooms adjacent to the laboratory. A biologist carried out a first evaluation and froze a 0.5 ml aliquot. The remaining of the sample was then given back to the volunteer with a specific freezing protocol using a kit designed by us. This kit allows the patient to freeze a sperm sample at home and be sent later to IVF centre.

Participants/materials, setting, methods: Samples frozen by volunteers(study) and aliquots frozen in parallel at the laboratory by expert biologists(control) were analysed before and after thawing. In both groups an evaluation of the following was carried out: sperm concentration and motility with Makler chamber, morphology according to Kruger criteria, vitality by using eosin-nigrosin staining, DNA sperm fragmentation with TUNEL test and bacterial growth after 24 hours of medium incubation. Wilcoxon signed-rank test was used to analyse differences among groups.

Main results and the role of chance: Regarding median progressive motility percentage recovered after thawing no significant differences between both groups were observed: 45.99% (range 2.2-119.9) versus 44.52% (range 18.2-90.9) respectively for control and study groups.

Nor significant differences among groups were found regarding median of vitality percentage: 86% (range 69-96) versus 79% (range 62-99); of morphology: 4% (range 1-7) versus 5% (range 1-9) and of DNA fragmentation rate: 9.14% (range 2.49-17.59) versus 5.18% (range 1.89- 17.16), respectively for control and study groups. After 24 hours of medium incubation, no bacterial growth in any sample was observed.

Limitations, reasons for caution:

After the shipment of the kit, it has to be delivered within 48 hours. Once frozen, the sample should reach the laboratory within 48 hours. A courier specialized in handling biological samples is required.

Wider implications of the findings: Semen cryopreservation protocols from commercial companies, when strictly followed by biologists, are so simple that could be carried out by non- experts obtaining similar results. Our freezing kit allows the patient to cryopreserve his sperm samples outside

the IVF centre in an efficient way, following a simplified cryopreservation protocol.

Trial registration number: Doesn't apply

P-033 Designing and testing a microfluidic chip for sorting spermatozoa from somatic cells to shorten the handling time of testicular biopsies for azoospermic males.

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Study question: Can a microfluidic chip be used for sorting spermatozoa from erythrocytes for use in intracytoplasmic sperm injection (ICSI) after a testicular biopsy?

Summary answer: Our microfluidic device can extract 95% of the spermatozoa from a 200µl sample, while removing 90% of the erythrocytes, within a 20 minute processing time.

What is known already: Men suffering from azoospermia can father a child, by extracting spermatozoa from a testicular biopsy sample. The main complication in this procedure is the presence of an abundance of erythrocytes. While larger cells can be removed in a density gradient filter, the isolation of the few spermatozoa from the erythrocytes is manually performed due to ineffectiveness of filtering methods, making it time consuming and labor intensive. Microfluidic chips can handle small volumes of liquid and, depending on the geometry, allow for manipulation of cells without physical filters.

Study design, size, duration: The sorting of spermatozoa and erythrocytes was performed using boar spermatozoa (KI Twente) and anonymized blood samples (University of Twente). 5 different extraction settings were tested, with n=3 per setting. An initial test was performed with a biopsy sample from a bull. We then determine the Collection efficiency (CE: collected spermatozoa /total spermatozoa in sample), Extraction purity (EP: collected spermatozoa /total collected cells) and Enrichment ratio (ER: spermatozoa concentration/sample spermatozoa concentration).

Participants/materials, setting, methods: The microfluidic chip contains two inlets from the sample and buffer vials and two outlets to the waste and collection vials. The chip is operated by a pressure pump. The sample flow is 5% of total flow, and 3-3.5-4-4.5-5% of total flow is routed to waste vial. Samples are flushed through the chip, imaged and counted for data processing. Viability is assessed via live/dead staining .

Main results and the role of chance: Our system can separate spermatozoa from erythrocytes despite their similar smallest dimension (both approximately 1-2µm). The hydrodynamic forces sort the cells based on their long axis (~10µm for erythrocytes and ~50µm for spermatozoa). For a sample of 200µl, the processing time of our set-up is 15-20 minutes. For the different settings we obtained a CE of 52±0.3% and EP of 81±8% with 5% of total fluid routed to waste vial, and up to 94 ± 8% and EP of 31±9 % for 3% fluid removal. The spermatozoa retained 88±6% (n=3) viability after sorting. An initial test with two coupled chips has been performed with a biopsy sample from a bull for removal of tissue cells. This initial test had not been optimized for erythrocyte removal. CE of spermatozoa after the first step was 89% with 65.1% of the larger tissue cells removed. The second step retained 43.9% of the spermatozoa, with another 3% of the larger tissue cells removed and 86.4% of the erythrocytes removed. Further optimization of the combined system should ensure similar results for erythrocyte removal as for the first set of experiments.

Limitations, reasons for caution: Samples from animals (boar and bull) were used in this study. Furthermore, the testicular biopsy was taken post-mortem from a young bull (1.5yo) by a researcher instead of an experienced lab technician, leading to a different sample composition than a biopsy of a living human patient.

Wider implications of the findings: This microfluidic design could be used in the clinic to reduce the processing time of testicular biopsies significantly by increasing the relative concentration of spermatozoa. This could lower cost of treatment for the clinic and increase the chance of a good outcome for the patients.

Trial registration number: Not applicable

P-034 Circulating cell-free-DNA quantification and sperm quality in men exposed to an experimental testicular and epididymal mild temperature increase which drastically alters spermatogenesis: a pilot study

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Study question: Could cell-free DNA (cfDNA) quantification in semen and serum samples become a new non-invasive biomarker of altered spermatogenesis?

Summary answer: Sera and seminal plasma cfDNA concentrations were increased during severe spermatogenesis alteration induced by mild temperature increase and were negatively correlated with sperm motility

What is known already: Studies provided evidence that a mild testicular and epididymis temperature increase have drastic effects on spermatogenesis and sperm DNA. Circulating cfDNA, presumably resulting from the release of genetic material from apoptotic/necrotic cells, has been previously reported as a diagnostic tool for female infertility, particularly in gynecological cancers and ovarian disorders. CfDNA quantification in follicular fluid has been reported as a biomarker for embryo selection during IVF. Furthermore, serum and seminal plasma cell-free mitochondrial DNA copy number was reported to be associated with human semen quality. However, circulating cfDNA quantification as a biomarker of altered spermatogenesis has not been investigated

Study design, size, duration: Five healthy fertile volunteers (age 25-35 years, having fathered at least one child) with no andrologic, medical or surgical history were recruited. An experimental mild testicular and epididymal temperature increase was induced by maintaining the testis in a suprascrotal position 15 hours daily for 120 days. The study was divided into three periods: before (baseline), during (0-120 days) and after (300 days) temperature increase. Semen and serum samples were collected before, during and after hyperthermia

Participants/materials, setting, methods: Semen samples were collected by masturbation. After liquefaction, semen volume, sperm motility (%) and total sperm concentration per ejaculate were investigated according to the WHO guidelines. Seminal plasma and serum cfDNA were measured by quantitative PCR with ALU-specific primers. Then, cfDNA quantification was normalized according to the total sperm cell concentration

Main results and the role of chance: Our results revealed an increase of seminal plasma and serum cfDNA during mild temperature increase, resulting in a significant decrease in total sperm output [sperm count Mean ± SEM: 2±1.94 million/ml at 120 days compared to 337 ± 99 (p = 0.03) and 218 ± 55 million/ml (p = 0.02) before and after hyperthermia). At baseline, seminal plasma cfDNA concentrations was 0.1 ± 0.02 ng/ml (normalised Alu-115 mean ± SEM). During temperature increase (day 120), seminal cfDNA concentration was 25.7 ± 13.1ng/ml (p = 0.087). After temperature increase (day 300) cfDNA concentration (0.16 ± 0.1 ng/ml) became similar to the baseline concentration.

In serum samples, cfDNA concentrations were 3.1 ± 1.4, 1146.5 ± 716.3 (p = 0.14) and 1.6 ± 0.4 fg/ml (p = 0.33) respectively before, during and after experimental temperature increase. Individual response per patient demonstrated a significant increase of seminal plasma and serum cfDNA during hyperthermia varying by a factor 18 to 26 000 and 54 to 2700, respectively, with highest ratio observed in azoospermia phenotype. In addition, we reported a negative correlation between motility and seminal plasma cfDNA (Pearson correlation, correlation coefficient r = - 0.64, 95% confidence interval:-0.87 to - 0.819, P-value = 0.01).

Limitations, reasons for caution: The low number of volunteers was the mainly limit of this study. These results must be validated in a large cohort of samples

Wider implications of the findings: Serum and seminal plasma cfDNA concentration were increased in severe altered spermatogenesis due to an experimental testicular and epididymal mild temperature increase. While preliminary, these results open new perspectives to identified non-invasive biomarkers of the spermatogenesis status and male infertility.

Trial registration number: not applicable

P-035 Evaluation of spermatozoa with normal and abnormal morphology using confocal Raman spectroscopy and multivariate data analysis

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Study question: Can confocal Raman spectroscopy (CRS) and multivariate analysis methods ensure selection of spermatozoa with intact nuclear DNA from among the spermatozoa with normal and abnormal morphology?

Summary answer: CRS provides for differentiation between spermatozoa with intact nucleus and spermatozoa with fragmented and defective nuclear DNA only when applying multivariate analysis methods.

What is known already: Sperm morphology does not always correlate with nuclear DNA integrity. CRS is a non-invasive, non-destructive analytical method for evaluating cell nuclear DNA.

Study design, size, duration: The study took place in 2016–2018. 23 We studied 161 spermatozoa (n = 161) taken from 21 healthy donors and assembled two sets of samples: morphologically normal sperm (*N* group, 125 samples) and morphologically abnormal sperm (*A* group, 36 samples).

Participants/materials, setting, methods: Sperm morphology was evaluated according to the World Health Organization guidelines using light microscopy. The Raman spectra were collected from the sperm nucleus region. After preliminary processing of the spectra, the data were analyzed using principal component analysis (PCA) and the one-class classifier DD-SIMCA.

Main results and the role of chance: *N* Group (125 morphologically normal spermatozoa) was divided into two classes, *NN* class (normal spectra, n =102) and *NA* class (abnormal spectra, n = 23). *A* group (36 morphologically abnormal spermatozoa) contained *AA* class (morphologically and spectrally anomalous spermatozoa, n = 19) and *AN* class (morphologically abnormal but spectrally normal spermatozoa, n = 17). It was concluded that the *NN* class is a uniform class and includes the *AN* class, which allowed the samples in these classes to be classified as normal, i.e. having intact nuclear DNA. Normal and abnormal spectra were present in both groups; however, normal spectra prevailed (82%) in the *N* group and the frequency of abnormal spectra was equal to that of normal in the *A* group.

Limitations, reasons for caution: The influence of laser radiation on living cells has not been investigated in full, which might limit the method application in clinical practice.

Wider implications of the findings: CRS along with multivariate analysis may serve as the method for investigating a single living sperm without damaging it before using that sperm for fertilization in IVF/ICSI.

Trial registration number: not applicable

P-036 Transfer of vitrified-thawed sperm from a patient with cryptozoospermia using hollow-core agarose capsules resulted in birth of a healthy infant

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Study question: Can a healthy baby be generated after embryo transfer with a few vitrified thawed sperm (cryptozoospermia) using agarose capsules and intracytoplasmic sperm injection?

Summary answer: Sperm was vitrified in hollow agarose capsules and thawed. A cultured vitrified blastocyst was thawed, and a transferred embryo resulted in pregnancy and childbirth.

What is known already: Small samples of spermatozoa from a patient with oligozoospermia or testicular sperm extraction (TESE) should be reliably recovered while maintaining survival after thawing. Small numbers of spermatozoa have been frozen in mouse and human zona pellucidae, and as small drops on CryoTop sheets. However, obtaining zona pellucidae from humans is difficult and recovering all of the thawed spermatozoa using the CryoTop procedure is laborious. Recently, a new method for freezing spermatozoa in agarose capsules has been reported.

Study design, size, duration: Repeated semen analysis of a man aged 25 years did not confirm any sperm. A single sperm was revealed in sediment. The man and his 22-year-old wife were referred to the Department of Urology, which resulted in a recommendation for TESE-intracytoplasmic sperm injection (TESE-ICSI). However, the patient wanted to use ejaculated sperm. In the first round of oocyte retrieval, sperm was frozen conventionally on two previous occasions before ICSI, but did not lead to pregnancy.

Participants/materials, setting, methods: We obtained written, informed consent to use agarose capsules and then finally vitrified 15 sperm from four ejaculations. As with ICSI, capsules were fixed using manipulators, and one or two spermatozoa were injected into one capsule. The capsules were transferred into a drop of cryoprotectant solution, and then placed on a CryoTop sheet, kept under liquid nitrogen (LN_2) vapor for 30 seconds, and placed in LN_2.

Main results and the role of chance: Vitrified-thawed frozen spermatozoa were injected into five of nine metaphase II oocytes (MII) and fresh spermatozoa were injected into four more. Of seven thawed spermatozoa, six were motile. The fertilization rate using frozen spermatozoa-ICSI was 80% (4/5), the blastocyst formation rate was 75% (3/4), the fertilization rate of fresh sperm-ICSI was 50% (2/4) and the blastocyst formation rate was 50% (1/2). All blastocysts were vitrified. After thawing, a single embryo produced using vitrified-thawed sperm was transferred to the wife of the patient during a hormone replacement cycle. A healthy boy weighing 3,120g was born at 40 weeks and 6 days of gestation by cesarean section.

Limitations, reasons for caution: This is the first reported instance of a healthy baby resulting from vitrified spermatozoa using agarose capsules in a background of cryptozoospermia. We will confirm the effectiveness and safety of agarose capsules, proceed with clinical trials, and apply this procedure to patients with TESE.

Wider implications of the findings: Conventional freezing of few sperm can result in a loss of sperm during transfer between containers or centrifugal washing during the procedure. On the other hand, freezing using agarose capsules allows immediate confirmation of spermatozoa status after thawing, which reduces the burden on the embryologist at the time of ICSI.

Trial registration number: Not applicable.

P-037 Effect of adipose tissue-derived mesenchymal stem cells conditioned medium on stressed and non-stressed human sperm: in vitro study

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Study question: Does adipose tissue-derived mesenchymal stem cells conditioned medium (AT-MSCs-CM) have an effect on human sperm parameters in the presence and absence of oxidative stress (OS)?

Summary answer: AT-MSCs-CM improves sperm vacuolization and DNA fragmentation while keeping other parameters stable, in stressed and non-stressed sperm.

What is known already: Male factor, is a major contributor to infertility, being responsible for approximately 50% of cases. OS results in sperm damage and reduction of IVF rates, thus it is identified as an important factor of male infertility; the present treatment options for it are either invasive or not cost-effective. AT-MSCs are known to have immune-modulatory and anti-oxidant effects through their secretions, hence raising the idea of their potential benefit to improve sperm parameters.

Study design, size, duration: A total of 30 men, in their fourth decade, with a normal sperm analysis, attending Azoury IVF clinic, between December 2017 and September 2018, were enrolled in the study. After obtaining a written informed consent from all patients, a questionnaire about age, previous diseases and habits was filled, and sperm samples were obtained.

Participants/materials, setting, methods: Sperm were divided into two groups: non-stressed and H_2O_2-stressed. Isolated AT-MSCs from healthy donors undergoing liposuction were seeded until 75% confluence and CM were collected at 24, 48 and 72 hours. The two groups were cultured with the indicated CM and a time course was performed followed by an evaluation for sperm motility, morphology, vacuolization, viability, DNA fragmentation and OS levels using light microscopy, Spermoscan kit, eosin-nigrosine staining, halosperm kit and nitroblue tetrazolium test, respectively.

Main results and the role of chance: The incubation of non-stressed and stressed sperm samples with AT-MSCs-CM for 24 hours was found to have the best impact on sperm vacuolization, DNA fragmentation and OS levels while preserving their motility, viability and morphology. Incubation with CM improved all sperm parameters except morphology in comparison to the non-treated group, with the best effect noted with CM collected at 24 hours for sperm vacuolization ($p<0.0001$) and DNA fragmentation ($p<0.0001$). When compared to fresh semen parameters (T0), samples cultured with CM 24h showed a significant decrease in sperm vacuolization ($p<0.0001$ and $p<0.001$ respectively) and DNA fragmentation ($p<0.0001$) while keeping other parameters stable.

Limitations, reasons for caution: Results were obtained from a relatively limited number of human sperm samples. It was also not possible to evaluate additional parameters due to the restricted number of sperm cells in the samples.

Wider implications of the findings: Findings from the present study contribute to the understanding of sperm physiology and would have relevance in the diagnosis and/or treatment of infertile patients, and hence can be regarded as an interesting strategy to improve IVF outcomes.

Trial registration number: N/A.

P-038 Sperm DNA fragmentation index (DFI) and alpha-glucosidase are good predictors for prognosis of sperm motility in oligoasthenozzospermic men, treated with carnitine and essential nutrients

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Study question: This study wanted to correlate DFI, seminal alpha-glucosidase and progressive sperm motility in a group of 175 oligoasthenozoospermic men treated with special test formulation

Summary answer: This study showed that increase of seminal alpha glucosidase and decrease of DFI positively impacts on progressive sperm motility, after the treatment with test combination.

What is known already: L-carnitine is concentrated in high energy demanding tissues and it plays an important role in transferring long-chain fatty acids into the mitochondria for beta-oxidation, producing energy. In addition, it modulates acyl-CoA /CoA ratio, stores energy as acetylcarnitine and reduces ROS. DNA damage, such as fragmentation and denaturation, can have adverse effects on fertilization and embryo development. Infertile men have a greater extent of sperm DNA damage and poor sperm DNA integrity than fertile men. Alpha-glucosidase is a normal constituent of human semen and produced mainly in the epididymis and it is also considered a marker of functional epididymis.

Study design, size, duration: This study was randomized, double blind, placebo controlled (DBPC) and examined the effect of combination (Proxeed Plus), containing L-C 2g and ALC 1g, as well as antioxidants, vitamins and minerals, in men with idiopathic oligo-asthenozoospermia (age group 18-50 years). The protocol was 2 months wash-out and 6 months treatment (T0, T3, T6), with test combination (125 patients) or placebo (50 patients).

Participants/materials, setting, methods: Men (age group 18-50 years) with idiopathic oligoasthenozoospermia, and history of difficulty conceiving >12 months were randomized to receive treatment or placebo in a double blind protocol. Analysis of ejaculate was done according to WHO 5th guideline. Progressive sperm motility (rapid, progressive) was done manually. DFI was evaluated by Halosperm kit (Halotech DNA, S.L, Madrid) and seminal alpha glucosidase was measured by a biochemistry analyzer (Contec Bc 300, Beijing, China).

Main results and the role of chance: The parameter values were: DFI (%): T0=38,50 (32,00-48,75), T3=35,50 (25.50-44,00) and T6= 31,00 (25,00-41,00); seminal activity of alpha-glucosidase (U/L) :T0 =2 5,40 (20.00-42.88) and T6 = 32.50 (23.00-42.83); the progressive sperm motility (%): T0 28,00(12,00-38,00), T3= 30,00%(25,50-44,00) and T6= 31,00%(20,00-41,00); these parameters showed significance of $p<0.001$ (DFI and motility) and $p<0.002$ (glucosidase) done by Wilcoxon rank test. Further the Spearman's rank-order correlation test showed that the increase of seminal alpha glucosidase ($R=0.246$; $p < 0.046$) levels influenced the progressive sperm motility. Thus the correlation of seminal plasma alpha glucosidase (AUC= 0.752) and progressive sperm motility showed that in man an increase of seminal alpha glucosidase of 12%, after six months therapy, would impact progressive sperm motility >10% with borderline significance (AUC= 0.726; $p<0.0048$). Whereas, if DFI drops by more than 3% (cut-off), after 6 months of therapy, it can be expected, with moderate accuracy, that men have sperm motility greater than 10% (AUC=0.793; $p<0.001$). In placebo group there was no significant difference in sperm motility, seminal aplha-glucosidase and DFI, between T0 and T6

Limitations, reasons for caution: None

Wider implications of the findings: This study demonstrated, after six months therapy, that increase of seminal alpha glucosidase positively impacted upon the patient progressive sperm motility. Further the decrease of DFI after therapy can be used as an independent predictor of progressive sperm motility higher than 10%

Trial registration number: PXB-001/B24

P-039 Comprehensive study of sperm head vacuoles from morphology to assisted reproductive outcomes

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Study question: Is it necessary to use other morphological examination that may be more relevant in regard to the promotion of assisted reproduction outcomes?

Summary answer: The sperm head vacuole reflect different sperm abnormalities declined viability and acrosome integrity. Therefore, intracytoplasmic sperm injection outcomes will be different in the semen samples

What is known already: Nowadays, the assessment of sperm parameters is performed according to standard sperm analysis. The current morphological examination does not provide any information about abnormalities in nuclei and genomic quality. Therefore, Vacuolization are formed from unnecessary organelles and cytoplasm which have not been removed during spermatogenesis. In the ICSI cycles, the use of such sperms have been associated to poor embryo and pregnancy outcomes. Moreover, the aim of this study was to assess whether the examination of sperm vacuole characteristics and its association with other parameters included DNA conformation and integrity, acrosome integrity, viability, and protamination status can predict ICSI outcomes.

Study design, size, duration: Semen samples were obtained from 98 men who visited Alzahra Educational and Remedial Center between October 2017 to February 2018. Infertile couples with male factor infertility (teratozoospermia) who were undergoing ICSI treatment were included. All semen samples had vacuolated spermatozoa. The classification of each semen sample was performed based on Vanderzwalmen's criteria as follows: grade I, no vacuoles; grade II, ≤2 small vacuoles; grade III, ≥1 large vacuole; grade IV, large vacuole with other abnormalities.

Participants/materials, setting, methods: To accurately measure the size and location of vacuole, the frequency, size, high magnification and scanning electron microscope methods were used for each patient. The chromatin integrity and condensation were assessed using toluidine blue and aniline blue staining, respectively. Viability and acrosome integrity was evaluated using triple staining. The protamine status was evaluated with chromomycin A3 staining. The assisted reproduction outcomes such as rates of fertilization, embryo development, biochemical pregnancy, and miscarriage were followed.

Main results and the role of chance: There are significant association between vacuole size and status of sperm chromatin condensation and packaging. So that, the results show a significant correlation between the vacuole size (large vacuole) and abnormal chromatin condensation of sperms ($p<0.05$). also, the presence of bright yellow fluorescence (CMA3-positive) was more frequently observed in spermatozoa with grades of III and IV than other groups ($p<0.05$), reflecting a higher percentage of abnormal chromatin packaging in these groups. These results were also investigated in the sperms with large nuclear vacuole.

However, a significant correlation was investigated between the frequency of vacuole and acrosome integrity and sperm viability. So that, the percentage of reacted acrosomes (blue/white) was significantly higher in spermatozoa with grades II-IV in comparison to normal group ($p< 0.05$). the viability of sperms was also declined in these groups ($p<0.05$).

The examination of sperm head vacuole was not significantly associated with fertilization rate ($p >0.05$) and embryo development rate ($p >0.05$). Whiles this association was significantly observed on biochemical pregnancy rate in the grade III and IV ($P <0.05$). Also, the odds ratio for miscarriage percentage was significantly higher (3.3: 1.1-9.6; $p=0.024$) in these groups in comparison with other groups.

Limitations, reasons for caution: The lack of evaluation of the gene expression involved in the protamination of the vacuolated sperms, which is closely associated with sperm quality and the small sample size are the limitations of this study.

Wider implications of the findings: The results of our study highlight the importance of follow up of more sperm parameters such as sperm head vacuole characteristics, because may reflect DNA damage and protamination defects.

Trial registration number: The results of this study indicated that our projects in Guilan University of medical sciences with No: 97320107 and .

P-040 Relationship between occupational exposures and seminal parameters: a prospective cohort study.

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Study question: Is there an association between occupational exposures and semen quality in men of subfertile couples undergoing assisted reproductive procedures?

Summary answer: Occupational exposures appear to reduce semen quality, specifically, decreasing concentration and sperm motility.

What is known already: Seminal parameters have been declining over time worldwide. This decline is likely multifactorial, and a variety of lifestyle factors, environmental and occupational exposures has been proposed to influence spermatogenesis and reproductive function, either positively or negatively. Occupational hazards are by far the best documented in reproductive epidemiological research. Generally, occupational exposures have been divided into physical exposures (heat, radiation), chemical exposures (solvents, pesticides), psychological exposures (distress), exposure to metals, and welding. It is important to know the effect of occupational exposures and to know what type they are in order to adequately inform patients.

Study design, size, duration: This prospective cohort study included 224 male patients undergoing conventional semen analysis for infertility investigation from January/2014 to December/2017, contributing with 840 seminograms (3.8 in average per patient). The study was approved by the institutional ethics committee and subjects were consented prior to participation.

Participants/materials, setting, methods: Semen analysis (3-5 days of abstinence) of conventional parameters (volume, sperm concentration, progressive motility and morphology) was carried out through computer-assisted semen analysis (CASA), and sperm DNA fragmentation (SCSA) was evaluated as a sperm function parameter. Exposures were assessed through questionnaires and were defined as being exposed to chemical (i.e. solvent, paints) or physical (i.e. extreme temperatures) or outdoor air exposures (i.e. particles) within the occupational framework. ANOVA/t-test measures were used to determine significant differences.

Main results and the role of chance: We observed that 20.1% of patients were exposed to any occupational exposures. The most frequent exposures were: chemical and outdoor air exposures (40.0%), only chemical (37.8%) and physical such as extreme temperatures (22.2%). We found statistically significant differences in means of sperm concentration according to the exposure: in not exposed, 62.7 mill/mL; exposed to chemicals and outdoor air pollutants, 55.8 mill/mL; exposed only to chemicals, 36.7 mill/mL and exposed to extreme temperatures, 13.6 mill/mL ($p = 0.040$). Also, we found statistically significant differences in progressive motility according to the exposure: in not exposed, 40.8 mill/mL; exposed to chemicals and outdoor air, 41.7 mill/mL; exposed only to chemicals, 30.5 mill/mL and exposed extreme temperatures, 31.7 mill/mL ($p = 0.043$). Volume, morphology and sperm DNA fragmentation were not significantly influenced by occupational exposures.

Limitations, reasons for caution: These analyses are crude and are not adjusted by potential confounders. Also, we considered exposure at any time during work life without taking into account duration or when the exposure occurred.

Wider implications of the findings: Occupational exposures are linked to decreased sperm quality but the mechanisms of action of exposure and the repercussion on reproductive physiology are yet to be understood. Our results open the way to better understand which parameters are more affected by different kinds of occupational exposure in a susceptible population.

Trial registration number: Not applicable

P-041 Optimizing sperm selection by magnetic activated cell sorting (MACS) for couples with idiopathic failed implantation

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Study question: Will sperm selection by magnetic cell sorting (MACS) optimize the reproductive outcomes for women with previous idiopathic failed implantation?

Summary answer: MACS seems like a beneficial intervention to optimize sperm selection and reproductive outcomes for women with previous idiopathic failed implantations.

What is known already: Reasons for Implantation failure are still elusive. Sperm selection criterion at Intra-Cytoplasmic Sperm Injection (ICSI) is shown to influence reproductive outcomes. In-spite of decades of research we have not found the best method to optimize sperm selection. MACS technique has shown to sort sperms with relatively normal sperm DNA fragmentation Index and there by improve the reproductive outcomes.

Study design, size, duration: This is a retrospective study of couples that underwent fertility treatments at our center in the year 2018. Patients with advanced maternal age, uterine anomalies, endometriosis and severe male factor infertility were excluded from this study. Women with previous failed idiopathic implantation were offered MACS and recruited in this study (n=52).
Participants/materials, setting, methods: Women with history of unexplained failed implantation were only included in this study (n=52). All women were under the age of 35 years and underwent controlled ovarian stimulation. Semen sample on the day of oocyte retrieval was subjected to MACS and oocytes were injected by ICSI. Embryos were cultured till blastocyst stage and vitrified. In a frozen embryo transfer (FET) cycle two blastocysts were transferred. Implantation rates, Clinical Pregnancy rates and Miscarriage rates were calculated.
Main results and the role of chance: This group of women had a failed implantation in the previous cycle and in this cycle with usage of MACS at ICSI showed a CPR of 77%, IR of 65% and MR of 11%. MACS seems like a beneficial intervention to optimize sperm selection at ICSI and in-turn ensuring an optimal reproductive outcome in couples with history of idiopathic failed implantations.
Limitations, reasons for caution: Retrospective data, Small sample size
Wider implications of the findings: MACS seems to be an active intervention for optimizing sperm selection criterion in specific defined groups and its role in routine use of MACS for all couples undergoing fertility treatments needs further evaluation.
Trial registration number: -

P-042 The potential of sperm retrieved by micro-TESE in fertilizing vitrified and warmed oocytes

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Study question: Do sperm retrieved by micro-TESE from men with reduced spermatogenesis have a potential to fertilize vitrified/warmed oocytes equally to donor sperm?
Summary answer: A trend for increased fertilization, cleavage and pregnancy rates were obtained using donor sperm compared to sperm retrieved by micro-TESE from men with reduced spermatogenesis.
What is known already: It is well documented that fertilization, cleavage, implantation and pregnancy rates using vitrified/warmed oocytes are equal to results obtained using fresh oocytes not exposed to cryopreservation. Testicular sperm retrieved by micro-TESE can be used fresh for fertilization of fresh, aspirated oocytes. However, such sperm often are cryopreserved and later on thawed for fertilization of fresh oocytes since it may be difficult to coordinate oocyte aspiration and micro-TESE. By micro-TESE testicular sperm are often obtained in a low number, and their potential to give rise to pregnancy might be reduced.
Study design, size, duration: Historical prospective study comparing fertilization, cleavage and pregnancy rates using testicular sperm obtained by micro-TESE or (when it was not possible to find testicular sperm) donor sperm. Forty consecutive couples undergoing micro-TESE during 2016-2018 due to Klinefelter's syndrome, maturation stop in the spermatogenesis, or failed sperm retrieval by conventional techniques with needle or TruCut were included.

Results obtained with cryopreserved, excess testicular sperm from the included patients were not analyzed.
Participants/materials, setting, methods: Three hundred and sixty two oocytes from 30 women were vitrified, and after warming 283 oocytes survived.

The women were stimulated with FSH in GnRH-agonist protocols and the aspirated oocytes vitrified using the Cryotech or Vitrolife techniques. The oocytes were warmed at the day of micro-TESE.

Fertilization and cleavage rates using sperm from the patients versus donor sperm were compared using the $\chi 2$-test, and pregnancy rates were compared using Fishers exact test.
Main results and the role of chance: Seventeen couples having 184 oocytes warmed and injected with own sperm obtained a fertilization rate (FR) of 64% and a cleavage rate (CR) of 53%. In comparison 13 couples having 99 oocytes warmed and exposed to donor sperm (control group) obtained a fertilization rate of 71% (NS) and a cleavage rate of 68% (p=0.019). No significant differences could be detected for subgroups with Klinefelter's syndrome (N=3; FR=62%, CR=57%), a history of cryptorchidism (N=4; FR=68%, CR=53%), or other reasons of non-obstructive azoospermia (N=10; FR=62%, CR=53%) compared to the control group.

A non-significant trend for an increased pregnancy rate was observed with donor sperm compared to own sperm [positive hCG in 69% (9 of 13) versus 35% (6 of 17) and clinical pregnancy in 46% (6 of 13) versus 35% (6 of 17)].

No differences could be observed by using the "vitrolife technique" for vitrification of oocytes compared to using the "cryotech technique". Excess blastocysts (10 fertilized with donor sperm and 7 fertilized with own semen) are still cryopreserved.
Limitations, reasons for caution: The study has not sufficient power for comparison of biochemical and clinical pregnancy rates between the groups. Furthermore, the subgroups with Klinefelter's syndrome, a history of cryptorchidism, or other pathologies are yet too small to make clear conclusions about differences between the respective subgroups and the control Group.
Wider implications of the findings: The study will be extended, and a new prospective study analyzing fertilization, cleavage and pregnancy rates using different combinations of cryopreserved and fresh testicular sperm, retrieved by micro-TESE, and fresh and cryopreserved oocytes will be designed.
Trial registration number: NCT 03809026

P-043 A low total motile sperm count in donor sperm obtained from commercial banks does not affect pregnancy rates after intrauterine insemination.

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Study question: Does the total motile sperm count of donor sperm obtained from commercial banks affect pregnancy rates after intrauterine insemination?
Summary answer: The absolute total motile sperm count found in thawed donor sperm on the day of insemination does not affect pregnancy rates after IUI.
What is known already: There is no consensus on the optimal sperm count for insemination with cryopreserved sperm. Samples sold by commercial banks typically represent just a portion of an ejaculate produced by donor who meets the banks standards for age, health and minimum sperm quality. Data from Denmark show that increasing the TMSC to greater than 19 million increased the likelihood of achieving pregnancy [1]. Another study found that frozen ejaculates with at least 20 million sperm yielded pregnancy rates similar to fresh samples containing more sperm [2].
Study design, size, duration: We performed a retrospective cohort study of single women and women in same sex relationships undergoing IUI at a single academic fertility center between January 2011 and March 2018.
Participants/materials, setting, methods: Our primary outcome is pregnancy rates per IUI, stratified by post washed TMSC. The data was analyzed in three groups: samples with less than 5 million, 5 to 10 million and greater than 10 million TMSC. Pregnancies are defined by a serum Beta HCG of greater than 5 mIU/ml. Chi-squared analyses and correlation coefficients were performed. IRB approval was obtained.
Main results and the role of chance: Of 9341 IUIs performed during the study period, 1080 IUIs were performed for single women and women in a same sex relationship using commercially available donor sperm. We found that there were no differences in the pregnancy rates per insemination based on TMSC. The pregnancy rates per cycle were 15/114(13.3%) for the group with less than 5 million, 34/351(9.5%) with 5 to 10 million and 61/609(10.0%) for samples with greater than 10 million TMSC, (p=0.52). We found no significant correlation (r= -0.072) between donor sperm TMSC and pregnancy after IUI (p= 0.46). Further, a reassuring beta-HCG level (>100IU/L) drawn 14 days after IUI was unrelated to TMSC (r= 0.0071, p=0.94).
Limitations, reasons for caution: A major limitation of our study design is that variables such medical history, BMI, and conception history were unavailable, thus our study represents all comers in an unselected sample using cryopreserved donor sperm.
Wider implications of the findings: Our data shows that the absolute total motile sperm count found in donor sperm on the day of insemination does not affect pregnancy rates after IUI. This result is useful in reassuring patients when freshly thawed donor sperm is found to have a lower TMSC.
Trial registration number: IRB approval MUHC NAGANO 2019-5254

P-044 Controversy Over The Use of Fresh Versus Frozen-Thawed Testicular Sperm In Men With Non-Obstructive Azoospermia Undergoing ICSI

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Study question: Does testicular sperm cryopreservation in non-obstructive azoospermia affect fertilization and pregnancy rates in ICSI cycles? And does sperm motility play role in the outcome?
Summary answer: No discernible differences in fertilization and pregnancy rates were found when using fresh versus frozen-thawed testicular spermatozoa, and sperm motility is a major determinant.
What is known already: Azoospermia is found in 10-15% of infertile men (1). Non-obstructive azoospermia (NOA) is one of the most challenging subsets, which is caused by dysfunction in the process of spermatogenesis (2). With the introduction of sperm retrieval techniques, such as testicular sperm extraction (TESE) and microsurgical testicular sperm extraction (mTESE), those infertile men were able to have children of their own (3). Integration of cryopreservation techniques in the TESE-ICSI procedures reduced the need for repetition of the TESE procedure in future attempts (4), as well as the need of synchronisation between the male and female cycle for oocyte retrieval (5,6)
Study design, size, duration: Retrospective analysis of consecutive ICSI cycles.

A total of 226 cycles of TESE-ICSI were performed from October 2017 until August 2018.

They included 63 fresh TESE sperm and 163 frozen-thawed TESE sperm .
Participants/materials, setting, methods: 226 male patients with NOA in whom testicular sperm was found after testicular sperm extraction. Study was conducted in the IVF Unit at Elshatby University hospital, Egypt

A testicular tissue was excised from the male participants for TESE under general anesthesia. All subjects under went this procedure for the first time. Samples were either directly used for ICSI or cryopreserved. ICSI was performed with at least one mature oocyte (MII) injected with testicular sperm.
Main results and the role of chance: A total of 226 cycles of TESE-ICSI were performed. They included 63 fresh TESE sperm and 163 frozen-thawed TESE sperm. P value less than 0.05% was considered significant. The fertilization rates were comparable between fresh and frozen TESE (67% and 55% respectively, p=0.101) as well as the pregnancy rates (42.6% and 39% respectively, p= 0.647). Afterwards, the sperm were categorized according to their motility which was found to positively affect the fertilization and the clinical pregnancy rates; fertilization rates of fresh TESE sperm using motile versus immotile sperm were (70% and 46% respectively, p=0.029) and of frozen TESE sperm using motile versus immotile sperm were (60% and 40% respectively, p<0.001).

Clinical pregnancy rates of frozen TESE sperm when using motile versus immotile sperm were (52.7% and 14% respectively, p<0.001).

However, the rates of fertilization and clinical pregnancy were comparable when using fresh motile versus frozen motile sperm (p=0.151 and 0.233 respectively).

Thus, the motility of the sperm is a main determinant of the fertilization and clinical pregnancy rates of TESE-ICSI procedures regardless the use of a fresh or frozen TESE.
Limitations, reasons for caution: The retrospective design of the study was a limiting factor as the presence of residual unknown bias could not be ruled out.
Wider implications of the findings:

Using cryopreservation in TESE procedures is an effective method to treat infertility in patient with NOA without minimizing neither the fertilization nor the clinical pregnancy rates. Selection of sperm should be based on their motility.This would spare the female an unnecessary ovarian stimulation and the male an avoidable TESE repetition

Trial registration number: Alexandria University , faculty of Medicine research committee

P-045 Roles for osteocalcin in proliferation and differentiation of spermatogonial cells cocultured with somatic cells

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Study question: Do osteocalcin affect in proliferation and differentiation of spermatogonial cells?
Summary answer: Osteocalcin improved proliferation and differentiation of spermatogonial cells.
What is known already: Osteocalcin can improved male fertility by Pancreas, bone testis axis.
Study design, size, duration: SCs and Leydig cells were isolated from neonate NMRI offspring mice and adult NMRI mice, respectively.
Participants/materials, setting, methods: SCs population were then enriched in a differential attachment technique and assessed for morphological features and identity. Then, SCs were cocultured with Leydig cells and incubated with osteocalcin for 4 weeks. Evaluation of proliferation and differentiation-related factors were surveyed using immunocytochemistry (ICC), Western blot, and quantitative real-time polymerase chain reaction (PCR). Finally, the rate of testosterone release to the culture media was measured at the end of 4th week.
Main results and the role of chance: Morphological and flow cytometry results showed that the SCs were the population of cells able to form colonies and to express ID4, α6-, and β1-integrin markers, respectively. Leydig cells were also able to express Gprc6α as a specific marker for the cells. Incubation of SCs/Leydig coculture with osteocalcin has resulted in an increase in the rate of expressions for differentiation-related markers. Levels of testosterone in the culture media of SCs/Leydig was positively influenced by osteocalcin. It could be concluded that osteocalcin acts as a positive inducer of SCs in coculture with Leydig cells probably through stimulation of testosterone release from Leydig cells and associated signaling.
Limitations, reasons for caution: this research should be done in human Sc.
Wider implications of the findings: Previously some researchers did in vivo study about Osteocalcin but we did in vitro and also in vivo in other studies.
Trial registration number: 123

P-046 Chemokines Alter Fallopian Tube Responses to DNA-Fragmented-Sperm by PCR Array

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Study question: Can DNA-fragmented-sperm make changes in chemotactic response of fallopian tube during reproductive process?
Summary answer: Data suggested that sperm DFI is an important factor which can alter the fallopian tube reaction in reproductive events.
What is known already: Normal physiological homeostasis in the female reproductive tract needs Pro-inflammatory chemokines that attract immune cells. Chemokines are one of the major compartments of immune system. Sperm and fallopian tube interaction in the female reproductive tract has an important role in fertilization, early embryo development, implantation and pregnancy. It was shown that the chemokines including CCLs and CXCLs have relevance in ovulation, sperm capacitation and fertilization. Studies also showed that seminal fluid presented at intercourse provokes expression of chemokines, appears to initiate adaptations in the female immune response that promote fertility.
Study design, size, duration: Semen samples with normal features from 30 patients who considered unexplained infertile were collected. After washing sperms, they were categorized to two groups according to their DNA fragmentation by TUNEL assay.

Fallopian tube epithelial cell was also cultured into the tissue culture flasks containing DMEM/F12 with 10%FBS medium up to 70% of confluence.

Participants/materials, setting, methods: Fallopian tube epithelial cells were co-incubated with sperms for 24h. Afterwards, cells were washed and RNA extraction was performed followed by cDNA synthetized. The control group was fallopian tube epithelial cells without sperm co-incubation. Finally, the mRNA expression level of chemokines was evaluated by PCR Array and compared between 3 groups (control, normal DFI, abnormal DFI, n=2×10).

Main results and the role of chance: Our findings are compatible with previous studies which show that chemokines play fundamental role in the interaction between sperm and female reproductive tract. Generally, CCLs and CXCLs had lower expression than the control group. However, the expression of CXCL13 and PPBP was higher in sperm groups than control. Moreover, the considerable point is that CXCL13 had higher expression in abnormal DFI group than in normal DFI group, similar to CX3CL1 ($P<0.05$). Unlike mentioned genes, CXCL10 and CXCL11 which have the same pattern of expression between different groups, had lower expression in abnormal DFI group than in normal DFI group.

Limitations, reasons for caution: Obtaining semen samples due to ethical and logistical issues was the major limitation of this study. Furthermore, *in vitro* co-incubation of fallopian tube epithelial cells with sperms may not directly represent the *in vivo* interaction.

Wider implications of the findings: Our results showed that abnormal sperm DFI seems to be established as a pathogen in female reproductive tract and significantly alter the chemokines in fallopian tube which prevent fertilization events in advance to other reproductive process.

Trial registration number: Not applicable.

P-047 Sperm DFI changes cytokines influences in fallopian tube using PCR array

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Study question: Can DNA fragmentation of sperm induce significant changes in immunological response of fallopian tube?

Summary answer: Sperm DNA fragmentation can change immunological response of fallopian tube.

What is known already: The interaction between the male and female gametes and embryos in the female reproductive system plays an important role in fertility, embryonic development, and implantation. Active immune systems throughout the female genitalia are against viral pathogens and bacterial agents which cause sexually transmitted diseases. Cytokines are one of the most important variables of immune cells that provide effective host protection. During fertilization sperms carry proteins which are allogenic to the female immune system. Therefore, cytokines play and important role in the fallopian tube specially in the presence of sperm.

Study design, size, duration: Fallopian tube epithelial cells were cultured into the tissue culture flasks containing DMEM/F12 with 10% FBS medium. Sperm samples from 20 donors with normal features were collected. The extent of sperm DNA fragmentation was measured by the TUNEL assay. Afterward, samples classified to two groups of normal and abnormal DFI. The third group was fallopian tube cells without sperm.

Participants/materials, setting, methods: Different sperms were co-incubated with fallopian tube cells for 24h. RNA extraction from cells was down fallowed by cDNA synthetized. Finally, PCR array was performed to evaluate of cytokine genes expression profiling. In addition, this data was validated by q-PCR.

Main results and the role of chance: The results of the data analysis indicated that the expression of some cytokines in the vicinity of sperm significantly changes. Sperm DFI is also effective in expressing cytokines, and the results has shown that the expression of cytokines in cells exposed to abnormal-DFI sperm compared to the cells exposed to sperm with normal DFI significantly changes.The present study on the effect of spermatozoa on cytokine production from the fallopian tube epithelial cells revealed that the expression of cytokines altered between different groups. Anti-inflammatory cytokines including IL4, IL6 and IL11 has the lowest expression in abnormal DFI group. However, other cytokines like IL18, IL23A and IL17F had different pattern of expression between the groups.

Limitations, reasons for caution: The major limitation of this study was obtaining semen samples due to ethical and logistical issues. On the other hand, *in vitro* culture system may not directly mimic of *in vivo* environment.

Wider implications of the findings: This study indicate that abnormal DFI can change the expression of cytokines which have essential roles in sperm preservation and fertilization. Therefore, this promising novel outcome might be true that alteration in immunological responses of fallopian tube can disrupt fertilization events.

Trial registration number: Not applicable.

P-048 AZFc-gr/gr Partial Deletion and Related DAZ/CDY1 Copy Number Change is Associated with Azoospermia in Iranian Men

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Study question: Is there any association between AZFc subdeletions and related *DAZ* and *CDY1* gene copies with Azoospermia among Iranian men?

Summary answer: AZFc-gr/gr partial deletions and two copy of *DAZ* with one copy of *CDY1* deletions are a risk factor for Azoospermia in the Iranian population.

What is known already: Microdeletions in the azoospermia factor (AZF) regions of the Y chromosome are well-known genetic causes of male infertility, resulting in impairment of spermatogenesis. However, the importance of partial deletions of AZFc region is controversial. This region harbors genes of multi-copy that are expressed in the testicular tissue. In the last decade, much research has focused on deletion frequencies and types of *DAZ* and *CDY* family members and their relationship to male infertility.

Study design, size, duration: 204 azoospermic and 200 fertile men were included in the study as case and control groups while subjects with abnormal karyotype, Yq microdeletions, obstructive azoospermia, hypogonadism, hypoandrogenism, chronic diseases or those reported to be heavy smokers and/or with alcohol intake were excluded from the study. The study was approved by the Royan institute Ethics Committee. Written informed consent was obtained from all study participants.

Participants/materials, setting, methods: Study subjects were evaluated for the presence of AZFc partial deletion (gr/gr, b1/b3 and b2/b3) using seven sequence tagged site (STS) markers through Multiplex PCR. *DAZ* and *CDY1* copy number status were determined using sequence family variant analysis through PCR-RFLP.

Main results and the role of chance: Amongst the three AZFc partial deletions, the frequency of gr/gr was significantly higher (6.4%; 13/204) in azoospermic group compared to control (1.5%; 3/200) ($P = 0.012$). Therefore, gr/gr deletion can be considered as a risk factor for male infertility in Iranian population. All gr/gr partial deletions were accompanied with loss of two of four *DAZ* and one of two *CDY1* copies. Among 13 cases with azoospermia and gr/gr deletions, 11 were of *DAZ1+DAZ2/CDY1a* or *CDY1b* copy deletions (84.6%) and 2 of *DAZ3+DAZ4/CDY1b* copy deletion (15.4%). All 3 fertile men with gr/gr deletions were of *DAZ3+DAZ4/CDY1a* or *CDY1b* deletion (100%). In conclusion, AZFc-gr/gr deletions are a risk factor for male infertility in the Iranian population, where accompanying *DAZ1+DAZ2* copy number deletions are more frequent among azoospermic men compared to *DAZ3+DAZ4*.

Limitations, reasons for caution: The researchers had not access to the spermograms of all members of fertile group (56/200). Assessment of

oligospermic men (moderate and severe cases) will help in showing the importance of findings. A larger sample size will enable us to statistically compare between the *DAZ/CDY1* copy number groups.
Wider implications of the findings: AZFc partial deletions are prone to be vertically transmitted to the male offspring and even expanded in size through ART. So, genetic counseling is recommended and offering PGD may prevent the condition in affected men. Present study implies that AZFc partial deletion screening needs to be considered in routine practice.
Trial registration number: -

P-049 Oxidation-reduction potential levels are not influenced by the presence of leukocytospermia

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Study question: Does leukocytospemia affect the level of seminal oxidation reduction (ORP) potential?
Summary answer: ORP levels are not influenced by the presence of leukocytospermia
What is known already: Oxidative stress (OS) is a major contributor to male infertility. It is caused by an imbalance between reactive oxygen species (ROS) and reductants in favor of ROS, negatively affecting sperm functions. OS can be directly measured through ORP using the MiOXSYS system which has been shown to predict male infertility. In semen, ROS are produced by sperm and leukocytes. It is not clear as to what is the extent of leukocytic contribution to ROS production in semen as this could influence the ORP result.
Study design, size, duration: This was a retrospective study. It included 3,968 patients presenting with male factor infertility to a tertiary medical center over a period of 18 months. The inclusion criteria were infertility > 1year in duration and normal fertility assessment of the female partner.
Participants/materials, setting, methods: ORP was determined using the MiOXSYS system (Aytu BioScience, Englewood, CO) which has a cut-off of 1.36mV/106 sperm (3,968 patients) while SDF testing was done using the Halosperm G2test kit (Halotech DNA SL, Madrid, Spain) with a cut-off of 30% (1147patients). Leukocytospermia was detected by means of the Endtz test in 241men. Statistical analysis was performed using MedCalc Statistical Software version 18.10 using non-parametric tests (Spearman Rank correlation, Mann-Whitney test, Fisher's Exact Test, ROC curve).
Main results and the role of chance: SDF showed positive correlation with ORP ($r=0.225$, $P<0.0001$) and negative correlation with the number of leukocytes ($r=-0.262$, $P=0.0383$). However, no significant correlation was detected between ORP and leukocyte count ($r=0.0195$, $P=0.7665$). While Fisher's Exact test was significant ($P=0.0408$) for leukocytospermia and SDF, no relationship was found for leukocytospermia and ORP ($P=0.1369$). ROC curve analysis showed that neither ORP could predict leukocytospermia, nor could the leukocyte count predict high (≥1.36mV/106 sperm/mL)/low (<1.36mV/106 sperm/mL) ORP levels.
Limitations, reasons for caution: The main limitations are the retrospective design of the study.
Wider implications of the findings: More studies are needed to verify the effect of ROS source on sperm function.
Trial registration number: NA

P-050 The use of phospholipase C zeta (PLCζ) analysis to identify candidates for artificial oocyte activation (AOA): a series of clinical pregnancies and proposed management algorithm

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Study question: Does PLCζ analysis constitute an efficient diagnostic and prognostic tool to inform clinical decisions when considering AOA treatment in patients with male-factor infertility?
Summary answer: PLCζ analysis is a helpful diagnostic and prognostic test to determine patient eligibility for AOA treatment and predict the likelihood of subsequent clinical pregnancy.
What is known already: PLCζ is a key sperm protein that triggers oocyte activation in successful fertilisation. In couples with male-factor infertility undergoing intracytoplasmic sperm injection (ICSI) there is a 1-3% rate of total fertilisation failure (TFF). TFF results from oocyte activation deficiency (OAD), where the abnormal expression of PLCζ in sperm alters calcium oscillations within the oocyte leading to fertilisation failure. AOA is the only known treatment for OAD using chemical stimuli to artificially reproduce calcium release and successfully trigger oocyte activation. Nevertheless, evidence on the diagnostic applicability of PLCζ analysis and pregnancy outcomes following AOA in couples with TFF remains scarce.
Study design, size, duration: This prospective study was conducted between October 2016 and March 2018 at Oxford Fertility, UK. We recruited 27 couples with known male-factor infertility due to either globozoospermia; previous total fertilisation failure of ≥8 mature oocytes; or a history of fertilisation failure caused by suspected impaired oocyte activation. Eight fertile controls with proven fertility were also recruited. Women whose partners were diagnosed with PLCζ deficiency underwent ICSI-AOA using protocols with added calcimycin to induce oocyte activation.
Participants/materials, setting, methods: Immunofluorescence staining and quantitative analyses of PLCζ in participants' sperm were performed. The relative fluorescence (RF) of PLCζ in sperm from infertile males was compared with those of randomly matched fertile controls. Comparisons of PLCζ expression between the two groups were established. Clinical outcomes following ICSI-AOA were recorded, including fertilisation rate and rates of biochemical/clinical pregnancy and live birth. Using Youden index analyses, cut-off values of PLCζ associated with successful ICSI-AOA outcomes were determined.
Main results and the role of chance: No significant age difference was identified between infertile patients (35.4 ± 5.2 years, mean ± SD, n=27) and controls (38.0 ± 2.8 years, n=8) ($p=0.36$). Overall, the proportion of sperm expressing PLCζ was lower in the infertile group ($78.16\pm3.54\%$, n=27) than in the controls ($91.65\pm1.00\%$, n=8) ($p<0.001$). Moreover, the mean total level of PLCζ in sperm from the infertile males was lower ($p<0.001$) than that of the fertile controls (RF 17.21 ± 1.45 and 24.61 ± 1.29 arbitrary units [a.u.], respectively). Among the 27 infertile males, immunofluorescence analysis identified 7 with PLCζ deficiency (significant reduction in percentage of sperm expressing PLCζ and in mean PLCζ fluorescence level). Of these, 5 opted to undergo ICSI-AOA treatment. Following ICSI-AOA, successful fertilisation was recorded in all 5 couples (fertilisation rate 56.8%) with a total of 1 biochemical pregnancy, 3 ongoing clinical pregnancies (>12 weeks' gestation) and 1 live birth at term gestation. Overall, we report a clinical pregnancy rate of 80% in couples whose oocytes were successfully fertilised using AOA. Following Youden index analysis of the PLCζ data from all infertile males, the cut-offs below which oocyte activation was likely to benefit from AOA were 71% for proportion of sperm expressing PLCζ, and 15.57 a.u. for mean PLCζ per sperm.
Limitations, reasons for caution: PLCζ immunofluorescence analysis does not use real-time assays, thus precluding the assessment of PLCζ levels in individual sperm cells chosen for ICSI-AOA. Moreover, AOA treatment was not used in infertile patients with similar PLCζ levels to fertile controls, limiting evaluation of the PLCζ analysis as a predictor of AOA outcomes.
Wider implications of the findings: This study demonstrates the applicability of PLCζ quantification to inform clinical decisions when considering AOA treatment for couples with OAD. Larger comparative studies are required to provide further evidence of the effectiveness of AOA. In addition, developing a recombinant PLCζ protein would circumvent the need for calcimycin in AOA.
Trial registration number: National Research Ethics Committee (REC) Reference Number: 10/H0606/65

P-051 Clinical outcome of ICSI cycles in a series of 112 patients with severe asthenozoospermia and ultrastructural flagellar defects: a 17-year experience

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Study question: Does the flagellar ultrastructural phenotype in patients with severe asthenozoospermia influence the outcome of ICSI ?
Summary answer: Reproductive outcomes, in terms of fertilization, implantation, embryo quality, miscarriage, pregnancy and live births are similar regardless the flagellar phenotypes.
What is known already: There are two main forms of flagellar pathologies: non-specific flagellar anomalies (NSFA) and homogeneous, suspected genetic, disorders as primary flagellar diskinesia (PFD) and dysplasia of fibrous sheath (DFS). In the literature, there is still a debate on the impact of the flagellar abnormalities on the ICSI outcomes that may be influenced by the nature of flagellar defect. However, the number of cases is still small for drawing conclusions.
Study design, size, duration: A large retrospective cohort analysis was performed on 112 severe asthenozoospermic patients using spermogram and sperm ultrastructural data from 2001 and 2017 in the reproductive units of Lille and Rouen University Hospitals.
Participants/materials, setting, methods: The 112 patients were classified as three groups according to the ultrastructural flagellar phenotype: NSFA n=67, PFD n=18, DFS n=27. In ICSI, 169 cycles were evaluated (NSFA n=114; PFD n=27; DFS n=28). Comparisons in outcomes were done using generalized liner mixed model included women as random effect and women age and ICSI rank as covariates. Semen parameters were compared between groups using Kruskall-Willis. P values<0.05 were considered significant.
Main results and the role of chance: Statistically significant lower motility, vitality and normal forms were associated with PFD and DFS defects respectively compared to NSFA group. The overall fertilization, pregnancy and delivery rates were of 57.6%, 31.7% and 25.5%, respectively. Fertilization rate was 58.4% in the NSFA, 52.6% in PFD, 61.7% in DFS. There were no statistically significant differences between the NFSA, PFD and DFS in terms of fertilization, implantation, pregnancy and delivery rates. Embryo quality, expressed as mean number of grade A defined as 4 cells on Day 2, ≥8 cells on Day 3, <10%fragmentation and equally sized mononucleated blastomeres, was 21.6% (NSFA), 16.7 (PFD) and 32.6% (DFS), respectively. Thus a trend in reducing the good quality embryos in PFD was observed but without significant statistical differences among the 3 groups (p= 0.44).
Limitations, reasons for caution: Although a large cohort of patients was enrolled, the study is retrospective. Further studies could consider a cohort of transferts in embryo development as blastocysts.
Wider implications of the findings: ICSI offers a valuable treatment for asthenozoospermic patients who desire biological offspring. Outcomes derived from this series study demonstrated that the use of ejaculated spermatozoa with entirely different flagellar ultrastructural defects (homogenous and heterogenous) has similar likelihood of success in ICSI. It is reassuring for the patients and clinical staff.
Trial registration number: Not applicable.

P-052 Protamine 1 (P1) as a prognostic indicator for human sperm quality and ICSI outcome

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Study question: Could Protamine 1 (P1) be used as a prognosis indicator for human sperm quality and ICSI output?
Summary answer: Protamine 1 revealed correlations with sperm parameters and pregnancy rate after ICSI treatment and therefore, it could be used for assessment of sperm quality.
What is known already: Abnormal protamination during the spermatogenesis increases the abnormality of human spermatozoa (Utsuno *et al*., 2014) and decreases the chromatin condensation (Aoki *et al*., 2005).

Protamine 1 considered as a main nuclear protein in mammals, it packages sperm DNA. In some mammalian species, the sperm contains only P1 (Balhorn, 2007). It acts as the main protector for sperm DNA, hence any defect in the P1 may increase the sperm DNA fragmentation and subsequently decrease the sperm quality and eventually impair the ICSI outcomes.
Study design, size, duration: This is a prospectively designed study which was carried out at the Department of Obstetrics and Gynecology, University of Saarland, Germany in 2018. One hundred twenty semen samples were collected from unselected male partners of couples consulting for infertility treatment and were included in this study. 84 of them underwent an intra-cytoplasmic sperm injection (ICSI).
Participants/materials, setting, methods: Semen samples were assessed and prepared according to WHO guideline 2010. Sperm nuclear protamine was extracted and analyzed using acid-urea –PAGE. Chromatin condensation and DNA fragmentation were evaluated by Chromomycin (CMA_3) and TUNEL assay respectively.
Main results and the role of chance: The mean and standard deviation of the analyzed parameters were as a follow:

Sperm concentration (58.61 ± 47.481 $X10^6$/ml); sperm motility (42.90 ± 20.272%); progressive motility (17.01 ± 13.014); morphologically abnormal spermatozoa (91.05 ± 6.597%); sperm DNA fragmentation (14.83 ± 9.430%); sperm protamine deficiency (34.81 ± 17.586%); protamine P1(466.03 ± 154.979 (ng/10^6 sperms) and P2(451.43 ± 148.827 (ng/10^6 sperms).

Protamine 1 showed significant positive correlations with sperm concentration and total motility (r= 0.320, p<0.01; r= 0.195, p<0.05; respectively) and correlate negatively with morphologically abnormal spermatozoa and sperm protamine deficiency (CMA_3) (; r= -0.193, p<0.05; r= -0.242, p<0.01; respectively). But, no significant relationship between P1 and other sperm parameters was found**.**

In addition, P1 concentration found to be significantly higher in the sperm of male partners of patients with negative pregnancy test (498.28 ± 152.46, ng/10^6sperm) in comparison to the sperm of the male partners of patients who did get pregnant (425.03 ± 151.43, ng/10^6sperm) (p<0.05).
Limitations, reasons for caution: The sample size.
Wider implications of the findings: The findings indicated that the P1 could be used as a prognostic factor for sperm quality. However, more studies with large sample size are needed.
Trial registration number: Basic Science.

P-053 Selection of vacuole free spermatozoa with the assistance of the hyaluronic acid-binding assay.

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Study question: Does the hyaluronic acid-binding assay has a positive effect on the selection of vacuole free human spermatozoa, comparison to the standard morphologically selected sperm method?
Summary answer: Hyaluronic-acid-binding assay selection is a good method to select spermatozoa in regard to the presence of vacuoles, especially for spermatozoa with more than two vacuoles.
What is known already: The selection of normal spermatozoa during ICSI does not enable the detection of nuclear defects. Sperm head nuclear abnormalities were identified earlier as vacuoles by motile-sperm organelle-morphology examination (MSOME). In terms of the links between the presence of vacuoles and embryo development, it was shown that the injection of morphometrically normal spermatozoa with no vacuoles was associated with significantly higher blastocyst rate, a smaller proportion of arrested embryos and higher pregnancy rates. According to previously obtained data

in assisted reproduction, it is of importance to reliably select vacuole-free spermatozoa.

Study design, size, duration: This is prospective and blinded observational study. Hyaluronic acid (HA) bound, standard morphologically (SM) selected (200x) and unselected sperm (control) were collected by different persons. The evaluation of vacuoles was performed by Nomarski high-power differential interference contrast (Nomarski/DIC) optics (600x up to 7.200x). The number of vacuoles in each sperm head was determined as an: absence of vacuoles, the presence of one vacuole, the presence of two vacuoles, the presence of more than 2 vacuoles.

Participants/materials, setting, methods: Fifteen human semen samples were prepared by 80% density gradient. From each sample, a minimum of 20 spermatozoa per method (HA, SM selection) were collected in separated PVP droplets. Additionally, 20 unselected sperm were collected from each sample designated as controls. All samples were blind-observed by the same person. Statistical significance was defined as $P<0.05$ One way analysis of variance and post-hoc Tukey-Test were performed. All statistical evaluations were carried out using SigmaStat Version 3.5.

Main results and the role of chance: After complete statistical analysis, the number of spermatozoa without vacuoles found in Hyaluronic acid bound selected ($p<0.001$) and standard morphologically selected ($p<0.01$) were significantly higher compared to the unselected spermatozoa. The number of sperm with 1 ($p<0.05$) or 2 vacuoles ($p<0.01$) and more than 2 vacuoles ($p<0.001$) was significantly higher in the unselected spermatozoa. Furthermore, in Hyaluronic acid bound selected spermatozoa the appearance of 2 and more than 2 vacuoles was significant lower compared to the standard morphologically selected spermatozoa ($p<0.05$).

Limitations, reasons for caution: The time necessary for the isolation of individual spermatozoa from each sample was one of the limiting factors. Therefore, only 20 spermatozoa per sample were collected. However, obtained results are encouraging and in order to obtain more concrete results, future research should be performed on a larger numbers of samples.

Wider implications of the findings: Both selection methods provide spermatozoa which containing less number of vacuoles, compare to the unselected samples, especially in the group with more than two vacuoles. Therefore, HA selection may be an effective method to identify spermatozoa with a higher fertilization potential in order to improve results in ART procedures.

Trial registration number: not applicable

P-054 The assessment of microdissection testicular sperm extraction (micro TESE) and intracytoplasmic sperm injection (ICSI) in couples with Klinefelter syndrome (KS)

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Study question: What are sperm retrieval rate (SRR) by micro TESE, fertilization rate, and pre- and post- implantation development in KS couples?

Summary answer: In KS patients with successful sperm retrieved, age was younger than failure group and their embryonic development using motile sperm was comparable to unexplained NOA.

What is known already: Micro TESE, in combination with ICSI, is presently used to treat infertility in cases of NOA including KS, which is the most common sex-chromosome disorder among infertile males, with a prevalence of 1 in 660 men and is a frequent cause of hypogonadism and infertility. There were few reports regarding ICSI outcome in the couples of KS and the aim of this study is to assess the prevalence and the significance including SRR by micro TESE and ICSI outcomes with embryonic development in KS couples.

Study design, size, duration: A retrospective study was conducted in 131 patients with KS and 649 NOA patients with 46, XY without past history (unexplained NOA; not including after orchidopexy, KS, cryptozoospermia, mumps orchitis, etc) who underwent micro TESE and ICSI for their wives between September 2013 to December 2018.

Participants/materials, setting, methods: A total of 1186 azoospermic patients were examined chromosomal analysis on cultured lymphocytes from peripheral blood. We evaluated SRR of micro TESE, two pronuclei (2PN) oocyte rates, blastocyst development rates, good-quality blastocyst (Grade 3BB and above on day 5 by the Gardner scoring) rates, and clinical pregnancy rates per embryo transfer (ET). We did not undergo preoperative hormonal therapy for KS patients. Statistical analysis was performed using unpaired t-tests and chi-squared tests.

Main results and the role of chance: We identified 131 KS out of 1186 azoospermic patients (11.0%). SRR of first attempt micro TESE in KS (55/102=53.9%) was higher than unexplained NOA (102/482=21.2%) ($p<0.01$). Spermatozoa were successfully retrieved in 5 of 29 (17.2%) KS and 19 of 167 (11.4%) unexplained NOA who had previously undergone micro TESE with no sperm found. No correlation was found between serum FSH, LH, and T level and testicular volume with the success of sperm retrieval. Patient age in successful micro-TESE for cases of KS is significantly younger (32.1± 4.8 years) than that in failed cases (35.2±5.5 years) ($p<0.05$) in first attempt micro TESE. 2PN oocytes, blastocysts development, and good-quality blastocysts rates were 54.2%, 40.7%, and 15.1% in KS and 53.9%, 45.1%, and 18.7% in unexplained NOA, respectively (no significant differences). Clinical pregnancy rates per ET were 33.6% in KS and 29.7% in unexplained NOA. Eighty-eight out of 100 (88.0%) cycles could be performed ICSI using motile sperm. With respect to motility of retrieved sperm, 2PN oocytes (56.9%), blastocysts development (43.7%), and good-quality blastocysts rates (16.1%) using motile sperm was significantly higher than that of using immotile sperm even after pentoxifyllin administration (41.0%, 6.7%, and 3.3%, respectively) ($p<0.005$) from KS couples.

Limitations, reasons for caution: We did not show the data using ejaculated sperm with KS. The natal outcome and development of these children has not been fully investigated. We need long-term follow-up of babies of the KS couples.

Wider implications of the findings: Micro TESE is particularly helpful for successful sperm retrieval in KS cases, however, a salvage micro TESE offers a less chance of finding sperm. In KS, the motile sperm retrieval from testicular tissue in micro-TESE is a critical key to succeed and rationale for good embryonic development and clinical pregnancy.

Trial registration number: Not applicable.

P-055 Perinatal outcomes using ejaculate versus surgical sperm retrieval in patients undergoing intra cytoplasmic sperm injection for male infertility – A retrospective analysis of 628 patients

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Study question: Does surgical sperm retrieval in patients with azoospermia influence the perinatal outcomes when compared to ejaculate in patients with non-azoospermic male infertility

Summary answer: There was no increased risk of preterm birth (PTB), low-birth weight (LBW) or congenital anomalies when surgically retrieved sperm was used compared to ejaculate

What is known already: Surgical sperm retrieval (SSR) followed by intracytoplasmic sperm injection (ICSI) is the main line of treatment for couples with azoospermia desiring a biological child. Various studies have reported either a decrease or no difference in pregnancy and live birth rates following ICSI with testicular sperm compared to epididymal sperm and ejaculate. However, perinatal and neonatal outcomes with ICSI using surgically retrieved sperm in azoospermic men and ejaculate in non -azoospermic infertile men are not well established

Study design, size, duration: 628 couples undergoing ICSI for male infertility between 2011- 2015 were included in this retrospective cohort study. The ejaculate group had 478 couples and the SSR group had 150 couples

Participants/materials, setting, methods: Data from all couples who underwent ICSI for male infertility using ejaculate or surgically retrieved sperm during the study period were analysed to compare the pregnancy outcomes (miscarriage, multiple gestation, preterm birth, live birth, mode of delivery and congenital anomalies). Logistic regression analysis was performed for perinatal

outcomes like PTB, LBW following singleton live births. A subgroup analysis for perinatal outcomes was conducted between testicular and epididymal sperms

Main results and the role of chance: The clinical pregnancy rate per embryo transfer was 212/465 (45.6%) in the ejaculate group and 74/146 (50.6%) in the SSR group (p value=0.15). The live birth rate per embryo transfer was 163/465 (35.05%) in the ejaculate group and 51/146 (34.9%) in the SSR group (p value=0.97). The mean gestational age at delivery was lower in ejaculate group (36.3 ± 2.3 weeks) compared to SSR group (37.29 ± 1.3). There was no difference in miscarriage rate, mode of delivery and congenital anomaly rates between the two groups (p value of 0.5, 0.16 and 0.16 respectively).

Logistic regression analysis for singletons showed no difference in PTB (epididymal sperm vs ejaculate OR 0.21, 95% CI 0.02- 1.66) (testicular sperm vs ejaculate OR 0.46, 95% CI 0.12- 1.65) or LBW (epididymal sperm vs ejaculate OR 0.42, 95% CI 0.09- 1.9) (testicular sperm vs ejaculate OR 0.80, 95% CI 0.27-2.3)

Subgroup analysis between epididymal and testicular sperm also showed no difference in PTB (OR 2.18, 95% CI 0.20- 22.94) or LBW (OR 1.87, 95% CI 0.31-11.02) for singletons.

Limitations, reasons for caution: The main limitation is that it is a retrospective study. An adjusted logistic regression analysis for perinatal outcomes could not be performed because of the small number of patients .Also,data was not available for male FSH, LH and karyotype.

Wider implications of the findings: No major differences were noted in perinatal or neonatal outcomes when ejaculate or surgically retrieved sperm was used. This helps in counseling couples prior to ICSI regarding the outcomes

Trial registration number: N/A

P-056 Post-wash total motile sperm count as the most accurate predictor for live birth after intrauterine insemination

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Study question: Is it possible to predict the success of a homologous or donor intrauterine insemination (IUI) cycle according to the male infertility?

Summary answer: The post-wash total motile sperm count (PTMSC) has more precise predictive value over standard WHO criteria at the time of homologous and donor IUI.

What is known already: The effectiveness of IUI depends on several factors. One of the most important is the sperm quality. World Health Organization (WHO) defined cut-off values to distinguished between normal and abnormal semen samples. Although this criteria classification has been widely used, there is a controversy among clinicians. Some of them claim that the most influential variable in the IUI is only the sperm motility. Others consider that pregnancy rates are influenced by concentration too. This lack of standardization could be solved with the PTMSC, a parameter that represents the exact number of progressive motile sperm that are available for the insemination.

Study design, size, duration: A retrospective cohort study. A total of 7435 subfertile couples undergoing IUI in the last 15 years were analysed. 5390 couples underwent IUI with homologous semen (IUI-H) and 2045 couples underwent IUI with frozen donor semen (IUI-D).

Participants/materials, setting, methods: All the infertile couples that underwent IUI. Three prediction models of pregnancy and livebirth were compared according to the WHO parameters, the sperm count and the PTMSC, calculated by multiplying the sperm concentration by progressive motile spermatozoa and the volume used in the insemination procedure. Logistic regression analysis and AUC-ROC curve processed by R package were used to evaluate the association between the three parameters and the likelihood of pregnancy and live birth in IUI.

Main results and the role of chance: The overall pregnancy rate (PR) per IUI-H cycle was 10.4 and 20.8% after IUI-D. The multiple PR was 5.4% in IUI-H and 7.8% in IUI-D, and the overall live birth rate (LBR) was 80.5% and 85.6%, respectively. Logistic regression analysis showed that the PR and LBR were not influenced by WHO criteria in none of the IUI procedures. Sperm count and PTMSC were significantly correlated with a positive clinical outcome in terms of PR after both IUI ($p<0.01$). AUC-ROC curves showed that PTMSC has a significantly greater predictive capacity of livebirth. The final predictive model had an AUC of 0.51 ($p<0.05$) in case of total sperm, and an AUC of 0.55 ($p<0.01$) in reference to PTMSC in IUI-H. The same trend was observed after IUI-D ($p<0.01$). The probability of achieving a live birth after IUI-H was higher when PTMSC was over 10 million (OR=0.85, 95% CI: 0.67 to 1.08). The mean value of PTMSC was significantly higher in IUI-H (13.85±9.28 vs 6.59±4.19, $p<0.05$). Patients with a PTMSC less than 5 million had a greater challenge in getting a live birth after IUI-D (OR=0.37, CI: 0.24 to 1.18).

Limitations, reasons for caution: A prospective design study is necessary to further confirm the correlation between PTMSC parameter and clinical outcome in IUI.

Wider implications of the findings: Due to PTMSC has shown to be better correlated with the PR and LBR than the WHO 2010 classification criteria, we should reconsider using the PTMSC parameter as the best prognostic tool to achieve a live birth after IUI-H or IUI-D.

Trial registration number: This is not a clinical trial.

P-057 Oxidation-reduction potential in semen correlates with semen parameters and DNA fragmentation but not necessarily with lifestyle in men visiting a fertility clinic

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Study question: Does lifestyle influence the redox potential in semen affecting semen quality via sperm parameters and DNA fragmentation in the total and vital fraction?

Summary answer: Oxidation-reduction potential correlates with semen motility, morphology and vital sperm DNA fragmentation, but not with physical activity, BMI, smoking, drug or alcohol abuse.

What is known already: A disbalance between reactive oxygen species and the antioxidant capacity can lead to oxidative stress (OS) in semen. A MiOXSYS™ analyser assesses easily the static oxidation-reduction potential (sORP). A bad lifestyle is hypothesized as being a major cause of OS, thereby inducing DNA fragmentation, membrane oxidation and apoptosis. Investigating lifestyle is mostly performed by self-reporting surveys. For physical activity, a validated Baecke questionnaire (Baecke et al.,1982) which coded three categories: occupation, sports, and leisure time, is frequently applied. Lifestyle adjustment in men with inferior semen quality, might reduce the need for more advanced assisted reproductive technologies.

Study design, size, duration: This cross-sectional study comprised a cohort of 225 patients between January 2017 and March 2018. Only complete semen samples were included with a minimum of 2 days of abstinence. The study was approved by the institutional ethical committee and written informed consent was obtained from each participant.

Participants/materials, setting, methods: In 206 men (18-65 years) semen parameters were evaluated according to WHO 2010 guidelines, OS was measured by MiOSYS™ (n=198) and DNA fragmentation was assessed on fresh semen by TUNEL assay in the total and the vital fraction using flow cytometry (n=179). A clinical male fertility diagnosis questionnaire (n=206) and the Baecke survey (n=95) were filled out. Spearman correlation and Mann-Whitney test were used to compare groups.

Main results and the role of chance: Significant negative correlations were found between the sORP and progressive motility (r= -0.24; p=0.0005), total motility (r= -0.23; p=0.0010) and morphology (r= -0.18; p=0.0112). While sperm concentration, total sperm count and semen leukocyte concentration showed no significant correlations. Moreover, sORP was significantly higher

(p=0.0001) in patients with one or more subnormal sperm parameter (4.23 mV/sperm concentration) than in normozoospermic men (0.77 mV/sperm concentration). Sperm DNA fragmentation in the vital fraction, and not in the total population, was significantly correlated with sORP (r=0.19; p=0.01). As far as lifestyle is concerned, no significant correlations could be found between sORP (M ± SD; 2.50±6.22 mV/sperm concentration) and body mass index (BMI; 25.3±3.96), physical activity (8.1±1.4), or mean age (34.8±8.26 years). Moreover, none of the other lifestyle factors (smoking, alcohol, drug abuse), had a significant influence on the oxidation-reduction potential in semen.

Limitations, reasons for caution: The study was limited by the limited number of participants who filled out the Baecke questionnaire. Self-reporting surveys can be subjective. Moreover, the type of diet was not included in the study.

Wider implications of the findings: Measurement of sORP can serve as an adjunct to routine semen analysis as a supplementary test to link oxidative stress and poor semen quality, identifying altered functional status of the sperm and thereby directing those men to appropriate therapeutic management.

Trial registration number: N/A

P-058 Relationship between oxidative stress in semen and the fertilizing capacity of the sperm - evidence from the conventional IVF model in couples with unexplained infertility

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Study question: Is there a relationship between oxidative stress in the native semen and the fertilizing capacity of the spermatozoa?

Summary answer: Oxidative stress in the native semen is negatively and significantly related to the fertilizing capacity of the spermatozoa.

What is known already: Oxidative stress (OS) is thought to affect the fertilizing capacity of the sperm. This is based on indirect evidence such as the association of OS with sperm parameters in normo and oligo-asthenospermia as well as the relationship between OS and the clinical outcome of ICSI. However, the relationship between OS in semen and the fertilizing capacity of the sperm should ideally be tested in the IVF model taking the fertilization rate (FR) in relation to OS as the primary outcome measure, preferably in couples with unexplained infertility where the fertilizing capacity of the sperm is in question.

Study design, size, duration: This prospective cohort study was conducted between September 2017 and December 2018. Couples with unexplained infertility were treated for one cycle of combined IVF/ICSI if 12 oocytes or more were retrieved. If good embryos resulted from IVF, 2 were transferred. If no fertilization occurred from IVF, 2 embryos resulting from ICSI were transferred. All remaining embryos were frozen. A total of 108 couples were enrolled but only 25 fulfilled the criteria and completed the study.

Participants/materials, setting, methods: A total of 575 oocytes were retrieved from the 25 patients attending our IVF Unit (mean ± SD = 20.5 ± 5.6 oocyte/cycle). In each patient, 3 to 5 oocytes were inseminated by conventional IVF procedure and the rest were treated with ICSI. Oxidative stress was determined in the native semen samples by measuring the oxidative reduction potential (ORP) using the MiOXYS system and the results correlated with the fertilization rate of the IVF-fertilized oocytes.

Main results and the role of chance: Out of the 575 oocytes retrieved, 108 were inseminated with conventional IVF (mean ± SD = 4.2 ± 1.4 oocytes per cycle) resulting in 36 embryos from 14 patients (FR = 33.3%), of whom 3 became pregnant (pregnancy rate = 21.4%). There was a negative correlation between the ORP in the native semen and the FR. The mean (± SD) ORP in the native semen in couples with =>50% IVF fertilization was 1.02 ± 0.1 mV/10^6 sperm/mL which is significantly lower than in couples with <50% fertilization (2.05 ± 0.7 mV/10^6 sperm/mL) (P<0.02). The sensitivity, specificity, PPV, NPV, +LR and –LR of the ORP assay in predicting =>50% fertilization were 70.0%, 60.0%, 53.8% , 75.0%, 80.0% and 20% respectively with a cut-off point at =<1.5692 mV/10^6 sperm/mL. In the other 11 patients where no fertilization occurred by IVF, embryos resulting from ICSI were transferred, resulting in 7 pregnancies (pregnancy rate = 63.6%). The mean (±SD) ORP in the native semen in patients pregnant with IVF-fertilized oocytes was also significantly lower than in patients pregnant with ICSI-fertilized oocytes (0.99 ± 0.1 mV/10^6 sperm/mL versus 2.68 ± 0.7 mV/10^6 sperm/mL; P <0.05).

Limitations, reasons for caution: The small sample size of this study can be considered a limitation but these early results can be further confirmed by larger prospective studies involving couples with various causes of infertility, although the criteria required for the recruitment of these couples are not easy to fulfill.

Wider implications of the findings: These results confirm the negative effect of OS in native semen on the fertilizing capacity of the sperm. Measuring ORP in native semen can be used in couples with unexplained or male factor infertility to help determine whether anti-oxidant therapy, IUI, IVF or ICSI is the best management option.

Trial registration number: Not applicable.

P-059 An evaluation of sperm parameters based on age; an analysis of almost 18 000 specimens.

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Study question: Is there an age influence on mean sperm parameters?

Summary answer: Age affects the semen volume, sperm concentration and motility in a non-linear fashion with the best sperm parameters available between the ages of 30-40 years.

What is known already: Normal ranges of sperm parameters in a man's ejaculate, were defined previously but do not take age into consideration (Cooper *et al.* 2009, Check *et al.* 1992). A negative correlation was found between age and routine semen parameters when using 40 years as a cut-off value (Verón *et al.* 2018). But to the best of our knowledge, no age-normograms of semen analysis results have been published.

Study design, size, duration: This is a retrospective study that includes 17,777 semen analysis from the MUHC reproductive center produced from 2004 to 2018. Mean data is presented with standard error bars. Data was analyzed as nonlinear polynomial regression analysis and p-values.

Participants/materials, setting, methods: Males who were referred to MUHC reproductive centre, for any clinical reason, and produced sperm for diagnosis. Sampled were produced by masturbation in the clinic and handed to the Andrology laboratory for CASA.

Main results and the role of chance: Analysis of the 17,777 computer-assisted and technician verified semen analyses (CASA) demonstrated that (graph): the volume, motility, count, progressive count, rapid motility and concentration increase to a maximum level between the ages of 30 to 40 after which they decrease again (P value < 0.0001; R^2>0.90 in all cases). Interestingly Krugger Tyberg % strict normal forms almost follow a linear relationship increasing as males age (P value < 0.0001; R^2=0.89). To test whether these results were a function of patients who presented to fertility centers and did not represent the general population we plotted only values with normal results based on the 2010 WHO parameters (not shown). Again a similar pattern was seen with the CASA results increasing until about 30 years of age. Remaining best until 40 years of age and then subsequently decreasing. This pattern among the normal results confirms the nonlinear relationship of sperm parameters and age.

Limitations, reasons for caution: This is a retrospective study, hence has inherent bias. However, being almost 18,000 specimens, likely the results reflect the general population. None were lost to follow up, since all could be extracted from our sperm database where they all specimens were maintained at initial calculation of results and entered prospectively.

Wider implications of the findings: Unlike female fertility which peaks in the 20s, as an evolutionary theory males could best protect based on strength, knowledge and status in their 30s. Best sperm at 30-40, allows males an advantage when multiple copulations are occurring with different individuals, as occur with chimpanzees, or possibly did with pre-humans.

Trial registration number: n/a

P-060 The correlation of sperm DNA fragmentation with Blastocyst grading and it's implantation potential

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Study question: Is sperm DNA fragmentation (SDF) has a correlation with Blastocyst's trophectoderm (TE) and Inner cell mass (ICM) grading, or with the implantation rate?
Summary answer: There is no correlation between SDF and blastocyst grading. Although there is an inverse correlation between abnormal SDF and the implantation rate in ICSI patients.
What is known already: Achieving pregnancy depends on sperm and oocyte quality. Sperm DNA integrity is a crucial parameter representing sperm quality. Abnormal SDF has a negative impact on pre and post implantation embryo development. Published studies focused on the correlation between SDF and Blastocyst formation with its morphological grading, based on the fact that embryonic genome activation starts between 4-8 cells stage of development.
Study design, size, duration: Retrospective cohort study included 189 couples, assigned to normal SDF group (121 cases), and abnormal SDF group (68 cases). Semen in both groups was processed by double layer density gradient (DG). All included cases underwent ICSI in Ganin Fertility Center from January 2018 to September 2018.
Participants/materials, setting, methods: All cases in this study reached the blastocyst stage with female age $\leq$37 years old and male sperm count $\geq$ 5 million. SDF test was done by TUNEL assay using benchtop flow cytometer with cutoff value 20%. Blastocyst morphological assessment was carried out by experienced embryologists according to Gardner's criteria. The data were collected and analyzed using IBM SPSS version 22 for Microsoft Windows. The difference is considered significant if the *p*-value is $\leq$ 0.05.
Main results and the role of chance: There were no significant differences in male age, female age or number of MII oocytes between the two groups. There was a significant difference between SDF values for normal and abnormal SDF groups (14.3% vs. 28.6%) ($p < 0.005$). The fertilization rate was (78.44% vs. 76.67%) ($p > 0.05$) for normal and abnormal SDF groups respectively. The blastulation rate was (65.52% vs. 58.71%) ($p < 0.05$) for normal and abnormal SDF groups respectively. On comparing the blastocysts grading we found that the good TE was (18.91% vs. 21.45%) ($p > 0.05$), Fair TE was (33.94% vs. 35.02%) ($p > 0.05$), Poor TE was (7.54% vs. 7.54%) ($p > 0.05$), Good ICM was (32.34% vs. 35.18%) ($p > 0.05$), Fair ICM was (26.73% vs.27.65%) ($p > 0.05$) and poor ICM was (1.59% vs. 1.74%) ($p > 0.05$) for normal and abnormal SDF groups respectively. Pregnancy rate was (66.3% vs. 51.6%) ($p > 0.05$) for normal and abnormal SDF groups respectively. However, the implantation rate was (47.96% vs. 33.20%) ($p < 0.05$) for normal and abnormal SDF groups respectively So, the abnormal SDF levels would affect the implantation potential without reflection on the blastocyst morphological grading.
Limitations, reasons for caution: This study is retrospective and the blastocysts assessment would have been better if done through PGT-A not only the morphological grading of TE and ICM.
Wider implications of the findings: If these findings are confirmed with larger prospective randomized control trials so, the usage of sperm selection techniques may improve the negative impact of abnormal SDF on the reproductive outcomes.
Trial registration number: not applicable

P-061 Unraveling the origin of azoospermia in male cystinosis patients.

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Study question: Is azoospermia in male cystinosis patients obstructive or non-obstructive in origin?
Summary answer: Azoospermia in male cystinosis patients is obstructive in origin.
What is known already: Cystinosis is a rare autosomal recessive metabolic disorder caused by mutations in the lysosomal membrane protein cystinosin (CTNS), which leads to intracellular lysosomal cystine accumulation. Depending on the disease severity, three forms are distinguished: infantile (most severe), juvenile (intermediate), and ocular (benign) form. The main clinical presentation in infantile cystinosis is the renal Fanconi syndrome, leading to end stage renal disease (ESRD) by the age of ten, if the patient is left untreated. Cysteamine, a cystine-depleting agent, beside renal replacement therapy, has improved the life expectancy of cystinosis patients. In contrast to females, male infantile cystinosis patients suffer from infertility.
Study design, size, duration: Three groups were included in a prospective case control study; adult cystinosis patients (three infantile, two juvenile, and one ocular male patients), control group (seven adult fertile male subjects), and a positive control group for obstructive azoospermia (ten adult vasectomized males who underwent vasectomy 4-8 weeks prior to enrollment). In addition, clinical data and testicular sections for five adult infantile male cystinosis patients were retrospectively included in the study (in total, eight infantile cystinosis patients).
Participants/materials, setting, methods: Scrotal ultrasound for screening for soft biomarkers of obstruction and semen analysis were performed in all prospectively included subjects, while sexual hormonal levels were evaluated only in the cystinosis patients. In addition, clinical data, semen analysis results, percutaneous epididymal sperm aspiration (PESA) results, sexual hormonal profile, and testicular sections from the retrospectively included cystinosis patients were analyzed. For the testicular sections, morphological evaluation and immunohistochemistry were performed.
Main results and the role of chance: All testicular sections taken from three infantile cystinosis patients (3/3) showed the presence of testicular sperm and normal spermatogenesis, with a Johnsen's score of 7 to 9. Besides, epididymal sperm was present in two other infantile cystinosis patients (2/2), obtained by PESA procedure, which was only performed in those two patients. Hence, all of the five investigated infantile cystinosis patients showed either testicular or epididymal sperm (5/5 or 100%), indicating that testicular function was preserved in those patients. In contrast, seven out of the total eight infantile cystinosis patients underwent semen analysis, and they all presented with azoospermia (7/7 or 100%), including four out of the five patients that showed testicular or epididymal sperm, while a semen sample from one infantile patient, who underwent testicular biopsy, could not be retrieved. Meanwhile, the two juvenile cystinosis patients (2/2) showed a reduced sperm count (15.9 and 6.4 million/ml), and the ocular cystinosis patient (1/1) showed normal sperm count (71.6 million/ml). Remarkably, the scrotal ultrasound results revealed a significant higher epididymis caput diameter (normalized to the testis volume) in infantile cystinosis group compared with healthy controls (1.63 ± 0.66 mm/cm^3 for infantile cystinosis vs 0.50 ± 0.18 mm/cm^3 for controls, $p<0.001$).
Limitations, reasons for caution: The study is performed in a small group of patients; however, the rarity of the disease makes it challenging to recruit more male cystinosis patients. Moreover, we could not investigate the presence of testicular or epididymal sperm in all included cystinosis patients due to ethical reasons.
Wider implications of the findings: The results obtained in the study further unravel the origin of the observed azoospermia in male infantile-type cystinosis patient by suggesting an obstructive cause. In addition, our research might provide a better age-dependent treatment to circumvent infertility in these patients.
Trial registration number: Not applicable

P-062 Male ageing negatively affects the chances of live birth in IVF/ICSI cycles for idiopathic infertility.

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Study question: Does male age have an effect on the clinical outcomes of IVF/ICSI cycles adjusted by female age in idiopathic infertility?
Summary answer: Male ageing negatively affects clinical IVF/ICSI outcomes in women independent of female age in idiopathic infertility couples.
What is known already: The effect of male age (MA) on the outcomes of infertility treatments is controversial and poorly explored. In contrast, fertility declines significantly with female age (FA) beyond the mid-thirties and reduced oocyte quality plays an important role. The negative effect of male age on sperm quality is largely associated with an increasing susceptibility to sperm DNA damage which has a negative predictive factor for clinical outcomes. Although increasing maternal/oocyte age may diminish the efficiency of DNA repair, studies disregard the need to control for female age/infertility making it difficult to clearly define the role of male ageing in infertile couples.
Study design, size, duration: This cohort study analyzed records from 24,411 IVF/ICSI cycles performed at Monash IVF in Australia between 1992-2017. Separate analyses were performed for (1) 2,425 cycles of couples with idiopathic infertility (IIG) and (2) the general study group (GG). The primary outcome was live birth and secondary outcomes were clinical pregnancy and miscarriage. Male and female age were examined as continuous and categorical (MA: <40, 40-44, 45-49, 50-54, ≥55; FA:<30, 30-34, 35-39, ≥40) predictors.
Participants/materials, setting, methods: Couples with primary/secondary infertility who underwent IVF/ICSI cycles with male partners classified as normozoospermic were selected. Women with moderate to severe endometriosis, tubal factors, polycystic ovarian syndrome, ovarian hyperstimulation syndrome, poor responders (≤3 mature oocytes retrieved) and women with more than 15 cumulus oocytes complexes retrieved or who used cryopreserved gametes were excluded. Binary logistic multilevel modelling was used to identify the effect of MA and FA on clinical outcomes after controlling for cofounding factors.
Main results and the role of chance: For the idiopathic infertility subgroup, there was a negative effect of MA and FA on live birth (LB) [MA-OR: 0.959 (0.941-0.978); FA-odds ratio (OR): 0.904 (0.880-0.928) *p<0.0001*]. Possible confounding factors such as number of embryos transferred, type of treatment (IVF/ICSI) and embryo transfer day (Day3/Day5), and the interaction between male and female age were not statistically significant (p>0.05). The GG analysis (n=22,670 cycles) revealed a negative effect of both MA [0.971 (0.966-0.976) *p<0.0001*] and FA [0.922 (0.914-0.929)*p<0.0001*] on LB. Secondary outcomes in IIG showed significant reduction in the odds of clinical pregnancy (CP) [MA-OR: 0.972 (0.955-0.989); FA-OR: 0.919 (0.897-0.942) *p<0.0001*] and increase in the odds of miscarriage with older age: MA [OR: 1.045 (1.009-1.083); *p=0.002*]; FA [OR: 1.113 (1.048-1.182); *p<0.001*]. The interaction between MA and FA was not significant (*p>0.05*). Worse outcomes were associated with more cycles [CP-OR: 0.964 (0.932-0.996) *p=0.03*; LB-OR: 0.956 (0.919-0.994) p=*0.023*] while more inseminated oocytes were associated with better outcomes [CP-OR: 1.063 (1.031-1.065) *p<0001*; LB-OR: 1.070 (1.035-1.105) *p<0.0001*].Analyses for age categories showed that a negative effect of MA on all outcomes was observed only when the female partners were ≥30 years (*p<0.05*) with no effect of MA in women younger than 30 years (*p>0.05*).
Limitations, reasons for caution: Due to the nature of this study, selection bias may have been introduced, including limited information in confounding factors. The study may also be limited in its generalisability due the strict selection criteria. Age as category could potentially result in residual confounding due to categorizing a continuous variable.
Wider implications of the findings: These findings provide evidence of an effect of male age on fertility outcomes, independent of female age. By adjusting for female age/infertility etiology, we have more clearly defined the role of male ageing in the infertile couple. This study provides information for counseling of couples with idiopathic infertility.
Trial registration number: not applicable

P-063 Impact of obesity and type 2 diabetes on male infertility: relevance to seminal oxidative stress

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Study question: This study investigates the possible effect mechanism of reactive oxygen species induced by obesity and diabetes on sperm parameters in obese and diabetic's subfertile male.
Summary answer: We observed elevated ROS levels in semen samples of type 2 diabetic and obese subjects compared with normal donors
What is known already: Increased amount of ROS levels in men affected by either diabetes or obesity could be considered prognostic factors in subfertile patients, by affecting the hypothalamus-pituitary-gonadal (HPG) axis, hypothalamic–pituitary–adrenal (HPA) axis and DNA fragmentation, Following the generation of ROS the HPA axis becomes activated and releases cortisol in response to stress and the cross-talk between the HPG and HPA axes, negatively affect testosterone secretion from Leydig cells to produce enough testosterone
Study design, size, duration: A case-control study was conducted in men (n = 150) attending the Infertility Center of Royan Institute between December 2016 and February 2017. Participants were categorized into four groups; 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 (Nob-DM=35), and obese and type-2 diabetic (Ob-DM=35).
Participants/materials, setting, methods: The semen analysis was performed according to the World Health Organization criteria. Oxidative stress, DNA fragmentation, sperm apoptosis, and total antioxidant capacity (TAC) were evaluated in semen samples of men. Serum glucose, HbA1c, cortisol, and testosterone levels were determined using the ELISA method.
Main results and the role of chance: Compared with the control group, sperm motility, progressive motility, and normal morphology were significantly decreased in the obese, Nob-DM, and Ob-DM groups (P<0.01). The obese, Nob-DM, and Ob-DM groups showed significantly lower levels of TAC and higher amounts of oxidative stress, early apoptotic sperm, and the percentage of DNA fragmentation as compared with the control group (P < 0.05). Testosterone concentration was decreased in the obese, Nob-DM, and Ob-DM groups when compared with healthy individuals (P < 0.05), whereas the cortisol level was significantly increased in the Nob-DM and Ob-DM groups in comparison to the obese and control group (P < 0.01).
Limitations, reasons for caution: The main limitation of this study was the lack of our knowledge about the patient's lifestyle (especially for physical activity). Although we tried to age-matched our studied groups, it was not possible even with adjusting the age between experimental groups.
Wider implications of the findings: hormonal regulators of male reproductive functions can be affected by the disruption of the balance between ROS production and the antioxidant defence mechanism in the male reproductive
Trial registration number: no

P-064 IGF2 and IGF1R mRNAs are detectable in human spermatozoa

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Study question: Are *IGF2* and *IGF1R* genes expressed in human spermatozoa? If so, are their levels related with sperm concentration and total sperm count?

Summary answer: *IGF2* and *IGF1R*genes are expressed in human spermatozoa. The levels of their transcripts correlated positively with the sperm concentration and total sperm count.

What is known already: *IGF2* and *H19* are two imprinted genes; the former is paternally expressed, whereas the latter is maternally expressed. In mammals, *h19* and *Igf2* have been shown to influence the embryo-placental development:*H19* inhibits its growth through the inhibition of the *Igf1r* transcription, *Igf2* stimulates it. Infertile patients with oligozoospermia have lower *H19* DMR methylation rate compared with fertile normozoospermic men. This may possibly impact on sperm *IGF2* and *IGF1R* expression. However, no study has evaluated whetherthese genes are expressed in human spermatozoa so far.

Study design, size, duration: This was a cross sectional study. Twenty-two semen samples from Caucasian patients attending the Division of Andrology, University Teaching Hospital "G. Rodolico", University of Catania, for semen analysis were consecutively recruited, from January 2018 to March 2018. No exclusion criterion was adopted.

Participants/materials, setting, methods: Spermatozoa were subjected to reverse transcription quantitative PCR (RTpPCR) using specific primers for the detection of IGF2 and IGF1R mRNA levels. They were then correlated with patient conventional sperm parameters using the Spearman (τ) and Kendall (rho)'s ranks, as appropriated for not normally-distributed variables. The Student-*t* test was adopted to assess differences of expression in oligozoospermic patients compared to those with a normal sperm count. A p value <0.05 was considered as statistically significant.

Main results and the role of chance: The patients enrolled in this study were 32.1±9.3 (15-54) years old. They underwent to sperm analysis for the following reasons: andrological control (n=14), varicocele (n=6), premature ejaculation (n=1), infertility (n=1). Most of the patients were not interested to fertility at the time of counseling. IGF2 and IGF1R mRNA were both detectable at the sperm level. A statistically significant positive correlation was found between IGF2 mRNA levels and sperm concentration (τ=0.403, p< 0.01; rho=0.587, p<0.005) and total sperm count (τ=0.347, p<0.024; rho=0.509, p<0.015). IGF1R mRNA levels correlated positively with sperm concentration (τ=0.595, p<0.001; rho=0.774, p<0.001) and total sperm count (τ=0.547, p<0.001; rho=0.701, p<0.001). No additional correlations were found between the levels of these transcripts and the other conventional sperm parameters. Among the entire cohort, on the basis of total sperm count, two groups could be identified: Group 1 (n=5; age: 29.6±8.2 years), made up of patients with a total sperm count <39 million/ejaculate and Group 2 (n=17; age: 32.9±9.7 years), made up of those with a total sperm count ≥39 million/ejaculate. Both IGF2 (1.7±0.9 vs. 3.6±1.5, p<0.05) and IGF1R (1.8±0.6 vs 3.3±1.2, p<0.05) mRNA levels were significantly lower in Group 1 compared to Group 2.

Limitations, reasons for caution: Although our data showed that sperm IGF2 and IGF1R mRNA levels significantly correlated with sperm concentration and total sperm count, larger sample size will be needed to confirm these results.

Wider implications of the findings: Due to the paternally-imprinting of the *IGF2* gene which makes spermatozoa the only source of IGF2 in the early phase of fertilization, sperm *IGF2* and *IGF1R*mRNA may be translated in the oocyte and influence fertility and embryo-placental development. *Ad hoc* studies are needed to evaluate this hypothesis in humans.

Trial registration number: Not applicable.

P-065 Proliferation and differentiation of fresh and frozen-thawed pre-pubertal mouse spermatogonial stem cells in a sequential organotypic culture.

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Study question: To assess the impact of a sequential organotypic culture on the ability of fresh and frozen-thawed pre-pubertal mouse spermatogonial stem cells (SSCs) to proliferate and differentiate in vitro.

Summary answer: Organotypic culture in a sequential medium didn't promote the in vitro progression of spermatogenesis beyond the round spermatid stage and maintained the balance between proliferation/apoptosis.

What is known already: Each year, approximately 2550 new cases of cancer are diagnosed in children and adolescents. The recent advances in oncology have drastically improved the survival rates (up to 80%). Cancer treatment have several adverse side effects, particularly on SSCs.In prepubertal boys, fertility preservation should be considered by testicular tissue freezing. In order to restore fertility in infertile cancer survivors, in vitro maturation of frozen SSCs seems to be a possible option. However, the spermatogenic yield remains actually very low even in mouse model.

Study design, size, duration: Fresh and frozen-thawed testicular tissues from 6.5 days post-partum (*dpp*) CD-1 male mice were cultured using an organotypic culture system. Two type of culture media were compared : (i) a two-step culture, 21 days in a StemPro-34 SFM medium supplemented with EGF, bFGF, GDNF, LIF, Kit-Ligand and CSF, then 30 days in the basal medium containing α-MEM, 10% KSR supplemented with retinol (10^{-6}M), (ii) a one-step culture using the basal medium during 30 days.

Participants/materials, setting, methods: Histological analyses were used for the distinct experimental conditions tested as well as for in vivo control mouse testes, to identify the in vitro differentiation stage of germ cells. Cell proliferation (PCNA/Ki67), germ cell apoptosis (TUNEL assay/cleaved caspase 3), immature germ cells (TRA98), round spermatids (CREM/acrosin) and spermatozoa (acrosin and α-tubulin) will be evaluated by immunohistochemistry or immunofluorescence.

Main results and the role of chance: The sequential culture condition did not alter the structural integrity of testicular tissue and the balance between germ cell proliferation/apoptosis. However, this protocol did not allow the in vitro progression of spermatogenesis beyond the round spermatid stage. Surprisingly, the proliferation phase before differentiation did not allow the spermatogonial cell line enrichment, but promoted their entry into meiosis. In addition, our data show that the use of the vitrification protocol seems to be more favorable to the progression of in vitro spermatogenesis than controlled slow freezing.

Limitations, reasons for caution: Our data on in vitro spermatogenesis using organotypic culture was only obtained in a mice model of pre-pubertal testicular tissue maturation. These experimental conditions should be tested to human pre-pubertal testicular tissue.

Wider implications of the findings: The enrichment of the spermatogonial cell line wasn't obtained after culture in the presence of GDNF, EGF, bFGF, LIF, CSF-1 and Kit-Ligand and the sequential culture protocol tested didn't promote the complete in vitro spermatogenesis.

Trial registration number: not applicable

P-066 Prospective analysis of dietary pattern influence on sperm quality on male partners of couples undergoing IVF/ICSI

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Study question: Does following the European Dietary Recommendations influence the sperm quality in men of subfertile couples attempting IVF/ICSI?

Summary answer: Adequate kilocalorie, total fat, saturated fat and vitamin B9 intake are correlated with better sperm quality.

What is known already: Some dietary pattern studies have concluded that diets characterized by high intakes of fruits, vegetables, whole grains, fish and low intake of meat are associated with better semen quality. Yet, whether following the European DRV (Dietary Reference Values) of specific nutrients is associated with semen parameters remains largely unexplored.

Study design, size, duration: This was a multicentric prospective cross-sectional study of 144 men from couples attending 3 fertility clinics in Spain. The study was designed to evaluate the influence of habitual dietary pattern and lifestyle on fertility outcomes.

Participants/materials, setting, methods: Men aged 25-49 years, 51.3% overweight or obese, 35.9% smokers, with complete dietary and lifestyle data

were analyzed. Diet was assessed via a food-frequency validated questionnaire. Semen quality was evaluated according to World Health Organization 2010 guidelines. Multiple logistic regression analysis was used to evaluate associations between consumption of each nutrient and the likelihood of having abnormal semen parameters, after adjusting for potential confounders.

Main results and the role of chance: Men who have an adequate kilocalorie intake have a higher percentage of sperm with mobility grade a than men with an inadequate kcal intake (11.2 ± 11.3 versus 7.8 ± 6.98 respectively,p = 0.010). Men who have an adequate intake of total fats have a total concentration of sperm higher than those with an inadequate fat intake (156 ± 142 million vs. 115.9 ± 93 million sperm respectively, p = 0.011). Those who have an adequate intake of saturated fats have a higher total concentration of sperm (182 ± 183 vs. 123 ± 95 million sperm respectively, p= 0.011) and a higher percentage of sperm with mobility grade a (13.6 ± 13.8 vs. 8.7 ± 8.1 respectively, p = 0.01). Men who have an adequate vitamin B9 intake exhibit a higher percentage of sperm with mobility grade a than men with an inadequate intake (12.1 ± 12 versus 7.7 ± 6.6 respectively, p = 0.01). No statistically significant relationship was observed for any of the other parameters analyzed.

Limitations, reasons for caution: The main limitation of the study stems from its cross-sectional nature, limiting our ability to determine causality.

Wider implications of the findings: Male adherence to a healthy diet could improve semen quality. Following the European Dietary Recommendations shows a positive correlation with semen parameters. Since observational studies may prove associations but not causation, the associations summarized in the present study need to be confirmed with well-designed RCTs.

Trial registration number: N/A

P-067 New medium from green tea extract, glutathione and vitamin C (GGC) for activation of human spermatozoa in vitro

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Study question: Are the antioxidants: green tea, glutathione and vitamin C have an effect on human sperm parameters during *in vitro* activation (IVA)?

Summary answer: Green tea, glutathione and vitamin C used together in a new culture medium (GGC) and they observed synergism effect to improve sperm motility during IVA.

What is known already: Several factors obstruct sperm motility such as high viscosity and lipid peroxidation in sperm membrane, so that the uses of *in vitro* media with aqueous nature and antioxidant content lead to decrease the viscosity and reduce the lipid peroxidation, allowing sperms move more freely. Polyphenols in the green tea extract are proved to be of the most powerful antioxidants. Glutathione and vitamin C are also antioxidants known to fight free radicals and reduce the oxidative damage. To date, the effect of these three antioxidants together on the outcomes of the *in vitro* sperm activation has not been evaluated.

Study design, size, duration: The study was conducted on 250 semen samples from patients with asthenozoospermia, diagnosed by seminal fluid analysis according to World Health Organization (WHO) 2010 standard criteria, their age between (21-45) years, they attended the infertility outpatient clinic of High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University in Baghdad form September 2017 to June 2018.

Participants/materials, setting, methods: Each semen sample divided to three groups: (G1) activated with ferticult flushing medium, (G2) activated with GGC medium using (10 *mM/ml* of Green tea extract, 10 *mM/ml* of Glutathione, 60 *μM/ml* of Vitamin C, sodium pyruvate 0.01*g/L* and 10% human serum albumin added to 1*L* Ringer solution), and (G3) activated with GGC medium (with double concentrations of Green tee extract, glutathione and vitamin C). All samples activated by swimming up technique.

Main results and the role of chance: Sperm motility, progressive sperm motility and normal sperm morphology were significantly (P<0.01) increased in G1 which were (85.22±0.86) (60.12±1.02) (56.39±1.33) respectively and in G2 which were (97.18±1.28) (93.49±1.34) (77.06±1.24) respectively, while significantly (p<0.01) decreased in G3 which were (24.59±0.66) (20.55±0.54) (22.10±0.83) respectively as compared with pre-activation which were (36.18±0.91) (29.95±0.1.07) (38.46±0.85) respectively. Sperm concentration was significantly (P<0.01) decreased in activated groups (G1=21.96±1.59, G2=22.91±1.63 and G3=19.91±0.66) as compared with pre-activation (40.05±2.06). Among the activated groups, G2 was demonstrate significant (P<0.01) increased in sperm motility, progressive sperm motility and normal sperm morphology as compared with (G1 and G3), While G3 observed significant (P<0.01) decreases in these three parameters as compared with (G1 and G2).

Limitations, reasons for caution: This study was to evaluate the sperm parameter after *in vitro* activation. Further studies are required to evaluate the effect of GGC medium on successful rate of intrauterine insemination (IUI) and *in vitro* fertilization (IVF).

Wider implications of the findings: The new GGC medium was appropriate to improve sperm motility and morphology of asthenozoospermic patients by *in vitro* sperm activation using direct swimming up technique. The increased concentrations of green tea extract, Glutathione and Vitamin C gave opposite effect on the sperm parameters post activation.

Trial registration number: not applicable.

P-068 Does dual ovulation trigger offer any advantage over single ovulation trigger in IUI cycles – An open label RCT

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Study question: Whether co-administration of hCG and GnRH enhances the pregnancy rate in intra uterine insemination cycles

Summary answer: No significant difference was observed in pregnancy/patient or pregnancy /cycle in hCG and GnRH agonist group vs hCG alone for ovulation trigger in IUI cycle

What is known already: IUI increases the number of oocytes and sperms available for fertilization. hCG is the standard drug used to trigger final oocyte maturation and ovulation. Prolonged LH like action of HCG leads to the development of OHSS. GnRH trigger is used for final oocyte maturation in patients with high risk of OHSS. Recently concept of dual trigger with single bolus of GnRH agonist with reduced hCG dose for trigger has been investigated in IVF high responders and have demonstrated significant improvements in ongoing pregnancy and live birth rates. But its use in IUI cycle has not been studied.

Study design, size, duration: Open label randomized control trial, conducted over a period of one year from August 2017 to August 2018 after ethical approval in a teaching hospital India. Block randomization (2:2) was done according to computer generated random table into control (hCG trigger) and study group (hCG and GnRH trigger) Allocation concealment was done by sequentially numbered opaque sealed envelopes. Total women enrolled were 399, randomization done in 92. 70 IUI cycles done in both the groups

Participants/materials, setting, methods: Infertile women planned for IUI enrolled after consent. Bilateral tubal block, vaginal infection, ovarian cyst, AUB, primary ovarian failure, uncontrolled thyroid and adrenal dysfunction excluded. Follicular monitoring done by TVS. Injection HCG 10000 IU or 5000 IU IMI with GnRH agonist 1 mg sc administered at 17-18 mm follicle. IUI done 36- 40 hours after trigger. Failed ovulation labelled if follicle not ruptured 48 hours after trigger. Luteal support given, reviewed after fortnight for pregnancy

Main results and the role of chance: Demographic factors between two groups were similar. HCG alone group had 59 women while dual trigger group had 57 women. 85% women in both group belonged to urban area. Unexplained infertility was the most prevalent in 42.86% IUI cycles. In 74.29% clomiphene citrate and in 25.71% cycles letrozole was used for ovulation induction. Follicle ruptured in 90% IUI cycles in both the groups. Single IUI done in both the groups in 92.8% cycles. There was no significant difference in the distribution of women on the basis of endometrial thickness in both the groups. Pregnancy/patient was seen in 10.16% cases in HCG alone group vs 8.7% in Dual trigger group (p= 0.797). Pregnancy/cycle was seen in 8.57% in cases of HCG group vs 7.14% in dual trigger group (p= 0.753). Present study did not show any advantage of dual trigger over single ovulation trigger in IUI cycle. There were no side effects observed in either of two groups

Limitations, reasons for caution: Small sample size is its limitation. Determinants of success in IUI cycles are multiple including duration of infertility, drugs used for ovulation induction, endometrial thickness,

sperm preparation techniques, follicle and Oocyte quality and number of times IUI done in one cycle etc. and do not depend only on ovulation trigger.

Wider implications of the findings: Study has generated enough interest and future research potential. It is proposed for further multi-centric randomized trials with simultaneous study for other determinants of IUI success with bigger sample size

Trial registration number: Ref/2017/10/15731

P-069 Soluble CD147 triggers differential calcium mobilization and sperm functions in human sperm

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Study question: How did CD147 regulate different sperm functions in male and female reproductive tract?

Summary answer: We revealed the differential localization of membrane-bound CD147 and the differential calcium mobilization elicited by soluble CD147 before and after capacitation for sperm functions.

What is known already: CD147 is expressed as membrane-bound and soluble form and is required for male and female fertility. In male mice, the membrane-bound CD147 shuttles from the principal piece to the mid-piece of sperm during epididymal maturation. In female mice, CD147 null cumulus-oocyte complexes show a significant decrease in the fertilization rate. In human, we showed the presence of CD147 in seminal fluid and follicular fluid. Further, we showed that the decreased level of CD147 is associated with the decreased sperm motility and defective acrosome reaction in asthenozoospermia patients. However, the mechanism underlying the effect of CD147 on sperm functions remain elusive.

Study design, size, duration: We collected sperm samples from normal donors and evaluated the localization of CD147 and the effect of soluble CD147 on sperm functions and calcium mobilization with or without capacitation.

Participants/materials, setting, methods: The localization of CD147 on sperm before and after capacitation was assessed by immunofluorescence staining. Effect of CD147 on sperm functions was examined by treating sperm samples with CD147 neutralizing antibody or with soluble CD147 (recombinant protein or conditioned media containing soluble CD147). Sperm motility and hyperactivated motility were measured by computer-assisted sperm analysis (CASA), acrosome reaction was evaluated by fluorescein-Pisum sativum agglutinin (FITC-PSA) assay. Calcium mobilization was recorded by calcium imaging.

Main results and the role of chance: In ejaculates, the localization of CD147 was observed in the mid-piece. The localization of CD147 shifted from the mid-piece to the head region upon capacitation. Immunodepletion of CD147 in non-capacitated sperm significantly reduced sperm motility. Soluble CD147 treatment in non-capacitated sperm did not trigger hyperactivation. Intriguingly, upon capacitation, soluble CD147 induced a 3-fold increase in the percentage of acrosome-reacted sperm. The degree of acrosome reaction induction was comparable to that induced by progesterone, a well-established acrosome reaction inducer. Soluble CD147 elicits calcium mobilization in both non-capacitated sperm and capacitated sperm but the magnitude of mobilization was 2-fold higher in capacitated sperm. These results suggest that the shuttling of membrane-bound CD147 and the differential calcium mobilization triggered by soluble CD147 may underlie the differential regulation of sperm motility and acrosome reaction by CD147.

Limitations, reasons for caution: The mechanism underlying the CD147-induced calcium mobilization await further investigation.

Wider implications of the findings: CD147 triggers calcium mobilization and regulates sperm functions. CD147 is a potential diagnostic marker for defective sperm motility and acrosome reaction in male infertility.

Trial registration number: Not applicable

P-070 Sperm motility factor using CASA is related to PGS outcome.

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Study question: Is there an involvement between embryo aneuploidy and sperm motility factor using CASA?

Summary answer: Even if the sperm morphology is normal, motility related-factors affect the chromosome abnormality of the embryo.

What is known already: General semen analysis is subjective technique depending on the laboratory. Because, detailed description of sperm motility is not possible without use of special equipment. As an attempting to assess sperm quality accurately, computer-assisted semen analysis (CASA) has been developed. Using the CASA system, the several parameters for sperm motility are quantified and detailed.

Many previous studies reported that the sperm morphology affects the chromosome abnormality of the embryo. However, there is a lack of research on sperm motility and embryo aneuploidy. Therefore, in this study, we identified the effect of sperm motility analysis using CASA system on the PGS outcome.

Study design, size, duration: This is a retrospective study included 38 PGS cases between January 1, 2016 and August 31, 2018.

Participants/materials, setting, methods: The subject of study is the patient who needs PGS at the doctor's discretion with normal sperm. We analyzed sperm motility of raw semen by CASA system and carried out aCGH to determine chromosomal abnormalities of embryos. Patients are divided to two groups, Group I (with all abnormal embryos) and Group II (with one or more normal embryos). Then, we compared CASA parameters between 2 groups. The data are statistically analyzed by T-test using SPSS.

Main results and the role of chance: Among 8 sperm motility parameters (STR, LIN, WOB, ALH, VCL, VAP, VSL and BCF), the average of STR (straightness), LIN (linearity), WOB (wobble) and ALH (lateral head amplitude) showed similar pattern between 2 groups ($p>0.05$). However, VCL(curvilinear velocity), VAP(average path velocity), VSL(straight line velocity), and BCF (beat cross frequency) are significantly lower in group I, than group II (28.75 ± 6.90 vs. 37.84 ± 6.28, 19.08 ± 5.22 vs. 25.59 ± 3.60, 13.80 ± 5.08 vs. 19.71 ± 3.62, 4.05 ± 0.95 vs. 5.07 ± 0.86, $p<0.05$).

Therefore, 4 parameters from CASA (VCL, VAP, VSL and BCF) are related to the aneuploidy ratios of IVF embryos.

Limitations, reasons for caution: More subdivided patients are required for further study.

Wider implications of the findings: In addition to sperm morphology, sperm motility of raw semen might also be related to the PGS outcome.

Trial registration number: N/A

P-071 Antioxidants for male subfertility: a systematic review and meta-analysis

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Study question: Do oral antioxidants improve the live birth rate when taken by subfertile men of a couple attending a reproductive clinic?

Summary answer: This review shows that antioxidant supplementation in subfertile men may improve live birth rates for couples, however the quality of the available evidence is low.

What is known already: The inability to have children affects 10-15% of couples worldwide. A male factor is estimated to account for up to half of the infertility cases with between 25% to 87% of male subfertility considered to be due to the effect of oxidative stress. Oral supplementation with antioxidants is thought to improve sperm quality by reducing oxidative damage. Antioxidants are widely available and inexpensive when compared to other fertility treatments, however most antioxidants are uncontrolled by regulation and the evidence for their effectiveness is uncertain. We compared the benefits and risks of different antioxidants used for male subfertility.

Study design, size, duration: For the update of this Cochrane systematic review we conducted a search according to the Cochrane Gynaecology and Fertility search strategy for randomised controlled trials (RCTs) from inception to February 2018. After thorough literature search and review, 61 studies were included and 44 were found to be eligible for quantitative meta-analysis including 6264 subfertile men. Two review authors independently selected trials and extracted data for meta-analysis.

Participants/materials, setting, methods: Randomised controlled trials (RCTs) were included that compared any type, dose or combination of oral antioxidant supplement with placebo, no treatment or treatment with another antioxidant. We excluded studies comparing antioxidants with fertility drugs alone and studies that included fertile men attending a fertility clinic because of female partner infertility. Statistical analysis was performed in accordance with the Cochrane Gynaecology and subfertility Group guidelines.

Main results and the role of chance: Antioxidants may improve both, live birth rate (OR 1.79, 95% CI 1.20-2.67) and clinical pregnancy rate (OR 2.97, 95% CI 1.91-4.63). However, the quality of the evidence is low and the live birth result was based on only 124 live births from 750 couples in seven relatively small studies. When studies at high risk of bias were removed from the analysis, there was no evidence of increased live birth (OR 1.38, 95% CI 0.89-2.16). Adverse events were often not reported. We were uncertain whether antioxidants made a difference to miscarriage rates, (OR 1.74, 95% CI 0.40-7.60), as only three studies reported on this outcome and the quality of the evidence was 'very low'. Eleven studies reported on mild gastrointestinal upsets, (OR 2.51, 95% CI 1.25-5.03). The quality of this evidence was rated as 'very low quality', therefore we were uncertain whether the use of antioxidants made a difference to this outcome). We were unable to draw any conclusions from the antioxidant versus antioxidant comparison as insufficient studies compared the same interventions.

Limitations, reasons for caution: The quality of the evidence ranged from low to very low quality due to poor reporting of methods of randomisation, failure to report on live birth rate and clinical pregnancy, often unclear or even high attrition, and also imprecision due to often low event rates and small overall sample sizes.

Wider implications of the findings: Subfertile couples should be advised that overall the current evidence is inconclusive based on the above mentioned limitations and more studies are needed to investigate this further.

Trial registration number: Not applicable.

P-072 TRIM42 interacted with TRIM27, is required for spermatogenesis and testicular tumor

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Study question: Is there an association between the expression of Tripartite motif protein 42 (TRIM42) in human spermatogenesis and testicular tumor?

Summary answer: TRIM42 as an E3 ubiquitin ligase, interacted with TRIM27 could play multiple roles in the regulation of spermatogenesis and testicular tumor.

What is known already: Male infertility and testicular tumor are a common disease in young adults and these incidences have risen dramatically over the past several decades in many countries. Tripartite motif (TRIM) family proteins are involved in various biological and cellular functions including differentiation, development, proliferation, apoptosis, oncogenesis, innate immunity and viral autophagy. TRIM27, a known cancer-associated protein, associated with prostate cancer, endometrial cancer, ovarian carcinoma, breast carcinoma and spermatogenesis in testis. The expression and precise underlying mechanisms of Trim42 have not been clearly in human testis during spermatogenesis and risk of testicular tumor.

Study design, size, duration: A total of 126 individuals with azoospermia or oligozoospermia , 35 testicular tumor cases and 56 healthy controls were recruited to this study at the Reproductive Medical Center of Peking University Third Hospital between January 2016 and June 2018.

Participants/materials, setting, methods: TRIM42 were analyzed with blood and testicular biopsy samples from non-obstructive azoospermia and testicular tumor patients. Trim42 mRNA and protein levels in human testes were investigated using RT-PCR,Q-PCR and western blotting, respectively. Immunofluorescent analysis was performed on testis sections by TRIM42 antibodies during the development of spermatogenesis. And its interacted with a variety of functionally proteins was explored by co-immunoprecipitation and immuno-colocalization *in vitro*.

Main results and the role of chance: Trim42 mRNA and protein were abundantly transcribed in male germ cells in human. Immunohistochemical results revealed TRIM42 was abundant in the nuclei and cytoplasm of spermatogonia and primary spermatocytes. Moreover, TRIM42 was diffusely localized in the cytoplasm of spermatids and round spermatids, and functions as an E3 ubiquitin ligase collaborating with an E2 ubiquitin conjugating enzyme UbcH6 *in vitro*. In addition, TRIM42 interacted with TRIM27 and shared a similar distribution pattern *in vitro*. Furthermore, TRIM42 and TRIM27 expression levels were significantly changed during the normal group, azoospermia group and the testicular tumor patients group.

Limitations, reasons for caution: Protein analysis was performed in testicular biopsy samples from only a small number of patients and controls. In future investigations, a larger sample size should be used and the role of the other genes involved in the azoospermatism should be analyzed.

Wider implications of the findings: TRIM42 was an E3 ubiquitin ligase and interacted with TRIM27 could play multiple roles in the regulation of spermatogenesis and testicular tumor in human. TRIM42 connecting with TRIM27 could be a predictive marker for chemoresistance in testicular tumor patients and also a candidate for a molecular-targeted agent.

Trial registration number: N/A.

P-073 The Impact of Four Sperm Preparation Techniques on Sperm DNA Fragmentation, Motility and Concentration: A Prospective Study

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Study question: Is there a difference in sperm DNA fragmentation rate when the same semen sample is processed by four sperm preparation techniques?

Summary answer: Both direct and indirect swim-up were more effective at selecting lower percentages of spermatozoa with fragmented DNA compared to the density gradient technique.

What is known already: In assisted reproductive technology (ART), different sperm preparation techniques are used to select a motile spermatozoa population from semen. However, it has been shown that motility may not reflect the molecular composition of the spermatozoon. Specifically, the DNA integrity of the motile sperm has been identified as important factor for fertilization, pregnancy and offspring health. Moreover, sperm preparation techniques could have differential effects on the DNA fragmentation rate of motile sperm. Therefore, several studies attempted to compare the outcomes of these techniques in terms of sperm motility, morphology, and DNA integrity without reaching a firm consensus.

Study design, size, duration: A prospective auto-controlled study was conducted on 33 semen samples from 33 participants attending the Azoury IVF clinic. After liquefaction, sperm concentration, motility and DNA integrity were assessed. Each sample was then divided into four aliquots and each aliquot processed by one of the following techniques: direct swim-up, indirect swim-up, density gradient or density gradient followed by a swim-up. At the end of each experiment, sperm concentration, motility and DNA integrity were assessed.

Participants/materials, setting, methods: In order to perform the four different sperm preparation techniques on the same sample, only fresh semen samples with a minimum volume of 1.5 ml, a sperm concentration of $\geq$ 20 x 10^6/ml, and sperm motility of $\geq$ 30% were included. Before and after processing, sperm concentration and motility were assessed according to the World Health Organization guidelines (2010) and sperm DNA integrity was assessed using Sperm Chromatin Dispersion (SCD) test.

Main results and the role of chance: In this prospective study, the mean age of the participants was 39.10 years ± 8.47, the median of sexual abstinence days was 3.0 days, and the mean of infertility duration was 5.68 years ±3.12. After semen processing, lower sperm concentrations, higher percentages of motile sperm, and lower percentages of spermatozoa with fragmented DNA were detected in the 4 sperm preparation techniques compared to the unprocessed semen. Lower sperm concentrations were obtained after direct swim-up ($p<0.001$), indirect swim-up ($p<0.001$), and density gradient followed by swim-up ($p<0.001$) compared to the density gradient technique. Further, a higher percentage of motile sperm were recovered after direct swim-up ($p<0.001$), indirect swim-up ($p<0.001$) and density gradient followed by swim-up ($p<0.001$) compared to density gradient. In addition, lower percentages of spermatozoa with fragmented DNA were collected after direct swim-up ($p<0.01$) and after indirect swim-up ($p<0.001$), when compared to the density gradient technique. Interestingly, significant positive correlations were found between the percentage of sperm with fragmented DNA in the fresh semen and the percentages of sperm with fragmented DNA after direct swim-up ($R=0.91$, $p<0.001$), indirect swim-up ($R=0.81$, $p<0.001$), density gradient ($R=0.77$, $p<0.001$), and density gradient followed by swim-up ($R=0.76$, $p<0.001$).

Limitations, reasons for caution: Further studies are required to determine if these effects will ultimately affect patient outcomes in an ART setting including fertilisation rates, embryo development and pregnancy outcomes. Furthermore, additional experiments are needed to elucidate the molecular mechanisms underlying the outcomes of these sperm preparation techniques.

Wider implications of the findings: The two swim-up methods (direct and indirect) were less time-consuming, cost less, and resulted in lower sperm DNA fragmentation compared with the density gradient technique. Thus, using direct or indirect swim-up techniques could be beneficial for infertile men especially those with high DNA fragmentation.

Trial registration number: not applicable

P-074 Sperm quality and male fertility on chronic carriers of hepatitis B virus

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Study question: Is there a negative impact of chronic Hepatitis B virus (HBV) infection on sperm quality and male human reproductive potential in vivo and in vitro?

Summary answer: Hepatitis B surface antigen significantly affects sperm mobility and vitality. However, no significant impact was observed on male fertility both in vivo and in vitro.

What is known already: Chronic HBV infection leads to an adverse impact on sperm parameters and male fertility. The ability of HBV to integrate into human germ cells and sperm genome has been confirmed by cytogenetic and molecular studies suggesting the possibility of vertical transmission of HBV to offspring.

Study design, size, duration: This is a case-control retrospective study including 57 ejaculates of patients investigated for couple's infertility, over a period of 10 years.

Participants/materials, setting, methods: Semen analysis was carried out according to WHO method. Studied samples were divided in two groups : G1 (HBs Ag positive patients ; n=31) and G2 control group (HBs negative patients, n=26). We excluded from our study population all azoospermic patients or patients with any factor that may alter sperm parameters.

Main results and the role of chance: The mean values of sperm mobility and vitality were significantly lower than those in G2 ($p=0.01$, $p=0.04$; respectively) . These perturbations could be related to the process of apoptosis in SPZ induced by HBs antigen with structural and molecular sperm modifications. A history of paternity following spontaneous pregnancy was noted in 32.2% of G1 patients against 38.4% in G2 patients ($p = 0.4$). We didn't notice any significant differences in the outcome of assisted reproduction techniques (ART) between the 2 groups ($p = 0.3$). However, HBV replication within embryos conceived from HBV-contaminated SPZ may occur during the fertilization period with a risk of vertical father-child transmission.

Limitations, reasons for caution: A potential limitation for this study is its retrospective design. Moreover, patients come from different geographical regions with different HBV genotypes.

Wider implications of the findings: DNA viral integration into the sperm genome could interfere with male reproduction and offspring health. It would be important to deepen this work by conducting a molecular study in average and high endemicity areas where chronic hepatitis B constitutes a public health problem.

Trial registration number: No trial registration number

P-075 DNA methylation profile of spermatozoa varies regarding to the method of sperm preparation (MACS selection vs. DGC)

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Study question: How does magnetic-activated cell sorting (MACS) of non-apoptotic spermatozoa change the DNA-methylation profile of spermatozoa compared to spermatozoa prepared only by density gradient centrifugation (DGC)?

Summary answer: Reduced Representation Bisulfite Sequencing revealed 99 differentially methylated regions (DMRs) and 800 differentially methylated positions (DMPs) between MACS selected and DGC-only prepared spermatozoa.

What is known already: MACS procedure enables the selection of non-apoptotic spermatozoa with improved quality, especially in term of DNA fragmentation. Studies have shown that using such spermatozoa in ICSI/IVF procedures can improve fertilization rate, embryo/blastocyst quality (especially in women aged over 30 years) and pregnancy rate. Despite known positive effect for selection of spermatozoa with lower DNA fragmentation other influences of MACS on sperm genetic/epigenetic status are not known. Epigenetic status (especially methylation) becomes recently an important subject of interest, because it was shown that also epigenetic information can be transmitted transgenerational without changing the information on DNA level.

Study design, size, duration: Our prospective sibling-oocyte study included 19 couples who were treated in 2018 with ICSI due to male infertility, more precisely due to teratozoospermia (defined by strict Kruger criteria). The women in the couple included in the study were not older than 36 years and were required to have at least 6 mature MII oocytes after controlled ovarian hyperstimulation. Half of MII oocytes were fertilized using spermatozoa prepared only with DGC and half with MACS.

Participants/materials, setting, methods: MACS was performed after DGC using MACS® ART Annexin V System (Miltenyi). The remaining spermatozoa that were not used for ICSI, were evaluated for morphology, DNA fragmentation (HaloSperm) and stored in liquid nitrogen for epigenetic status in term of methylation using Reduced Representation Bisulfite Sequencing (RRBS). To detect differentially methylated regions (DMRs) and positions (DMPs) between DGC and MACS samples a two-dimensional Kolmogorov-Smirnov test (for DMRs) and Mann-Whitney U test (for DMPs) were used.

Main results and the role of chance: Of all stored samples, samples from 7 patients were used for RRBS analysis, of which 5 were successfully analysed. The comparison of MACS-selected and DGC-only prepared spermatozoa of these 5 patients showed that the percentage of morphologically normal spermatozoa (14.0±10.8 vs. 13.2±10.0;P=0.335) and of spermatozoa with fragmented DNA (39.4±14.8 vs. 47.3±22.8;P=0.183) were similar between the groups, although both of DNA-fragmentation indexes were abnormal. Fertilization rate and the quality of embryos was not significantly different, although there was a trend towards higher blastocyst rate in MACS-group (25.0% vs. 52.6%;P=0.097). The RRBS analysis identified 99 DMRs and 800

DMPs. 43 DMRs and 392 DMPs were hypermethylated in DGC-group, while 56 DMRs and 408 DMPs were hypomethylated. When DMRs were annotated to genes, 8.8% of them were annotated to promoters, 11.8% to exons, 33.0% to introns and 46.4% to intergenic regions. In case of DMPs, 5.3% were annotated to promoters, 5.4% to exons, 39.3% to introns and the rest to intergenic regions. When DMRs were annotated to CpGs, 16.1% were annotated to CpG islands, 6.5% to CpG shores and 5.0% to CpG shelves, while in case of DMPs, 5.3% were annotated to CpG islands, 18.0% to CpG shores and 7.1% to CpG shelves.
Limitations, reasons for caution: Due to protamine the DNA in spermatozoa is highly condensed, which makes RRBS analysis difficult and can negatively influence the quality of obtained sequencing data. Higher number of samples should be analysed to confirm obtained results.
Wider implications of the findings: The sperm DNA methylation profile could be used as additional test to assess the quality of spermatozoa. It could be also correlated to quality of embryos/blastocysts and could serve as prognostic predictor for laboratory outcome of IVF/ICSI cycles.
Trial registration number: 'not applicable

P-076 Targeted methylation of MAEL promoter leads to double-strand breaks and activation of transposable elements in human hypospermatogenesis

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Study question: Does the targeted methylation of MAEL promoter have a direct impact on human genome instability?
Summary answer: Targeted methylation of the specific MAEL promoter region (-188 to +294) might induce DNA instability by de-repressing transposable elements.
What is known already: The mouse *Maelstrom (Mael)* localizes in perinuclear nuage and participates in the piRNA-mediated defense system to protect the germ line from retrotransposons. The male MAEL knockout mice are sterile with phenotype presenting small testicles and intra-testicular meiotic arrest. With high throughput analysis in human testes, we have identified significantly higher methylation levels of 26 consecutive CpGs in the MAEL promoter in patients with hypospermatogenesis(HS) and non-obstructive azoospermia(NOA), compared to patients with normal spermatogenesis(NS). Our previous report has shown that aberrant methylation of MAEL promoter is associated with de-repression of LINE-1.
Study design, size, duration: The targeted DNA methylation system (TDM) has been created to study the consequences after methylating the MAEL promoter region(from -188 to +294) in human NCI-H358 cells. We use germ cell-enriched testicular cells from HS patients versus NS group to study the transcript levels of MAEL and LINE-1.
Participants/materials, setting, methods: MAEL and LINE-1 expression levels in TDM cells were determined by quantitative real-time RT-PCR and compared with un-methylated cells. We checked the double-strand breaks (DSBs) by immunofluorescence with histone γ-H2AX in TDM cells compared with un-methylated cells. The quantification of γ-H2AX foci number is calculated and analyzed. In patients with hypospermatogenesis and non-obstructive azoospermia, the mRNA transcript levels of testicular tissue were determined by quantitative real-time RT-PCR compared to normal spermatogenesis.
Main results and the role of chance: After target methylation of MAEL promoter region, MAEL expression levels of TDM cells declined dramatically compared to un-methylated cells ($P < 0.01$). Inversely, LINE-1 transcripts were significantly higher in TDM cells compared to un-methylated cells ($P < 0.01$). Similar results were found in the human testicles. The MAEL transcript levels were significantly lower in HS patients than the NS group($P < 0.05$). Otherwise, the increasing LINE-1 transcript levels were determined in the HS group versus the NS group ($P < 0.05$). Back to in vitro TDM model, there were significantly more γ-H2AX foci numbers detected in TDM cells than in un-methylated cells ($P < 0.05$).
Limitations, reasons for caution: It is difficult and ineffective to use human intra-testicular germ cells to study the epigenetic regulation of spermatogenesis. Therefore, we conducted the targeted DNA methylation system of MAEL promoter in human NCI-H358 cells to explore the direct impact of MAEL methylation on DNA integrity.
Wider implications of the findings: Our report has extended the knowledge of epigenetic regulation of MAEL promoter in human hypospermatogenesis and provide clues for future treatment of male infertility.
Trial registration number: not applicable

P-077 Adaptation of four Sperm DNA Fragmentation techniques to study ICSI-selected sperm shows a bias between this subpopulation and the whole ejaculate

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Study question: Is it possible to adapt current techniques to detect if ICSI-selected sperms are different from the whole ejaculate in terms of Sperm DNA Fragmentation (SDF)?
Summary answer: Adaptation of SDF detection techniques to study ICSI-S is possible and shows that this subpopulation has differences in SDF compared to the whole ejaculate.
What is known already: During ICSI procedures sperm cells are selected according to morphology and motility. Multiple authors suggest that ICSI-selected sperm (ICSI-S) may represent a subpopulation of the ejaculate with different chromatin integrity quality. This could explain those controversial results found when comparing SDF and ICSI clinical outcomes.

As current procedures do not allow the analysis of the injected sperm cell, the best approach would be to study those cells with good morphology and motility candidates for ICSI selection. The study of this ICSI-S could give us more information about the real impact of the male factor during an ICSI cycle.
Study design, size, duration:

This methodological and prospective study included 15 infertile patients attending our centre seeking fertility advice from January 2017 to May 2018. All patients signed the corresponding informed consent and the study was approved by the Parc Taulí Hospital Ethics Committee.
Participants/materials, setting, methods: A swim-up (SU) procedure was performed on every ejaculate (E) sample. These samples were analysed using of four different SDF techniques: Neutral Comet, Alkaline Comet, SCD test and TUNEL assay.

ICSI-S were selected and collected in groups of 10-20 to finally be fixed in (1) 1µL agarose drops on agarose pre-treated slides for Neutral Comet, Alkaline Comet and SCD techniques or (2) with Carnoy solution for TUNEL assay.
Main results and the role of chance: A total of 2650 sperm cells were selected using ICSI criteria and fixed depending on the technique later performed. Sperm visualization rates on the slide were 98% for Neutral Comet; 93% for Alkaline Comet; 98% for SCD and 96% for TUNEL, respectively, with a total amount of 2552 ICSI-S visualized. From these ICSI-S, 81%; 79%; 79% and 100%, respectively, could finally be analysed (2126 sperm cells in total). SDF on E and SU samples was analysed with these four techniques simultaneously to ICSI-S samples.

SDF measured with Neutral Comet did not show differences between E (69.8%±7.2), SU (68.2%±7.9) and ICSI-S (67.1%±17.4) samples. However, Alkaline Comet, SCD and TUNEL showed a statistically significant reductions in SDF of SU compared to E. Furthermore, these techniques detected an even major reduction in ICSI-S compared to E. SDF percentages for E, SU and ICSI-S samples were 41.4%±12.0, 32.3%±6.9 and 13.6%±11.5 for Alkaline Comet; 22.8%±9.2, 16.5%±10.3 and 3.0%±6.3 for SCD test; and 18.2%±4.3, 12.6%±3.8 and 1.0%±1.6 for TUNEL assay.
Limitations, reasons for caution: Methodological adaptations to study single sperm cells selected for ICSI has only been done on four SDF detection techniques. Other potential sperm quality biomarkers should be assessed using these, or similar, methodologies to analyse more parameters that can contribute to the male factor in ICSI procedures.
Wider implications of the findings: To our knowledge, this is the first study of SDF on ICSI-S with specifically adapted techniques. As ICSI-S presented reduced SDF in three techniques, we propose sperm quality biomarkers to be

tested on ICSI-S instead of on the ejaculate to improve the evaluation of the male factor in ICSI cycles.
Trial registration number: Not applicable

P-078 Multicolor Flow Cytometry as a screening tool for human sperm

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Study question: Is Multicolor flow cytometry (MFC) a relevant tool for the screening and sorting of defective sperm populations?
Summary answer: MFC is an innovative tool capable of detecting simultaneously, membrane and mitochondrial integrity, oxidative stress and DNA fragmentation, opening novel concepts to understand sperm damage.
What is known already: Classical seminal analysis underestimates the true prevalence of male factors in infertile couples, providing limited diagnostic and predictive information. These limitations leave patients and clinicians in need of additional tools and better diagnostic biomarkers, to evaluate factors that conventional protocols ignore; especially sperm abnormalities at biochemical and molecular level, which can explain the direct causes of infertility. MFC, with his capacity to evaluate simultaneously multiple sperm compartments/functions, represents the missing link between clinical observations and the intrinsic physiological mechanisms in spermatozoa and will guide the development and application of new diagnostic alternatives.
Study design, size, duration: As a model to test the power of the technique we studied the effect *in vitro* on human spermatozoa of 3 sunscreen UV-filters that have been reported to mimic the action of hormones and to affect adversely fertility in animal models. A total of 6 seminal samples (n = 6) from normozoospermic donors were used. Non-exposed and exposed sperm suspensions prepared by swim-up were analyzed considering a number of 10.000 cells for each condition.
Participants/materials, setting, methods: Sperm suspensions were incubated for 30' with the sunscreen UV-filters, 4-methylbenzylidene camphor (4-MBC), 3-Benzylidene camphor (3-BC) and Homosalate (HMS) at 10µM. An untreated suspension and 2 additional controls containing an un-coupler of oxidative phosphorylation, Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) 50µM and a pro-radical, H_2O_2 200µM were incubated simultaneously. Membrane integrity (Live/Dead fixable blue Stain), mitochondrial membrane potential (MitoTracker Deep Red), oxygen radicals production (CellROX Green) and DNA fragmentation (TUNEL) were evaluated by multi-color Flow Cytometry.
Main results and the role of chance: No significant alteration was found in the membrane integrity values for any of the conditions (mean% ±SD, $*p \leq 0.05$): 91.25±9.12 (Cntrl), 91.15±6.10 (4MBC), 88.38±11.74 (3BC), 80.65±11.74 (HMS). Regarding mitochondrial status, only sperm exposed to HMS revealed a statistically significant rise of the sperm numbers showing a decreased mitochondrial membrane potential: 8.98±3.23 (Cntrl), 12.30±05.87 (4MBC), 10.03±07.66 (3BC), 21.42±08.25* (HMS). Concerning oxygen radicals production, only sperm exposed to 3BC and HMS showed a statistically significant increase in the production of reactive oxygen species (ROS): 05.20±03.38 (Cntrl), 06.47±02.86 (4MBC), 18.81±04.58* (3BC), 28.12±08.41* (HMS). Contrary to what is reported in the literature, none of the conditions showed signs of DNA fragmentation, not even when showing high levels of ROS production: 03.53±0.60 (Cntrl), 03.30±1.18 (4MBC), 2.98±0.48 (3BC), 02.82±0.47 (HMS). Validation of this finding was provided by Sperm Chromatin Structure Assay (SCSA) showing no change in the DNA fragmentation index (DFI) associated to the treatments regarding to the control (DFI%): 0.56% (Cntrl), 0.48% (4MBC), 0.67% (3BC), 0.5% (HMS). MFC is therefore a novel tool for the screening of defective sperm populations, being able to simultaneously identify crucial parameters in individual sperm.
Limitations, reasons for caution: This study was carried out under *in vitro* conditions using model substances at pharmacological levels. The system needs to be tested for potentially harmful chemicals in physiological range to determine its clinical applicability.
Wider implications of the findings: The capacity of MFC to evaluate simultaneously multiple sperm functions in individual sperm opens new insights for sperm analysis. Scoring sperm populations, this assessment provides a more accurate in depth analysis compared to conventional methods. MFC has the potential to revolutionize research and diagnosis in spermatology.
Trial registration number: N/A

P-079 The effects of HIV infection and antiretroviral therapy on semen analysis parameters.

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Study question: Do HIV infection and antiretroviral therapy duration have a negative impact on sperm parameters?
Summary answer: HIV-infected patients receiving ART have increased sperm DNA fragmentation and a greater number of abnormal sperm and have a negative effect on sperm motility.
What is known already: The introduction of antiretroviral therapy (ART) has allowed couples in which the male partner is HIV-positive to safely reproduce. However, it is still unclear whether HIV and ART affect ejaculate volume or sperm motility, concentration, and morphology [Nicopoullos, 2004, Nicopoullos, 2010, Pilatz, 2014). It has been suggested that ART may increase sperm DNA fragmentation (Savasi, 2018). Therefore, the lack of consistent data on the specific features of reproductive disorders in HIV-infected males receiving ART prompted this study.
Study design, size, duration: A prospective case-control study was conducted in 115 men who sought fertility assessment and reproductive medical assistance.The main group consisted of 51 males with HIV-infection;the control group comprised 64 HIV-seronegative subjects with normal sperm concentration, motility,and morphology.A total of 97and 111 sperm samples, respectively, were evaluated.The inclusion criteria for the main group were a diagnosis of HIVinfection and the use of ART.The inclusion criteria for the control group were HIV-negativity and normal semen analysis results.
Participants/materials, setting, methods: The HIV infection status of study subjects was evaluated using data on the disease stage and phase, viral load level, CD3+ CD4+ , CD8+ lymphocyte counts, immunoreactive score, and ART duration.

The semen analysis parameters were assessed in accordance with the World Health Organization 2010 criteria (WHO, 2010).

The proportion of sperm with DNA fragmentation was obtained using the TUNEL method.
Main results and the role of chance: The median age of men with HIV-infection was 36 years(interquartile range:33-39).The median duration of HIV-infection at the time of study enrollment was 5 years(2-9 years).All subjects received combination ART: 30 men(48.8%) received nucleoside reverse-transcriptase inhibitors(NRTIs) in combination with non-nucleoside reverse-transcriptase inhibitors(NNRTIs), 16 patients)(31.4%) were on NRTIs and protease inhibitors(PIs), 4 males (7.8%) received NRTIs with integrase inhibitors(IIs), and 1 subject(2%) took three NRTIs.The median duration of drug therapy was 1.5 years.Normozoospermia was revealed in 48.4 % of the HIV-infected subjects. Abnormal semen analysis results included teratozoospermia (40%), oligo-astheno-teratozoospermia(22%), and astheno-teratozoospermia (16 %).The proportion of abnormal sperm was significantly higher in HIV-infected subjects than in HIV-seronegative healthy males(p=0.0001).Correlation analysis demonstrated a moderate negative relationship between the number of abnormal sperm and the CD4+ lymphocyte count (r = -0.362; p=0.026); statistically significant negative relationships were obtained between disease duration and the sperm concentration (r=-0.242;p=0.020), as well as the number of progressively motile(Category C) sperm(r=-0.241; p=0.024). We also observed a positive correlation between duration of HIV infection and the number of immotile sperm (r=0.220; =0.040).ART duration had a negative correlation with the number of progressively motile(Category C) sperm (r=-0.224; p=0.036).Sperm DNA-fragmentation was significantly more pronounced in HIV-infected patients than in healthy HIV-negative males(p=0.001).

Limitations, reasons for caution: no
Wider implications of the findings: no
Trial registration number: not applicable

P-080 Is it time to switch to automated semen analysis? A comparative double-blind study between 2 recent sperm analyzers and manual semen analysis

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Study question: How do CASA and electro-optical automated semen analyzers compare to the manual analysis performed according to WHO 2010 guidelines?
Summary answer: The most recent automated semen analyzers represent a trustworthy alternative to manual semen analysis when used by a well-trained staff
What is known already: Despite the WHO efforts to standardize manual semen analysis,the technique still lacks reproducibility and accuracy.This is due to semen heterogeneity itself,but also to inter/intra-operator variability.

Several automated sperm analyzers have been developed and improved over the last 20years,based on various technological approaches i.e. image analysis(CASA)or analysis of electro-optical signals.Their main objectives were to improve lab's workflow and the precision/accuracy of semen analysis via objective and high-throughput cell analysis.

Although most studies have reported modest or acceptable agreement between automated and manual semen analysis,their design and conclusions were heterogeneous and many andrology labs still remain reluctant to invest in this technology.
Study design, size, duration: This prospective, double-blinded, monocentric study comparing 3 diagnostic methods was conducted between February and May 2018. Manual semen analysis was used as gold standard. Results were then compared with those obtained with each of the 2 automated semen analyzers, i.e. Sperm Class Analyzer® (CASA) and SQA-Vision® (electro-optical).
Participants/materials, setting, methods: 102 consecutive patients presenting at the andrology laboratory for a routine semen assessment were included in the study, provided sperm sample volume was >2.5ml. Only patients with azoospermia were excluded. Manual semen analysis was performed strictly according to WHO 2010 guidelines. Each sample also underwent automated analysis with both CASA system (SCA®, Microptics) and electro-optical analyzer (SQA-Vision®, MES) by different staff blinded to each other. Staff was specifically trained, and both automates underwent daily QC.
Main results and the role of chance: Mean patients' age was 35±7.6 years. Mean abstinence delay was 4.5±2 days. Mean sperm concentration, progressive and total motility were not statistically different between the 3 methods (64.8 vs 68.1 vs 53.1 M/ml; 39.7% vs 41.3% vs 39.2%; 60.3% vs 54.8% vs 59.3%, p>0.05 for manual analysis, SQA® and SCA® respectively). Mean proportion of typical forms was significantly higher with SQA® (13.5%) than with manual (8.1%) and SCA® (8.1% and 8.6% respectively, p<0.01). However, very high specificity was found for the detection of teratozoospermia by the two automated systems (98.8% and 96.4% for SQA® and SCA® respectively).

The correlation coefficient between automated and manual measures was excellent for sperm count (r=0.953 and 0.933 for SQA® and SCA® respectively), fair for sperm progressive motility (r=0.624 and 0.697) and moderate for sperm morphology (r=0.39 and 0.55).

Both sperm analyzers had good repeatability regarding sperm concentration, progressive/total motility, and sperm normal morphology, with lower coefficients of variation than manual analysis in most cases.

Out of the 102 samples analyzed, 16 (15%) had a different overall interpretation of sperm analysis according to the method used, i.e. automated or manual.
Limitations, reasons for caution: Staff training is of utmost importance when using automated semen analysis in order to limit the detection bias associated with high viscosity or the presence of round cells or debris. The relevance of these systems in severe oligospermia cases should also be specifically tested.
Wider implications of the findings: Although each andrology lab should test and validate sperm analyzer locally before using in daily routine, these results pave the way for a larger implementation of automated semen analysis. Cost-effectiveness studies are needed to evaluate the overall benefit of automated semen analysis.
Trial registration number: Not applicable

P-081 Biallelic mutations in CFAP65 lead to human primary ciliary dyskinesia and male infertility

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Study question: There are only ~20 primary ciliary dyskinesia (PCD) genes known to cause male infertility. Novel causative genes still remain to be elucidated.
Summary answer: Biallelic loss-of-function mutations in *CFAP65* might be the genetic causes of PCD and male infertility in two unrelated families.
What is known already: So far, about 40 genes were identified to be responsible for ~70% human PCD cases. However, as not all PCD investigations explored the sperm parameters, only about 20 PCD causing genes are reported to cause male infertility. CFAP65 has once been reported to be a possible causative gene for MMAF, while the validation study and subsequent functional investigations were not performed.
Study design, size, duration: We conducted genetic analysis using whole exome sequencing (WES) in 42 individuals with PCD and male infertility from 40 unrelated families.
Participants/materials, setting, methods: WES was performed in 42 individuals with PCD and male infertility from 40 unrelated families. Mutation screening of *CFAP65* was performed using WES in a cohort of 637 non-PCD individuals, including 219 asthenozoospermia, 195 non-obstructive azoospermia, and 223 fertile controls. Ultrastructural and immunostaining analyses of patient's spermatozoa were performed to characterize the impact of the variant.
Main results and the role of chance: One homozygous nonsense mutation (NM_194302, c.G5341T:p.E1781X) and two compound heterozygous mutations (c.C2284T:p.R762X and c.1751delC:p.P584fs) in *CFAP65*, which may lead to protein degradation, were identified in two unrelated PCD and male infertility families, respectively. The mutations were absent in 637 non-PCD individuals. Immunostaining showed that CFAP65 localized in the acrosome and flagellar midpiece of normal spermatozoa. Ultrastructural and immunostaining analyses of the spermatozoa from one patient showed highly aberrant sperm morphology with severe defects such as disruption of mitochondrial sheath, defect of acrosome and absence of central pair complex and annulus.
Limitations, reasons for caution: The evidence of the effect on patients' cilia caused by *CFAP65* mutations was insufficient, further investigations with screening large numbers of patients with PCD and male infertility and the generation of knockout mouse models are required.
Wider implications of the findings: We firstly identified *CFAP65* might be a cause of PCD and male infertility, and proposed that *CFAP65* could play an essential role in cilium/flagellum assembly and acrosome formation in humans. This finding has important clinical implications for genetic and reproductive counseling of affected families.
Trial registration number: None.

P-082 A microfluidic device to investigate chemotactic behavior of spermatozoa

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Study question: Can chemotactic behavior of spermatozoa be investigated using a hydrogel-based microfluidic device?
Summary answer: Hydrogel-based microfluidic device was successfully fabricated in which spermatozoa showed chemotactic behavior towards the chemical attractant.
What is known already: Chemotaxis is a movement changing behavior of organisms when there is a chemical gradient in their environment. Sperm

chemotaxis is the process where spermatozoa move towards an oocyte with help of its secretions. Therefore, it has been thought that chemotaxis plays a key role during fertilization. The content of the chemoattractants is uncertain, however, progesterone was proposed to be one of them. Sperm chemotaxis has been investigated with microfluidics, thereby easily mimicking the environment and controlling the parameters. Often these devices are made of polydimethylsiloxane or polymethylmethacrylate. Here we developed a novel hydrogel-based microfluidic device to assess the sperm chemotaxis.

Study design, size, duration: This study was performed with boar spermatozoa that were taken from Varkens KI Twenthe (Fleringen, the Netherlands). Sperm samples ($20x10^6$ cells/ml) were used within 1 to 4 days. Our microfluidic chip consists of 2 side channels and 1 middle channel which has 5 side chambers on each side and 6 inlets/outlets in total. To perform the chemotaxis experiments, 10µl sperm cells samples were injected into the middle channel.

Participants/materials, setting, methods: The microfluidic device was made from a gelatin/agarose mixture and covalently bonded to a glass slide with a surface coating of APTES (3%) and glutaraldehyde (10%) solutions. A fluorescein sodium salt solution (0.005%, MW: 332g/mol) was used to optimize the diffusion time, since its molecular weight is comparable with progesterone's (MW: 314g/mol). Progesterone (1µM) was injected into a side channel and empty chips were used as a control. Results were analyzed using CASA and RStudio.

Main results and the role of chance: Gelatin/agarose mixture (8 wt%:1 wt%) was preferred to fabricate hydrogel-based chips, since pure agarose chips caused cell death and gelatin was more compatible but has a melting point lower than 37°C which is a problem since chips were heated to that temperature during experiments. By using a cell counter plugin in ImageJ, the spermatozoa were counted. We defined a ratio called 'chemotaxis ratio'. It is the proportion of the number of cells in progesterone applied side of the middle channel vs. the other side of middle channel. If this ratio is bigger than 1, it means that spermatozoa moved towards to chemoattractant. Results showed that chemotaxis ratio was between 2 and 1,3. When progesterone was applied to the opposite side the chemotaxis ratio was between 1,6 and 1,3. For our control group, it was 0.97. This confirms that the chip did not affect the cell movement. Additionally, compared to the control chips, there was a significant difference in cell movement and accumulation. Therefore, progesterone has an effect on spermatozoa's chemotactic behavior and our chip can be used to quantify this.

Limitations, reasons for caution: During this study, boar spermatozoa were used. There may be other chemicals which have not been investigated that have affects the spermatozoa behavior other than progesterone. Furthermore, the counting of spermatozoa was done manually with ImageJ cell counter, making it prone to errors.

Wider implications of the findings: The progesterone experiments and developed hydrogel-based microfluidic chip are helpful to understand chemotaxis behavior in mammal's reproductive system and the chemoattractant effect on spermatozoa. Moreover, this study might be useful for sperm selection in the clinical researches.

Trial registration number: Not applicable.

P-083 Heavy metal level in human semen and oxidation-reduction potential.assessment by MiOXSYS.

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Study question: The purpose of this study is to merge and analyze correlations between heavy metals and oxidation-reduction potential (ORP) measurements in human semen with normal and low fertilities .

Summary answer: There were positive correlations between Lead concentration and ORP in human semen with normal and low fertilities .

What is known already: Cadmium (Cd), lead (Pb), selenium (Se),copper (Cu) and molybdenum (Mo) are well-known reproductive toxicants which humans are exposed to occupationally and environmentally, and can lead to negative effects on the sperm functions. Both are pervasive in the human environment and accumulate in the human body over a lifetime. Heavy metals have a strong capacity to induce oxidative stress in body cells by disintegration of the lipid membrane.

Study design, size, duration: The presented study concerned 50 patients (examined group) and 50 fertile donors (control group) aged 25-35, and was conducted in 2018 in the 'Ovum - Reproduction and Andrology' Non-Public Health Care Unit in Lublin, (Poland).

Participants/materials, setting, methods: All semen samples were analyzed for heavymetals (Cd,Pb, Se,Cu,Mo) by flame emission atomic absorption spectrophotometer and ORP by the MiOXSYS System.The results of the study obtained were subjected to statistical analysis. T Mann Whitney U test was applied to compare the levels of heavy metals and ORP in the examined groups. The relationships between concentrations of heavy metals and ORP were tested using r-Pearson correlations.

Main results and the role of chance: The study showed that the levels of Pb (Z=−3.480; p=0.029) and Cd (Z=−4.980; p=0.018) were higher in the plasma of males with reproductive disorders, compared to the control group. There were no statistically significant differencies between the levels of Se, Cu, and Mo in examined groups. Higher ORP values were presented in infertile semen samples when compared to those of healthy (Z=−2.970; p=0.027). In the case of males with reproductive disorders, possitive correlations were observed between Pb(r=0,155, p=0,039)., Cd (r=0,189, p=0,031) and ORP. There were no statistically significant correlations in the other cases.

Limitations, reasons for caution: Limitation of this study is the small sample size.

Wider implications of the findings: This data suggests that Pb and Cd may be a cause of oxidative stress in infertile patients.

Trial registration number: 188/ES

P-084 Prevalence of human papilloma virus in semen and sperm DNA fragmentation

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Study question: The aim of the present study was to investigate the implication of HPV infection on sperm DNA fragmentation.

Summary answer: HPV seminal infection is probably responsible of a higher DNA fragmentation index.

What is known already: HPV infection is a common sexually transmitted disease, related to genital warts and cancer. In males, HPV DNA has been found in the seminal fluids. HPV is commonly present in semen samples, modifying spermatic parameters as volume, count, motility, viability, viscosity, pH. However, whether the presence of HPV in semen is actually associated with sperm DNA fragmentation has yet to be elucidated.

Study design, size, duration: From January to December 2017, 303 infertile couples were analysed at the Human Reproduction Unit of Ioannina University Hospital. A total of 250 clinical and laboratory data from male partners were included in the study. Sperm DNA fragmentation was compared among those with or without HPV in spermatozoa.

Participants/materials, setting, methods: To detect the presence of HPV a real time polymerase chain reaction assay was performed. All patients underwent a sperm chromatin dispersion semen analysis. The amount of DNA fragmented sperm, expressed in DFI, was valuated using the sperm chromatin dispersion test. In each sample 300 spermatozoa were counted at fluorescent microscope. DNA fragmentation index was compared among HPV positive and HPV negative men.

Main results and the role of chance: 39 (15,6%) of the total semen samples were HPV positive. Overall, 28/250 (11,2%) and 11/250 (4,4%) patients had high risk HPV and low risk HPV, respectively. The amount of DNA fragmented sperm between HPV positive and negative males was different. SDF values were higher (P = 0.005) in infected men compared to those without HPV.

Limitations, reasons for caution: Main limitation is the relatively small sample size.

Wider implications of the findings: Seminal HPV infection was frequently associated with lower sperm quality. Few studies focused attention on the relationship between HPV and sperm DNA fragmentation. The results suggest that HPV infection probably impairs sperm DNA quality. These observations point out the importance of testing seminal HPV presence in everyday clinical practice.

Trial registration number: None.

P-085 Ultrastructural analysis of lyophilized human spermatozoa

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Study question: The aim of our study was to evaluate the ultrasctructure of lyophilized spermatozoa using transmission electron microscopy.
Summary answer: Lyophilization of spermatozoa using glycerol-containing media increase ultrastructural damage. Also, the use of equipment with fixed pressure and temperature control impairs the result.
What is known already: Freezing-thawing spermatozoa is an excellent alternative for male fertility preservation. The use of liquid nitrogen is associated to detrimental effect on sperm structure/function and has some drawbacks such as maintenance cost, storage space, transportation difficulties and risk of contamination. Lyophilization might be an alternative since there is no need for storage and transportation is simpler. As it is associated to virus inactivation, the risk of contamination will be eliminated. The feasibility of this method has been reported in animal studies, but in human, only a scarce data has been published without evaluation of the ultrastructure.
Study design, size, duration: We performed an observational study with 21 healthy men who had been subjected to assisted reproductive technology treatment, from August 2014 to August 2015, and donated their remaining semen for the study. The study was approved by the Research Ethics Committee.
Participants/materials, setting, methods: A total of 30 samples were analysed according to the WHO criteria. Samples were divided into two aliquots: cryopreservation and lyophilization and cryopreservation only. Samples were frozen using Freeze Medium or Sperm Freeze Solution. Aliquots for lyophilization were placed in vials loaded into a lyophilization equipment under a pressure 50-100 µbar and temperature at -50°C . Samples were kept at 4°C until rehydration. After rehydration and thawing, samples were analysed under transmission electron microscopy.
Main results and the role of chance: After rehydration, aliquots were fixed for analysis in transmission electron microscopy. Analysis of the aliquots showed that the spermatic morphology was intact, but without motility.

Samples lyophilized with *Freeze Medium* (Irvine Scientific) and *Sperm Freeze Medium* (Vitrolife) cryoprotectants were severely compromised. Spermatozoa heads had ruptured plasma membranes, absence of acrosome, nucleus with heterogeneous and decompressed chromatin. Midpiece mitochondria were degenerate. Longitudinal columns of dense fibers were absent in the flagellum. Also, degenerate or disorganized structures were observed in axonemes. Samples freeze-dried with glycerol-containing media were not lyophilized successfully, which led to organelle damage.

Control group that was frozen with cryoprotectant showed some lesions in midpiece, degeneration of some mitochondria and ruptured plasma membrane in some points. Most spermatozoa showed an intact plasma membrane, nucleus and acrosome. In the flagellum main structure structure with plasma membrane, longitudinal columns of dense fibers and conserved semicircular fibers.

Fresh spermatozoa showed intact head and plasma membrane, nucleus with compact and homogeneous chromatin, normal acrosome and subacrosomic space. In midpiece, we observed mitochondria and external dense fibers. Transverse sections of axonemes from flagellum showed plasma membrane involving the sheath of dense fibers, nine pairs of peripheral microtubules plus 2 pairs of central microtubules.
Limitations, reasons for caution: Due to equipment limitations on pressure and temperature control during lyophilization we understand that the use of more complex equipment might aloow better results. Therefore, more studies are necessary in order to optimize lyophilization technique.
Wider implications of the findings: Lyophilization is expected to be an alternative for sperm preservation, however new studies are necessary to improve the results before it can be offered in clinical basis.
Trial registration number: not applicable

P-086 Sperm telomere length is correlated with sperm quality in normozoospermic patients

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Study question: Is sperm telomere length (STL) correlated with sperm quality in infertile patients?
Summary answer: Sperm telomere length was significantly correlated with total sperm count and sperm fragmentation only in normozoospermic patients.
What is known already: Telomeres play an important role in human reproduction, including an active intervention during gametogenesis, fertilization and preimplantation embryo development. Since short telomeres are associated with cellular disfunction, it has been hypothesized that short STL is associated with poor sperm quality and male infertility. Recent studies have investigated this association in order to ascertain the suitability of STL as a new biomarker of sperm quality. However, published results are not consensual, differing in the infertile population characteristics under analysis regarding the type of sample (raw semen or swim-up samples), sample size, patients age range, seminal characteristics and infertility diagnosis.
Study design, size, duration: The present study was performed in swim-up samples containing rapid progressive motile sperm, to deliberately exclude sperm not used in assisted reproductive technology treatments. Samples were obtained from 78 treatment cycles of In-vitro Fertilization or Intra-cytoplasmatic Injection, between October and December 2018. Patients were randomly selected, and posteriorly subdivided into groups according to their seminal evaluation: 33-normozoospermic, 35-oligozoospermic, 9-asthenozoospermic, 35-teratozoospermic, 4-oligoasthenozoospermic, 8-oligoteratozoospermic, 7-asthenoteratozoospermic, and 3-oligoasthenoteratozoospermic, for a detailed statistical analysis.
Participants/materials, setting, methods: Spermiogram analysis was performed in raw semen samples. In the corresponding swim-up samples, average STL was measured by quantitative polymerase chain reaction, DNA fragmentation was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labelling assay, chromatin maturity was evaluated by aniline blue staining, chromosomic alterations were evaluated by fluorescent in situ hybridization and the oxidative profile was evaluated by Slot blot. Statistical analysis determined the correlation between STL and the different sperm quality parameters evaluated.
Main results and the role of chance: When analyzing the totality of the patients, no significant correlations were found between STL and the broad panoply of sperm quality parameters, except for sperm DNA fragmentation. A negative significant correlation between STL and sperm fragmentation in the post-acrosome region was observed, either in the totality of the patients (n=38, r=-0.371, p=0.022) or in normozoospermic patients (n=22, r=-0.464, p=0.03). Evaluation of the different fragmentation staining patterns has been recently discovered. Our results reinforce the importance of pattern differentiation in order to obtain a full characterization of sperm DNA fragmentation. The post-acrosome region is demarcated as having decondensed chromatin, making it more prone to fragmentation, which may favor STL shortening. In

addition, a positive significant correlation between STL and total sperm count was observed only in normozoospermic patients (n=33, r=0.361, p=0.039). Sperm concentration may be affected by short STL due to induced apoptosis when STL reaches a critical point. However, altered seminal parameters erase this correlation, indicating that sperm concentration can be altered independently of STL. The present results thus suggest that although STL cannot be considered a general indicator of sperm quality, it might have a biological significance.

Limitations, reasons for caution: Additional studies with an increased sample size in the different clinical groups will help to validate the present statistical results.

Wider implications of the findings: Our study clarifies that different sperm sample characteristics may lead to discrepant results when evaluating the importance of STL in male infertility. We conclude that STL is not a suitable biomarker of sperm quality. However, given the importance of telomeres in reproduction, STL may still be associated with treatment prognosis.

Trial registration number: not applicable.

P-087 Abnormal sperm mitochondrial membrane potential in "normozoospermic WHO patients": outcome in ICSI patients

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Study question: Is abnormal sperm mitochondrial membrane potential (MMP) at Andrositol Test (AT) related to ICSI outcome in infertile couples presenting normozoospermia according to WHO, 2010 parameters?

Summary answer: In infertile ICSI couples presenting normozoospermia by WHO, 2010 parameters, abortion rate was found to be significantly higher in "abnormal" than in "normal" AT group.

What is known already: Poor sperm motility and morphology according to WHO parameters, interfere with fertilization and ICSI outcomes. "Idiopathic male infertility" in WHO normozospermia still represents a difficult area where complex and expensive tests have been proposed. Andrositol® Test (Lo.Li. Pharma) is a simple and economic diagnostic procedure capable to qualitatively evaluate sperm MMP ("normal", "medium" and "poor") and allows to identify subfertile normozoospermic patients.

Study design, size, duration: The study is a retrospective review of semen samples investigated in 39 infertile couples undergoing ICSI over a 16 months period.

Participants/materials, setting, methods: 39 sperm samples with "normal" (WHO, 2010) sperm parameters from couples with "tubal factor" undergoing ICSI treatment, in a private fertility clinic, were analyzed by AT. Two groups were identified: group A, with "normal" AT and group B with "abnormal" AT. No differences were present in clinical parameters, stimulation and laboratory figures. Implantation, pregnancy and abortion rates were calculated.

Main results and the role of chance: Implantation and pregnancy rates were similar, on the contrary, abortion rate was found to be significantly higher (33%) in "abnormal" AT group (Group B) than in "normal" AT group (Group A) (16%). Since the clinical and laboratory parameters were similar in both groups, the increased abortion rate in Group B could be tentatively attributed to a post blastocyst negative factor related to abnormal sperm MMP.

Limitations, reasons for caution: Retrospective design and small sample size limit our results.

Wider implications of the findings: Whether confirmed in future studies, inositol supplementation should be considered, in vivo or in vitro, in male patients undergoing ICSI techniques presenting "abnormal" AT.

Trial registration number: The protocol was approved by the local Institutional Review Board.

P-088 Sperm p53 concentration: a potential new biomarker for environmental pollution. Preliminary data.(EcoFoodFertility Project).

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Study question: We evaluated the p53 protein concentration in sperm cells to test whether environmental pollution can affect expression levels of this protein.

Summary answer: We found a significant difference in p53 levels between two homogeneous groups by age and lifestyle, residents in two areas with different environmental impact.

What is known already: The World Health Organization (WHO) has placed among its priority objectives the understanding of the relationships between the sources of pollution and the effects on human health, which they represent a cause of surprisingly high mortality and morbidity.

International scientific literature shows that strong environmental impact may jeopardize the stability and integrity of cellular DNA. This impairment can affect cellular functions, such as the uncontrolled growth of cells with the development of neoplastic process, until the final process of cell death (apoptosis). P53 seems to play a key role in these mechanisms, as it coordinates cell fate.

Study design, size, duration: 237 healthy males, 18-36 years old, were observed according to their stable residence in low environmental impact area (LEIA) or High environmental impact area (HEIA) of the Campania region (Southern Italy) in a period between July 2014 and June 2018. The study group has been divided into Group A: 109 permanent residents in LEIA, aged 19 - 36 years; Group B: 128 permanent residents in HEIA, aged 18 - 35 years.

Participants/materials, setting, methods: All partecipants were no smokers, not habitual alcohol drinkers, no professionally exposed to environmental pollutants, without varicoceles, prostatitis, urethritis or chronic diseases. Semen analysis was assessed according standard criteria of WHO Manual, fifth edition (2010). All semen samples were examined 30 minutes after collection and immediately processed for the p53 protein assay, using ELISA test, proposed by Raimondo et al. (2010)[1]. Quantitative dosage of p53 protein was expressed in ng / million of spermatozoa.

Main results and the role of chance: We have observed not significant differences in ejaculate volume between group A and group B. About sperm concentration, 44,9% (40/109) of group A samples were normozoospermic while only 20,3% (26/128) of group B samples were normozoospermic. The quantitative dosage of p53 protein has been corrected in relation to sperm concentration number and shows statistically significant differences between two groups: group A had a minimum value of 0,29 ng/million of sperms and a maximum value of 4,05 ng/million of sperms, with a mean value of 1,74 ng/million of sperms; group B had a minimum value of 0,69 ng/million of sperms and a maximum value of 14,36 ng/ million of sperms, with a mean value of 6,45 ng/million of sperms.Statistical analysis of the two groups was performed using Fisher's correlation and then Student's test and we obtained a $p < 0.0005$.The differences observed in this study underlines the efficacy of quantitative dosage of p53 protein in identify a cell suffering due to environmental pollution.

Limitations, reasons for caution: This is a preliminary observational study on a small number of samples. Reasons for caution could be due to unknown confounding factors.A large number of tests are need to confirm our results.

Wider implications of the findings: p53 protein is well known as "guardian of the genome" for its key role in determining the fate of the cell following DNA damage. Quantitative dosage of p53, seems to give valuable informations on the degree of damage to sperm DNA by environmental pollution, suggesting it as potential new biomarker.

Trial registration number: it's a retrospective observational study.

P-089 Association between tobacco smoking and genetic variations of protamines genes PRM1 and PRM2

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Study question: Does Tobacco smoke cause genetic variations or polymorphisms in the DNA sequence of protamine genes PRM1 and PRM2?
Summary answer: Tobacco smoke has no effect on genetic variations of PRM1 and PRM2 genes
What is known already: The epigenetic regulatory process as much as DNA demethylation in spermatozoa affect the Protamination process during spermatogenesis. This protamination is important for the protection and stability of the paternal genome and its fertilization capacity during its passage and delivery to the oocyte.

Tobacco smoke is one of the most risk environmental factors that altered the male reproductive function by reducing semen parameters and causing impairment in sperm DNA packaging. Because of its harmful compounds and reactive intermediates like ROS and reactive nitrogen species, smoking may induce a number of genetic and epigenetic changes.
Study design, size, duration: A prospective controlled trial carried out between December 2017 and December 2018 at the Department of Obstetrics and Gynecology, University of Saarland, Germany.. 141 semen samples were included in this study.
Participants/materials, setting, methods: Semen samples of 98 heavy-smokers (G1) and 43 non-smokers (G2) were prepared and purified according to WHO guideline 2010 and sperm nuclear DNA was extracted using Isolate II DNA/RNA/Protein kit then concentration and purity were verified with Nanodrop ND-2000c. PRM1 and PRM2 genes were amplified by PCR and sequenced using Sanger sequencing technique. Protamine deficiency (CMA3+) and sperm DNA fragmentation (sDF) were assessed by Chromomycin CMA3 and TUNEL assay respectively.
Main results and the role of chance: The sperm concentration, progressive motility, normal morphology, CMA3+ and sDF in G1 were (70.46±55.59 mill/ml; 15.78±11.66%; 4.32±2.93%; 33.30±22.33%, 26.68±19.77% and the corresponding values for G2 were (98.56±64.63mill/ml; 31.42±22.24%; 12.91±12.76%; 20.35±13.34%; 14.23±13.07%, respectively). Four single nucleotides polymorphisms (SNP) were identified: rs737008 for PRM1, and rs2070923, rs1646022 and rs424908 for PRM2. In G1, sperm parameters were significantly lower ($p<0.05$) than G2 and CMA3+ and sDF were significantly higher ($p<0.01$).

By testing the association between SNP alleles and each group to find differences in allele distribution among G1 and G2, none of the SNPs were reported significant. Besides, by investigating the associations between these SNPs and the studied parameters, none of the SNPs showed a significant association with any of the phenotypes.
Limitations, reasons for caution: The size number of the sample
Wider implications of the findings: Smoking induces an epigenetic change that alters the gene expression without changing the DNA sequence of PRM1 and PRM2 genes.
Trial registration number: Basic Science

P-090 Male infertility in Ghana: an untold phenomenon. How well known is male infertility among men and women in a Ghanaian metropolis

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Study question: What is the level of knowledge on male infertility among men and women in a Ghanaian metropolis and what factors influence this knowledge?
Summary answer: Most (66%) men and women in Ghana have limited knowledge about male infertility, being married and educated enhanced knowledge on male infertility
What is known already: Research has shown there is little knowledge about infertility, and specifically male infertility, in most sub-Saharan African communities. A patriarchal system is predominant in many African communities and men often do not see themselves as a possible cause of infertility in a family. However, it is known that male and female factors contribute equally to a couple's inability to have a child. A recent study performed in Cameroon (West Africa) has shown that 76.8% of couples who presented at a fertility center had male-factor infertility (abnormal semen analysis) (Noumi et al. 2011).
Study design, size, duration: This study was a cross-sectional quantitative study. A representative sample of two hundred male and female respondents from the Ghanaian metropolis were asked to complete a structured questionnaire, which was also used to access their knowledge of facts about male infertility. The study spanned from June to August 2017.
Participants/materials, setting, methods: Men (n=98) and women (n=102) above 18 years, with a mean age of 32 years, were recruited for the study using a multistage sampling method. Participants completed nineteen questions which accessed their knowledge levels about male infertility and identified factors that affected it.
Main results and the role of chance: Overall, majority (66%) of the men and women had poor knowledge about male infertility. They were not able to identify the cause and risk factors of male infertility. They did not know what it was nor about modern treatment options to alleviate male infertility. Women (62%) seemed to be more knowledgeable about the physiology of male infertility than men. Married men and women had better knowledge generally about male infertility (Odds Ratio = 5.7, $p=0.001$). Education significantly boosted knowledge about male infertility (Odds Ratio = 14.7, $p=0.001$). The higher the level of education, the more knowledgeable the individual was about male infertility.
Limitations, reasons for caution: Information on the reproductive health history of respondents could have influenced the knowledge of male infertility. However, this study did not capture this information.
Wider implications of the findings: Many studies conducted into the area of infertility, focus on women's infertility . This appears to have fueled the general perception that women (female factor) are the main reason for infertility. Insights from this study may help to rectify this misconception and reduce the stigma women in childless marriages face.
Trial registration number: not applicable

P-091 Impact of obesity and bariatric surgery on erectile function, reproductive hormones, testicular function, and sperm DNA fragmentation

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Study question: What is the impact of obesity and bariatric surgery on semen parameters, erectile function, reproductive hormones and sperm DNA fragmentation index (DFI)?
Summary answer: Bariatric surgery can revert the deleterious effects in reproductive hormones and DFI, but can result in worsening of semen parameters on 6-month follow-up.
What is known already: Obesity has major importance as a global health issue, and although there is a paucity of data regarding its influence on male fertility, growing evidence in the literature suggests that obesity is capable of altering reproductive hormones levels and erectile function. Effects of bariatric surgery, one of the most effective treatments for obesity, on classic semen parameters and DNA fragmentation index (DFI), however, have not been properly established.
Study design, size, duration: 2-phase prospective non-randomized study, with participants divided into 2 treatment groups and 1 control group. The study took place in a university-based teaching hospital, with patients' recruitment over a 2-year period. The first phase performed as a cross-sectional study with

obese and fertile patients, and the second phase carried out as a 6-month prospective case-control study with obese patients submitted to clinical or surgical treatment.

Participants/materials, setting, methods: Fertile controls (N=32) and 42 obese patients waiting bariatric surgery (body mass index greater than 35kg/m^2, N=42) were submitted to complete urological evaluation, International Index of Erectile Function (IEEF-5) score, semen analysis (SA), DNA fragmentation index (DFI – Alkaline comet assay) assessment, and dosing of sexual hormones. On phase 2, 22 obese patients (Group A1) were submitted to bariatric surgery and 20 remained on conservative treatment (Group A2). All patients were invited to 6-month revaluation.

Main results and the role of chance: Obese men presented higher median levels of estradiol (33.3 vs. 22 pg/mL, p=0.003), LH (6.3 vs. 4.1 IU/L, p=0.0004) and FSH (4.8 vs. 3.2 IU/L, p=0.0060), and lower levels of total testosterone (TT, 272.5 vs. 413 ng/dL, p=0.0008) than eutrophic fertile men. Additionally, they present lower ejaculated volume (1.5 vs. 2.5mL, p<0.0001), sperm concentration (43 vs. 82 millions/mL, p=0.0183), total motility (54.5 vs. 72%, p=0.0004), progressive motility (27.5 vs. 54%, p<0.0001), Kruger morphology (2 vs. 3%, p=0.0098), and higher sperm DFI than eutrophic fertile men (48 vs. 24%, p=0.0092). On phase two, mean weight loss on patients submitted to bariatric surgery was 38.2 kg. Group A1 patients showed a decrease on sperm concentration (53.5 vs. 105.2 millions/mL, p=0.0572), total ejaculated sperm count (58.2 vs. 169.8 million, p=0.0098) and total motile sperm count (34.5 vs. 104 million, p=0.0036). TT levels (648.9 vs. 291.1 ng/dL, p<0.0001) and free testosterone levels (276.2 vs. 183.7, p<0.0001) dramatically increased after surgery, while FSH, LH and estradiol were not different from baseline. Moreover, they exhibited a reduction in sperm DNA fragmentation. No differences were observed in group A2. IIEF-5 score was not different nor changed with treatment between groups.

Limitations, reasons for caution: Despite all patients were waiting to be submitted to bariatric surgery, selection bias cannot be completed excluded in a non-randomized study. Additionally, the small sample size and short follow-up is a result of the difficulties involving studies containing invasive procedures and sperm samples from patients not concerned about fertility.

Wider implications of the findings: Improving knowledge in the effects of bariatric surgery in semen parameters and hormone levels of severely obese patients may provide better counseling on those seeking fertility. Obese patients may be advised that bariatric surgery may bring additional barriers to paternity, at least in a short-term setting.

Trial registration number: Not applicable

P-092 Attenuation of sirtuin 3 down-regulates mitochondrial complex I and V via inhibition of voltage dependent anion channel 1,3 proteins to promote asthenozoospermia in rat

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Study question: Does sirtuin (SIRT) 3 influence mitochondrial respiratory function in isolated asthenozoospermia (AS) in rodent spermatozoa?

Summary answer: A drop in SIRT 1,3 immuno-expression may attenuate Voltage Dependent Anion Channel (VDAC) 1, 3 proteins to impair mitochondrial activity and acrosomal integrity in AS.

What is known already: Mitochondria generate the bulk of cellular ATP via oxidative phosphorylation (OXPHOS), supported by reducing equivalents generated by tricarboxylic acid cycle and fatty acid b-oxidation. Sperm mitochondrial dysfunction and *oxidative stress* are viewed as possible reasons for *AS*, characterized by reduced sperm motility. *D*erangement of SIRT1 / peroxisome proliferator activated receptor gamma coactivator 1 alpha/SIRT3 induce imbalance of reactive oxygen species (ROS) and antioxidant defense to promote dysfunctional electron transport chain; deleterious to sperm capacitation. Although previous studies have shown a physiological relevance of SIRT1/SIRT3 in maintenance of testicular bioenergetic capacity, the specific mechanism underlying this association is unknown.

Study design, size, duration: Experimental study; Mature Sprague-Dawley rats (n = 23) weighing ~330 -360g were treated with Ornidazole (ORN) at a dose of 400 mg/kg body weight daily by oral gavage for 12~14 days to mimic AS. Control set (n = 11) received vehicle (sodium carboxymethylcellulose (CMC-Na)) in water at same volume. The study was approved by Animal Ethics Committee (IAEC-37/PC/2018/9) of Institute of Reproductive Medicine, Kolkata.

Participants/materials, setting, methods: Effect of ORN on sperm parameters (count, motility) and testicular morphology was studied by Makler's chamber, and haematoxylin-eosin staining respectively. Testicular bioenergetics was evaluated on basis of intracellular ROS, oxidative stress, mitochondria membrane potential, VDAC and sirtuin proteins, OXPHOS complexes (C1–C5) and intracellular ATP concentration by flow cytometry, immunohistochemistry, immunoblot, immunofluoroscence, and bioluminescent kit respectively. Data presented as mean ± standard deviation were compared with Student's t-test. $P < 0.05$ was considered as significant.

Main results and the role of chance: Sperm proportion and progressive motility decreased significantly ($p < 0\ 001$) in ORN-treated rat (18.2 ± 3.3%) compared to control (51.7 ± 3.1%) supported by atrophied seminiferous tubules with increased number of vacuoles. A significant increase (p<0.001) in intracellular ROS (0.92 ± 0.38 *vs* 0.65 ± 0.34) production along with simultaneous reduced percentage of MitoTracker Deep Red FM (MT-DR FM)-positive sperm in ORN-treated (88.85 ± 4.81) set was documented in midpiece compared to control (13.75 ± 6.49, p< 0 001) suggesting mitochondrial dysfunction in asthenozoospermic rat. Increased expression (p<0.01) of hypoxia inducing factor-1a was observed in nuclei of pachytene spermatocytes and to a lesser extent in the adjacent round spermatids supported by immunoblot expression. Among OXPHOS proteins, complexes I, and V were significantly decreased (p<0.05) by ~70% indicating decreased oxidative capacity in the treated set. Significant down-regulation in SIRT 1, 3 revalidated by immunofluoroscence document reduced localizations (p<0.01) of both in midpiece. The drop in ATP level (p<0.001) was evident and this was followed by a compromised acrosomal status of the spermatozoa by effective diminution of VDAC 1, and 3 localization in ORN-treated set.

Limitations, reasons for caution: The main limitation of this study was the absence of direct quantification of SIRT1 and SIRT 3 enzymatic activity due to the lack of an appropriately sensitive method and hence the regulatory pathway could not be assessed.

Wider implications of the findings: The present findings may provide a valuable background for studying the regulation of SIRT3 during spematogenesis and its relevance as a sensor of Leydig cell redox state and energy status. SIRT 3 agonists may provide a novel prospect for impacting mitochondrial function in AS to improve sperm motility in future.

Trial registration number: NA

P-093 Micro-Anatomical features and arteriovenous communications of Spermatic Cord in Varicocele Patients

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Study question: To record and video document, micro anatomy of the spermatic cord of infertile individuals undergoing microsurgical varicocelectomy

Summary answer: Fine arteriovenous communication were observed between internal spermatic artery and vena- comintantes. Lymphatics were seen forming notable boundary between internal spermatic vessels and vasal complex.

What is known already: Varicocele is known to be associated with sperm disorders but some individuals with significant varicoceles remain fertile. This cannot be fully explained by current knowledge. A limited number of studies have addressed the micro-anatomy of spermatic cord especially in Asian varicocele patients. We have already published that in our population, the blood in varicocele veins has higher oxygen saturation as compared to other veins. This may be due to micro-anatomical peculiarities, so further studies are required to investigate the microsurgical anatomy of varicocele.

Study design, size, duration: Sixty consecutive patients between 20 – 45 years age, diagnosed with infertility and varicocele, undergoing microsurgical varicocelectomy at a tertiary & teaching hospital over a period of three years were included in the study, after informed consent. IRB approval was obtained before initiation of the study.
Participants/materials, setting, methods: In all participant, semen parameters and scrotal color Doppler ultrasonography (CDUS) were recorded preoperatively. During surgery, micro-anatomy of the spermatic cord was carefully noted at 10x to 25 x magnification on karl Zeiss operating microscope. Important findings were documented and still as well as video recordings were made. A single surgeon performed all procedures. The data was processed by SPSS for descriptive statistics.
Main results and the role of chance: The internal spermatic veins were dilated in all patients (mean diameter 3.2 +- 0.7). The external spermatic, external pudendal and Gubernacular veins were dilated in 75%, 19% and 70% patients respectively. Collateral venous channels were noticed in 28% patients. The main testicular artery was found in close proximity, behind internal spermatic veins in 65% patients, whereas in 8.3% patients the artery was found to be lying anteriorly or elsewhere in the anterior compartment in 16.7% patients. Venae comitantes were found surrounding the main testicular artery and communicating with each other in all cases. There were communications between venae comitantes and the adjacent internal spermatic veins in 87% patients. Most importantly we found fine arteriovenous communications between internal spermatic artery and vena- comitantes in 12 % cases. We also noted a separately identifiable group of lymphatic channels forming an almost bloodless boundary between the internal spermatic vessels and the vas and vasal vessel complex. Relevant pictures and videos were recorded for presentation.
Limitations, reasons for caution: We have investigated varicocele patients at a single centre and most patients were from the same geographical region. Further studies on patients from various geographical regions and races as well as a larger number of patients are required to confirm our findings.
Wider implications of the findings: a: The location of lymphatic channels at the boundary between internal spermatic vessels and vasal vessel complex appear of significance as a land mark during microsurgery.

b : The arterio-venous anastomosis at sub inguinal level, between testicular artery and adjacent veins may help to understand the pathophysiology of varicocele.
Trial registration number: not applicable

P-094 Is there an association between clinical outcome and aneuploidy of embryo in patients with severe male factor infertility?

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Study question: We aimed to evaluate embryo aneuploidy in severe male factor infertility (oligozoospermia, severe- oligozoospermia, TESE-sperm) by preimplantation genetic testing (PGT-A) based on identified clinical outcome.
Summary answer: There was a significant difference in clinical outcome but no significant difference of embryo aneuploidy in severe male factor groups with PGT-A.
What is known already: Several studies presented that patient using testicular sperm showed higher implantation and pregnancy rates than patients with severe oligoasthenoteratozoospermia syndrome and also that sperm are susceptible to damage during passage through the male reproductive tract. Increased DNA damaged sperm may be able to fertilize an oocyte; however, this may result in low embryo development and pregnancy rates. Another study reported that severe male factor infertility showed similar incidence of aneuploidy and there was no statistical significance. There is an increasing concern of possible rise of embryo aneuploidy in patients with poor sperm quality in severe male factor.
Study design, size, duration: Our center performed 13,258 IVF-ICSI cycles from January 2016 to November 2018. Among them only 474 cycles with severe male factor were analyzed in this retrospective study. From those, only 59 cycles were performed with PGT-A. The clinical pregnancy rate was identified in severe male factor patients without PGT-A. Furthermore, embryo aneuploidy was evaluated in severe male factor groups after PGT-A. Statistical analysis was performed using t-test and chi-squared test where appropriate, significance at $p<0.05$.
Participants/materials, setting, methods: In this study, the clinical pregnancy rate was evaluated in Non-biopsied IVF-ICSI cycles. Female factor with repetitive implantation failure, recurrent miscarriage and increasing of maternal age was considered for PGT-A for embryo aneuploidy. All IVF-ICSI cycles were divided into three groups. Group 1: Oligozoospermia ($\geq 0.1\times10^6$/ml~ $< 15\times10^6$/ml), Group 2: Severe-oligozoospermia ($< 0.1\times10^6$/ml), Group 3: TESE sperm (sperm retrieved from non-obstructive azoospermia patients).
Main results and the role of chance: The clinical pregnancy rate of Group 2 was significantly lower than Group 1 and 3 (Group1(43.98%(141/62) vs Group 2 (29.33%(75/22); p=0.036 and Group 2 (29.33%(75/22)) vs Group 3 (44.86%(185/83)); p=0.021), but not in Group 1 (43.98%(141/62) and Group 3 (44.86%(185/83)); $p>0.05$, in non-PGT-A. In addition, there was no statistically difference in both the maternal age (Group 1: 36.00±4.13 (n=141), Group 2: 35.74±4.27 (n=75), Group 3: 36.00±4.50 (n=185); $p>0.05$), and the paternal age (Group 1: 38.00±5.06 (n=141), Group 2: 37.52±4.50 (n=75), Group 3: 39.00±5.76(n=185); $p>0.05$) in between all groups. In embryo aneuploidy rate of three groups, there was no significant difference in both trophectoderm biopsy cycles (Group 1 : 67.00%(n=100) vs Group 2 : 75.00%(n=8); $p>0.05$ and Group 2 : 75.00%(n=8) vs Group 3 : 56.67%(n=30); $p>0.05$ and Group 1 : 67.00%(n=100) vs Group 3 : 56.67%(n=30); $p>0.05$) and blastomere biopsy cycles (Group 1 : 79.57%(n=93) vs Group 2 : 89.29%(n=28); $p>0.05$ and , Group 2 : 89.29%(n=28) vs Group 3 (84.21%(n=19); $p>0.05$ and Group 1 : 79.57%(n=93) vs Group 3 : 84.21%(n=19); $p>0.05$). There was also no significant difference in both the maternal and paternal age ($p>0.05$).
Limitations, reasons for caution: Our center performs only limited PGT-A cycles and also severe male factor cycles are especially rare in total IVF-ICSI cases. Due to this small sample size, number of analyzed cycle for embryo aneuploidy by PGT-A in severe male factor was very restricted.
Wider implications of the findings: This study revealed that severe male factor infertility does not influence on the embryo aneuploidy. Consequently, PGT-A procedure may not be essential for IVF-ICSI cycles in severe male factor infertility while producing a good quality of embryo may be more important for enhanced reproductive clinical outcomes.
Trial registration number: Not applicable.

P-095 Effect of raised male body mass index (BMI) on IVF success rate in egg donation cycles

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Study question: Does increased male BMI affect IVF outcome measures such as fertilization (FR), clinical pregnancy rate (CPR) and implantation (IR)?
Summary answer: Higher male BMI is associated with significant reductions in CPR and IR. No effect is observed on FR and number of embryos obtained.
What is known already: Fertility rate is reduced in couples with obese men who have a body mass index (BMI, Kg/m2) ≥30. Obese men can have lower serum testosterone and higher estradiol levels that can affect spermatogenesis. Higher BMI was also shown to relate to poorer semen parameters (lower ejaculatory volume, oligoozoospermia, abnormal morphology and increased sperm DNA damage). Given that oocytes donors are carefully selected from a population of young and healthy women, the use of donor oocytes allows minimizing the effect of oocyte competence and represent a model to analyze different parameters of the male partners and their effect in IVF outcomes.
Study design, size, duration: We retrospectively analyzed the outcomes of 317 egg donation cycles (OD) performed in October 2014-January 2019. Our population includes cycles with 6-9 vitrified/warmed oocytes/cycle inseminated by ICSI with fresh ejaculated semen. PGS cycles were excluded. Only

data relative to fresh embryo transfers were included since the complete cumulative data was not yet available. We considered a BMI threshold value of 25. Data shown as average±SD were analyzed with Fisher's exact, Chi-square or Student-t tests.

Participants/materials, setting, methods: The population was divided into two groups. Group-1 includes men with BMI <25 (n=155 OD, BMI=23.24±1.14; 1110 oocytes received). Each recipient received on average 7.2±1.07 oocytes/cycle. Group-2 includes men with BMI≥25 (n=162, BMI=28.33±3.53; 1188 oocytes). Each recipient received 7.3±1.00 oocytes/cycle. In groups 1 and 2 donor age was respectively 25,50±3.58 and 25,35±3.55 (NS). The female recipient age was respectively 44,04 ±3.23 and 43,47±3.78 (NS). No significant different were found in male age into two groups.

Main results and the role of chance: Respectively in groups 1 and 2, the number of surviving and injected oocytes was 989 and 1053; FR was 72.2% (714/989) and 71.7% (755/1053) (NS) and no difference was found in the number of embryos obtained. A not quite significant difference was found in beta-positive patients/starting cycle (PR): 66.5% (103/155) in group-1 and 55.6% (90/162) in group-2 (P=0.051). Significant differences were found in clinical pregnancy/starting cycle [CPR: 57.4% (89/155) and 42% (68/162) in groups 1 and 2 (p=0.007)] and IR [39.8% (106/266) and 28.9% (81/280) in groups 1 and 2 (p=0.0088)]. However, the female recipient BMI was statistically different: 21.68±2.91 (Group-1) and 23.41±3.65 (Group-2) (p=0.0001). We then selected a subpopulation of normal-weight women (female-recipient BMI range 18.4-24.9): no significant differences in fertilization rates between men with BMI<25 (male BMI=23.27±1.13, N=131) and ≥25 (male BMI=28.14±3.22, N=115) (men age: 45.26±5.71 and 44.68±6.13, NS; women age=44.1±3.08 and 43.24±3.97, respectively, NS). However, PR, CPR and IR were significantly different between men with BMI<25 and BMI≥25 [PR: 68.7% (90/131) and 54.8% (63/115) (p=0.026); CPR: 60% (76/131) and 40.9% (47/115) (p=0.0104); IR: 39.5% (89/225) and 28.5% (57/200) (p=0.0187)]. It must be said that also within normal-weight women BMI was significantly different (female BMI=21.19±1.62 and 21.87±1.63, p=0.0012).

Limitations, reasons for caution: It could not separate male BMI and female recipient BMI variations therefore the latter is a confounding factor in evaluating clinical outcomes. Moreover, we lack data about men's diet/lifestyle and serum levels of FSH and LH. These parameters would add knowledge relative to the role of BMI in male fertility.

Wider implications of the findings: Semen quality could be affected by male diet/lifestyle which in turn affects BMI. Preimplantation genetic screening could add information relative to the embryo competence to implant and develop into a live-birth. The follow up of children is crucial for understanding the possible inheritance of specific conditions related to parents BMI.

Trial registration number: not applicable

P-096 A randomized clinical trial comparing intracervical insemination and intrauterine insemination for donor sperm treatment in the natural cycle.

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Study question: Are six cycles of intracervical insemination (ICI) non-inferior to six cycles of intrauterine insemination (IUI) in donor sperm treatment in the natural cycle in terms of ongoing pregnancy?

Summary answer: ICI in the natural cycle is inferior to IUI in the natural cycle in donor sperm treatment in terms of ongoing pregnancy rate.

What is known already: Both ICI and IUI in the natural cycle are performed as first line treatments in women who are eligible for donor sperm treatment. IUI is more costly than ICI, due to the involvement of sperm processing. High quality data on the effectiveness of ICI versus IUI the natural cycle in terms of ongoing pregnancy are lacking. A large retrospective cohort study showed that ICI and IUI in the natural cycle in donor sperm treatment resulted in similar ongoing pregnancy rates of 40% after six cycles.

Study design, size, duration: We performed a multicenter, non-blinded, non-inferiority randomized controlled trial in six fertility clinics in the Netherlands and Belgium. Based on the retrospective cohort study we assumed a live birth rate of 40% after six cycles of IUI. To assess a non-inferiority margin of 12%, we needed to include 416 women.

Participants/materials, setting, methods: Women scheduled for donor sperm treatment were eligible, regardless of the indication for treatment. The primary outcome was ongoing pregnancy leading to a live birth within eight months after randomisation. Secondary outcomes were multiple pregnancy, miscarriage, time to ongoing pregnancy and pregnancy complications.

We calculated relative risks (RR) and risk difference (RD) and 95% CI. We analysed the data on an intention to treat basis.

Main results and the role of chance: Between June 2014 and January 2019, we included 417 women, of whom 209 women were randomly allocated to ICI and 208 to IUI. At the moment of writing this abstract, we have access to 89% of the data regarding ongoing pregnancy. Women's age was on average 34 years (SD ± 4) in both groups. Of the 417 women included, ten women (5%) allocated to ICI and 10 women (5%) allocated to IUI did not start treatment mostly due to personal reasons. Ongoing pregnancy occurred in 43 women (21%) in the ICI group and 74 women (36%) in the IUI group (RR 0.58 (95% CI 0.42 to 0.80)). ICI was inferior to IUI; the left boundary of the 95% confidence interval was minus 0.23 and crossed the pre-set absolute difference of 12% (RD of -0.15 (95% CI -0.23 to -0.06)).

Limitations, reasons for caution: The current follow-up is based on 89% of the data. Full data will be available before June 2019. These data will also include timing of ICI and IUI and quality of frozen- thawed donor sperm.

Wider implications of the findings: In women undergoing donor sperm treatment in a natural cycle, ICI results in lower ongoing pregnancy rates than IUI. Therefore, IUI should be the preferred treatment.

Trial registration number: NL4309 **(**NTR4462)

P-097 Human testicular sperm vitrification and storage through Sperm vitrification device versus conventional slow freezing cryopreservation: a prospective randomized sibling oocyte study

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Study question: Does SpermVD provide an efficient approach for testicular sperm preservation before oocyte retrieval for men suffering from azoospermia and undergoing testicular biopsy?

Summary answer: ICSI outcomes were comparable between spermVD and slow freezing techniques. Moreover, the duration of micro-injection procedure was significantly shorter in SpermVD group.

What is known already: In order to avoid iterative testicular surgery, an efficient and validated cryopreservation method of testicular sperm retrieved by microTESE followed by ICSI in asynchronous procedures can be proposed for men suffering from azoospermia. Testicular sperm vitrification has been recently used as an alternative to slow freezing technique, but so far no prospective randomized studies with sibling oocytes were conducted to compare these techniques.

Study design, size, duration: We conducted a single-center prospective randomized sibling oocyte study on 16 ICSI cycles in men suffering from azoospermia and with micro-TESE between January and December 2018.
Participants/materials, setting, methods: Half of spermatozoa from positive testicular biopsies were frozen with each of 2 freezing methods: slow conventional and vitrification .Sibling MII oocytes were microinjected by sperm coming from these 2 methods. MII oocytes were allocated by randomization to one of the 2 freezing groups, to achieve a balanced distribution. The sperm were thawed on the day of oocyte retrieval. Comparisons were done using paired T-test and sign-test . p-value was considered significant when < 0.05
Main results and the role of chance: A total of 66 surgical TESE were performed during the period out of which 16 ICSI cycles were conducted with cryopreserved sperm from the positive testicular biopsies.

64 MII oocytes were allocated in the slow freezing group versus 68 in the vitrification group

Men age was 35.7 ± 3.7 years and female age 33.6 ± 4.3 years. Female BMI was 22.3 ± 2.2 kg/m2. The recovery rate was 90.3%. There was no significant difference in fertilization rates between the two groups : slow freezing and vitrification (53.9% vs 67.1% respectively ; p=0.15). However, a significant difference was observed in the duration of microinjection with a reduced time in the vitrification group versus the slow freezing group (7.8 min versus 29.7 min, $p< 0.05$). There was no significant difference in blastulation rates (43.9% vs 50.0% respectively ; $p>0.05$). The choice of transferred embryos was based on the embryonic morphology, 80% of transferred embryos were obtained from spermVD group and yielded a 62.5% pregnancy rate.
Limitations, reasons for caution: These preliminary results need to be confirmed by a large trail with possible focus on non-obstructive azoospermia cases where the amount of spermatozoa is low, the synchronous procedure often recommended, and where SpermVD might be an accurate device for convenient asynchronous microTESE planning.
Wider implications of the findings: The spermVD device is an efficient and safe carrier and seems to be appropriate for the asynchronous preservation of a small number of spermatozoa allowing us to plan asynchronous micro TESE before oocyte retrieval and perform rapidly safe delayed ICSI.
Trial registration number: The study was approved by Unilabs IRB

P-098 Analysis of sperm nuclear integrity and screening for DNAH1 gene mutation in infertile Tunisians patients with MMAF syndrome (Multiple Morphological Abnormalities of the sperm Flagella)

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Study question: The impact of nuclear sperm integrity in patients with MMAF syndrome and genetic defects could assess the chances of success in assisted fertilization?
Summary answer: Patients with MMAF syndrome have impaired nuclear sperm which affects assisted fertilization and mutations in DNAH1 gene is not the main cause of this syndrome.
What is known already: MMAF syndrome is a rare but severe form of teratozoospermia defined by the presence in the ejaculate of immotile spermatozoa with several abnormalities of the sperm flagellum including short, coiled, absent and flagella of irregular caliber. Mutations in *DNAH1*, an axonemal inner dynein arm heavy chain gene, have been shown to be responsible in 28% of MMAF patients (Ben Khelifa et al., 2014). Recently, Tang et al., 2017 have identified others mutations in CFAP43 and CFAP44 genes, encoding cilia- and flagella-associated proteins, in patients with MMAF syndrome. Otherwise, few data regarding the nuclear integrity were reported in the literature.
Study design, size, duration: This is a case control study carried out on 52 Tunisian infertile patients with MMAF syndrome and 25 fertile men with normal semen profiles included as a control group. Patients were recruited between January 2015 and September 2018.
Participants/materials, setting, methods: Semen samples were analyzed according to the World Health Organization criteria (2010). Sperm DNA fragmentation was evaluated by terminal desoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) assay. Chromatin condensation was assessed by aniline-blue Staining. Fluorescence *in situ* hybridization for chromosomes X, Y and 18 was performed to study the chromosomal meiotic segregation. We performed PCRs and Sanger sequencing for mutations screening of *DNAH1* associated to MMAF syndrome.
Main results and the role of chance: Semen morphology analysis shows a mosaic of multiple morphological abnormalities of the flagella with an average rate of 46.73% short flagella, 16.22% bent flagella, 22.07% coiled flagella and 10.90% absent flagella which describe a MMAF syndrome. We also showed a high percentage of microcephalic heads (37.41 ± 19.80%) and acrosome abnormalities (42.75 ± 16.3%). The mean DNA fragmentation index (DFI) was significantly higher in patients compared to controls (25± 4.61% vs 10± 3.77%; $p<0.05$). The results of aneuploidy frequencies showed a significant difference between both groups (15.89±7.18% vs 1.52±0.25%; $p<0.05$) predominant in the mean disomy rate of sex chromosomes (8.43±4.63% vs 1.05±0.56%; $p<0.05$).Whereas, the difference was not significant for the rate of aniline blue-reacted spermatozoa in patients compared to the control group (13 ± 2.21% vs 12 ± 3.22%, $p> 0.05$). For the molecular analysis, DNAH1 gene showed no mutation in exon 23, 31, 74 and 78 for our patients.
Limitations, reasons for caution: A limitation of this study is the unavailability of Next-Generation Sequencing Technology (NGS) to look for others associated genetic causes.
Wider implications of the findings: In these cases, we have demonstrated impaired sperm nuclear quality, which will affect the results in ART. There is no mutation in screening of *DNAH1* gene that's why we plan to complete the mutational research on other exons of *DNAH1*or in other genes like *CFAP43 and CFAP44*.
Trial registration number: NONE

P-099 IVF/ICSI outcomes in HIV- vs hepatitis-B positive men. An analysis of 249 fresh and frozen treatment cycles in a prospective case-control study

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Study question: What are the IVF/ICSI outcomes for HIV-positive men within serodiscordant couples using frozen washed sperm and do they differ from control men with hepatitis B?
Summary answer: IVF/ICSI treatment in serodiscordant couples with HIV-positive men had higher cumulative miscarriage rate compared to controls but a similar cumulative live-birth rate.
What is known already: IVF/ICSI with washed sperm for treatment of HIV-positive men with seronegative partners has shown to be an effective treatment with no higher risk of viral transmission to the partner or the child. Previous studies suggest that the antiviral therapy for HIV might have a negative impact on sperm quality although it is unclear if the IVF/ICSI treatment outcome is compromised in comparison to other couples that undergo IVF/ICSI with the washed-sperm procedure.
Study design, size, duration: Prospective case-control study of serodiscordant couples with HIV-positive men that underwent IVF/ICSI treatment with washed-sperm procedure between Januari 2004 and December 2017. All HIV-positive men were under antiviral treatment at the time of IVF/ICSI and had undetectable serum viral load. The control group consisted of couples with hepatitis-B positive men that underwent IVF/ICSI with washed sperm under the same period. HIV-positive men were categorized to investigate a possible impact of antiviral treatment on sperm quality.
Participants/materials, setting, methods: 53 serodiscordant couples with HIV-positive males underwent 82 fresh and 39 frozen cycles. IVF/ICSI was performed with frozen/thawed sperm that had been washed using double gradient centrifugation. In the control group 56 couples with hepatitis-B positive men underwent 92 fresh IVF/ICSI cycles and 36 frozen. The antiviral treatment

in HIV-positive men included combination of Nucleoside/Nucleotide analog-(NRTI) and Non-nucleoside reverse-transcriptase inhibitors(NNRTIs) in 22 cases or a combination of NRTI with other medication in 31 cases.

Main results and the role of chance: Couples with HIV-positive men were similar to controls in regards to infertility duration (3.76 vs 3.70 years, p=0,9), woman's age (33.40 vs 31.85, p=0.07), woman's BMI (24.42 vs 24.25,p=0,82), man's age (39.19 vs 38.24, p=0.45), smoking in women (17.65% vs 14.29% , p=0,63) or men (40.43% vs 45.45%, p=0,60) .

The treatments resulted in similar yields of mature oocytes (7,62 vs 8,09, p=0,63) and number of fertilized oocytes (5.38 vs 5.43, p=0,94). The cumulative miscarriage rate was higher, but not significantly, in the HIV-group (18,8 vs 11% P=0,31). The cumulative live-birth rate did not differ between the groups (54.72 vs 51.79, p=0,75). HIV virus was not detected in any of the sperm samples at the time of treatment. No partner seroconversion or child infected were reported.

The percentage of motile sperms was significantly lower in NRTI/NNRTI patients than in patients treated with NTRI combined with another medication (36.41 vs 54.48%, p<0.001). However sperm concentration and total sperm count were similar between the treatment categories (57.93 vs 59.13 million sperm/mL, p=0.92 and 190.6 vs 175.8 million sperm,p=0,75, respectively).

Limitations, reasons for caution: Although our center is the only performing treatments for serodiscordant couples in Sweden, the size of this cohort is relatively small. Further research is required to confirm our results.

Wider implications of the findings: Our analysis indicate that IVF/ICSI treatment after sperm washing in serodiscordant couples with HIV-positive men has similar outcomes to that of couples that undergo a similar procedure of sperm washing and ICSI due to viral infections. The type of antiviral regimen might affect the sperm motility in HIV-positive individuals.

Trial registration number: Not applicable

P-100 Adult testicular somatic cell feeders do not facilitate human embryonic stem cell differentiation towards male gametes.

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Study question: Does the addition of testicular somatic feeders enhance the differentiation of human embryonic stem cells (hESCs) towards male gametes?

Summary answer: HESCs seeded on testicular somatic feeders did not show higher differentiation efficiency compared to hESCs cultured on Matrigel.

What is known already: For men with non-obstructive azoospermia, creation of functional gametes *in vitro* starting from patient-specific pluripotent stem cells might offer the possibility to father genetically related children. Recent studies reported mouse ESCs (mESCs) differentiating towards functional male gametes resulting in live offspring. Easley et al. (2012) obtained human haploid germ cells from hESCs, but elongated spermatids were not produced. So far, complete *in vitro* spermatogenesis from hESCs has not been reported.

Study design, size, duration: Two hESC lines were cultured for 10, 15 or 20 days on Matrigel using the conditions described by Easley et al. in 2012 (controls). Undifferentiated hESCs were co-cultured on top of inactivated testicular somatic cells for 20 days (exp1). Alternatively, hESCs that have been cultured for 10 days on matrigel (pre-differentiated hESCs) were co-cultured on top of adult testicular somatic cells for another 10 days (exp2). Gene and protein expression and hormonal production were evaluated.

Participants/materials, setting, methods: Testicular tissue from three adult patients undergoing bilateral orchiectomy as part of a prostate cancer tratment was digested and cells were FACS-sorted. The somatic fraction ($EPCAM^-/HLA^+$) was mitotically inactivated. Differentiation efficiency of hESC lines (n=2) was evaluated by immunocytochemistry for the germ cell markers VASA, BOLL, CREM and ACROSIN and by qRT-PCR for VASA, GFRA1, TP1 and ACROSIN. The function of the testicular somatic cell feeder was assessed by testosterone (T) and inhibin B quantification.

Main results and the role of chance: When hESCs were cultured on Matrigel, VASA expression was detected after 10 days of culture and significantly increased after 15 and 20 days. The pre-meiotic marker BOLL and post-meiotic marker ACROSIN were rarely expressed. However, qRT-PCR results showed that GFRA1 and the post-meiotic markers TP1 and Acrosin increased during culture. Although no T was detected, Inhibin B production was detected at the end of the Matrigel culture.

When undifferentiated and pre-differentiated hESCs were seeded on top of inactivated somatic cell feeders, cells either formed colonies in between the somatic cells or formed massive cell clusters on top of the feeder cells. VASA was detected at the end of undifferentiated cell co-culture (20 days). BOLL was barely expressed and the post-meiotic marker CREM was not found.

At the end of the pre-differentiated cell co-culture, VASA was detected. BOLL and CREM were barely expressed.

When VASA positive populations were compared, the highest number of VASA positive cells could be found in the Matrigel culture (1.9-3%) compared to the pre-differentiated cell co-culture (0.2-1.5%) and undifferentiated cell co-culture (0.2-1%).

Limitations, reasons for caution: One medium composition was used during the entire culture period, although spermatogenesis is a very complex multi-step process. hESCs are less naive than mESCs, which might explain the success of the mouse studies.

Wider implications of the findings: This study demonstrated that the presence of an adult testicular somatic feeder did not enhance the differentiation of hESCs. Fetal testicular feeders might be a better environment for initial differentiation.

Trial registration number: N/A

P-101 The effect of advanced paternal age on sperm parameters and on the outcome of in vitro fertilization treatments

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Study question: How does advanced paternal age (APA) affect sperm parameters and the outcome of intracytoplasmic sperm injection (ICSI) cycles?

Summary answer: Based on our study, APA negatively affects initial sperm count and motility as well as blastulation rate, however, not affects clinical outcomes of ICSI cycles.

What is known already: Over the last decade, delayed childbearing has become a trend in developed countries. It is proven that advanced maternal age (AMA) plays a dominant role in determining the success of *in vitro* fertilization (IVF) treatments. However, the impact of APA on IVF outcomes is still poorly known. Recent studies found that a man's age can affect testicular functions and sperm parameters. Paternal aging was also found to be associated with increased incidence of DNA damage, chromosomal aberrations and embryonic aneuploidy. These can lead to decreased fertilization and blastulation rate or increased miscarriage rate and thus a decreased live birth rate.

Study design, size, duration: In this retrospective study, we collected data on all couples with female age less than 35 years at our private fertility clinic between January 2013 and December 2018. A total of 135 fresh embryo transfer cycles were analysed, and two groups were formed based on the paternal age: <40 years and ≥40 years. Cycles with preimplantation genetic testing were excluded from the analysis.

Participants/materials, setting, methods: In the <40 years group (average age 34.00±3.79) 101 fresh blastocyst transfers were carried out, while in the ≥40 years group (average age 43.18±2.47) 34 blastocyst transfers. Evaluation of all semen parameters were done according to WHO standard criteria (2010). All embryos were fertilized by ICSI using fresh, frozen or testicular sperm extraction sample. The measured IVF outcomes were implantation rate, clinical pregnancy rate and miscarriage rate.

Main results and the role of chance: The mean maternal age did not differ in the two examined groups (31.43±3.32 in the <40 years group and 31.76±4.29 in the ≥40 years group). The mean initial sperm count was significantly higher in the <40 years group than in the ≥40 years group (58.87% vs. 44.38%, p=0.0464) and sperm motility was also significantly higher in the <40 years group (49.98% vs. 37.18%, p=0.0054). The fertilization rate was slightly higher in the <40 years group (70.11%) but not significantly different from the ≥40 years group (66.67%, p=0.4371). We found a significantly higher blastulation rate in the <40 years group compared to the ≥40 years group (60.87% vs. 56.18%, respectively, p=0.02). The clinical pregnancy rate (42.57% (43/101) vs. 61.76% (21/34), p=0.0734) and implantation rate (41.22% (54/131) vs.

52.17% (24/46), p=0.1689) did not differ significantly in the examined groups. We also did not find a significant difference in case of miscarriage rates (22.22% (12/54) in <40 years group vs. 16.66% (4/24) in ≥40 years group, p=0.7634).

Limitations, reasons for caution: The main limitations of this study are small sample size and inclusion of retrospective cases.

Wider implications of the findings: Based on our results, the quality of sperm seems to decline with age, which demonstrates that man's fertility also has a defined lifetime. However, ICSI can compensate for this. With our findings, we would like to draw man's attention to the increasing health risks associated with late childbearing.

Trial registration number: not applicable

P-102 PREVALENCE OF MYCOPLASMA HOMINIS AND UREAPLASMA UREALYTICUM INFECTION IN CRYOPRESERVED SPERM FROM DONOR SPERM BANK

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Study question: What is the prevalence of Mycoplasma Hominis and Ureaplasma Urealyticum infection in cryopreserved donor sperm?

Summary answer: Mycoplasma Hominis and Ureaplasma Urealyticum may contaminate sperm bank imported sperm straws with a prevalence of 3.5% and 5.2% respectively, in normal sperm parameters post-thaw.

What is known already: Between 8 and 35% of male infertility is associated with infection of the genital tract. Even if many data are available for symptomatic infections, it is difficult to estimate the impact of asymptomatic infections on fertility. The asymptomatic infections can reduce fertility through alteration of spermatogenesis and reduction of sperm motility. Mycoplasma Hominis and Ureaplasma Urealyticum reduce sperm quality acting on the nuclear chromatin of the spermatozoa and on the embryonic development. Although the effects of these microorganisms are known, sperm banks do not always scan for them if donors do not show the classic symptoms of infection.

Study design, size, duration: This laboratory - based cross sectional study was performed on straws of cryopreserved sperm from 114 donors imported from a Danish sperm bank between January 2015 and December 2018. For each sperm donor two straws of the same collection were requested, one was kept in quarantine and the other was evaluated for conventional parameters, in accordance with the WHO 2010 Guidelines, and for Mycoplasma Hominis and Ureaplasma Urealyticum infection as required by Italian regulation.

Participants/materials, setting, methods: For each sperm donor one straw was kept in quarantine and the other one was evaluated for quality conventional parameters and for infection by bacteriological culture. In cases of positivity of the culture with concentrations $^{3}10^{4}$cfu/ml (MYCOPLASMA IST2-Biomerieux SA-France) a confirmation test Real-Time PCR, using 2 ml of DNA(Roche diagnostics GmbH, Mannheim, Germany), was performed.

Main results and the role of chance: After thawing the sperm parameters was normal according to WHO guidelines 2010. The cryo-survival rate was over 50% of the initial motility, no leukocytospermia was found. In the donor sperm, the prevalence of infection were, respectively, 3.5% (4/114) for Mycoplasma Hominis only, 5.2% (6/114) for Ureaplasma Urealyticum only and 3.5% (4/114) for both pathogens. The samples positive for at least one of the two microorganisms were not used for reproductive treatment and were eliminated as required by Italian regulation.

Limitations, reasons for caution: only donor semen samples have been analyzed in this study. No data about sperm donor pregnancies during other donations or abortion rates were evaluated.

Wider implications of the findings: The sperm from donor sperm banks can be contaminated with Mycoplasma Hominis and Ureaplasma Urealyticum, with possible negative effects on oocyte fertilization and embryonic development. These results demonstrate how scanning for these microorganisms is opportune during the recruitment of donors, thus improving the outcome of donation treatment.

Trial registration number: Not applicable

P-103 Sperm DNA Damage have an Effect on aneuploidy-euploidy of Embryos in ICSI-CGH Array Cycles

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Study question: What is the effect of sperm DNA damage on embryo chromosome aneuploidy and the level of semens MDA, ROS and TAC parameters in ICSI-CGH array cycles?

Summary answer: sperm DNA damage increase embryo chromosome aneuploidy. Also DFI have negative effects on semens MDA, ROS and TAC parameters.

What is known already: Studies have shown that sperm DNA fragmentation is higher in infertile men. There is clinical evidence to show that sperm DNA damage could be a marker of sperm quality and extensive data exist on the relationship between DNA damage and male fertility status. Sperm DNA fragmentation can be the most common reason for the transmission of anomalies of the father's DNA to a child that seen in a high percentage of sperms in infertile men.

Study design, size, duration: 40 patients with recurrent implantation failure (RIF) were selected. both women and men appeared to have no problem. All women were between 25-35 and stimulated with GnRH agonist. 2 groups were defined, 1: DFI > 20 % and 2: DFI < 20%. Intra-cytoplasmic sperm injection was also performed and then the day 3 embryos were subjected to blastomere biopsy and evaluated by Genomic Comparative Hybridization (CGH array).

Participants/materials, setting, methods: semen parameters in two groups were analyzed by CASA for motility and morphology and live ratio. Also, the level of total ROS, TAC, DNA fragmentation and MDA were evaluated by DCFH(by Fluorimetry) , ELISA kit, TUNEL assayby flowcytometry and ELISA respectively. Day 3 embryos were evaluated for aneuploidy by aCGH method. Also the correlation between embryo aneuploidy and DFI and semen parameters were evaluated in both groups.

Main results and the role of chance: The results of this study showed that sperm with high DFI (DFI> 20) significantly increased the number of the aneuploidy embryo than sperm with low DFI (P <0.001). Also, by increasing the DFI, the level of MDA significantly increased (P <0.001). The level of ROS and TAC were increased and decreased respectively but they was not significant by DFI (P >0.001). but there is no relationship between sperm ROS, TAC and MDA with day3 embryo aneuploidy.

Limitations, reasons for caution: the main limitation of the present research was the lack of embryo transfer at blastocyst stage.

Wider implications of the findings: These data indicate that sperm DNA damage have a significant effect on embryo chromosome aneuploidy. Embryo selection by aCGH should be considered in couples with high DNA fragmentation.

Trial registration number: NA

P-104 DNA damage after in vitro exposure to very low concentration of combusted cerium nanoparticles (CoCeO2NPs) and to CoCeO2NPs + benzo(a)pyrene in rat and human gametes

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Study question: Do *in vitro* gametes exposure to very low concentration of $CoCeO_2$NPs and to $CoCeO_2$NPs + Benzo(a)pyrene induce significant and similar DNA damage ?

Summary answer: *In vitro* gametes co-exposure to $CoCeO_2$NPs + Benzo(a)pyrene induced statistically higher DNA damage than exposure to $CoCeO_2$NPs alone in rat and human.

What is known already: Cerium dioxide nanoparticles (CeO_2NPs) are widely used as diesel additive and are released in the air after engine combustion. The Organization for Economic Cooperation and Development included CeO_2NPs in the priority list of nanomaterials requiring urgent evaluation. *In vitro* exposure of human and mouse sperm and cumulus oocyte complexes (COC) to very low concentrations of pristine CeO_2NPs induce significant DNA damage. Benzo(a)pyrene (BaP) is also released in the air by diesel combustion and is known to induce DNA damage in sperm and COC. Nevertheless, pristine CeO_2NPs are modified by combustion; the impact of $CoCeO_2$NPs exposure and to $CoCeO_2$NPs+BaP remains unexplored.

Study design, size, duration: Pristine CeO_2NPs were extracted from Envirox™ diesel additive and combusted at 850°C, (average combustion temperature in a diesel engine), to obtain $CoCeO_2$NPs. Crystalline structure of $CoCeO_2$NPs was analysed by X-ray Diffraction. Rat gametes were sampled in epididymis and oviducts after euthanasia of five mature males and fourty 4 weeks old females (after ovarian stimulation). Human frozen sperm from fertile donors were purchased from Germetheque biobank (France).

Participants/materials, setting, methods: Human and rat gametes were exposed *in vitro* to very low concentrations of CoCeO2NPs [1 to 1.10^3 $\mu g.l^{-1}$] and human sperm to 1 $\mu g.l^{-1}$ $CoCeO_2$NPs + 25µMol BaP during 1 hour in Ferticult© Medium + 1% DMSO at 37°C, 5% CO2. DNA damage was analysed by alkaline comet assay (ACA) and quantified by Olive Tail Moment (OTM) in oocytes and follicle cells and by % Tail DNA in sperm.

Main results and the role of chance: In rat oocytes and follicle cells, exposure to 1 $\mu g.l^{-1}$ $CoCeO_2$NPs induced significantly higher DNA damage (mean±SEM OTM = 10.26±0.36 and 4.91±0.29, respectively) compared with unexposed controls (2.21±0.18 and 0.72±0.05, respectively) and with higher $CoCeO_2$NPs concentrations ($p < 0.0001$). In rat sperm, exposure to 1 $\mu g.l^{-1}$ $CoCeO_2$NPs induced significantly higher DNA damage (mean %Tail DNA±SEM = 21.23 ± 0.37) compared with unexposed control (11.06±0.24) and with higher $CoCeO_2$NPs concentrations ($p <0.0001$). In human sperm, exposure to 1 $\mu g.l^{-1}$ $CoCeO_2$NPs also induced significantly higher DNA damage (mean % Tail DNA±SEM = 32.06 ± 0.54) compared with unexposed control (11.96 ± 0.30) ($p < 0.0001$); co-exposure to 1 $\mu g.l^{-1}$ $CoCeO_2$NPs + 25µM BaP induced significantly higher DNA damage (mean % Tail DNA±SEM = 33.63 ± 0.67) compared with 25µM BaP alone (28.57 ± 0.54) and 1 $\mu g.l^{-1}$ CoCeO2NPs alone (24.62± 0.52) ($p < 0.0001$). As in our previous results, obtained in human sperm after *in vitro* exposure to various concentrations of pristine CeO_2NPs [10 to 1.10^4 $\mu g.l^{-1}$], DNA damage was inversely proportional to the $CoCeO_2$NPs concentrations; we might hypothesize that our results are related to the lower aggregation states at low concentration, inducing a higher surface contact between cells and $CoCeO_2$NPs.

Limitations, reasons for caution: These results cannot be extrapolated to *in vivo* toxicity of $CoCeO_2$NPs after inhalation, but demonstrate that interactions between $CoCeO_2$NPs and germ cells induce significant DNA damage. Additional data should be needed to assess DNA damage in rat COC after *in vitro* exposure to very low $CoCeO_2$NPs concentrations and BaP.

Wider implications of the findings: The co-exposure to very low concentrations of $CoCeO_2$NPs and BaP showed for the first time a cumulative genotoxic impact on human sperm. Potential impacts of diesel exhaust exposure in couples and pregnant women are a major concern for public health, highlighting the need for *in vivo* studies.

Trial registration number: Animal experiment agreement N° 15447-2018061110211950.

P-105 The curious incidences of male factor infertility and number of ICSI cycles in the UK.

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Study question: What is the correlation between male-factor subfertility and the rate of IVF/ICSI cycles in the UK from 1995 to 2016?

Summary answer: The rate of IVF cycles using ICSI does not reflect the drop in male factor infertility throughout the years.

What is known already: The WHO guidance on normal semen analysis has changed over the years, resulting in a significant change of what is considered abnormal. Severe male infertility can justify the use of IVF/ICSI procedures. Specialist-Urology referral is currently reserved for patients for whom ICSI is not available. However, increasing numbers of men diagnosed with male factor issues are seeking advice and further investigations. Experienced clinical andrologists investigating these men know that treatable conditions often can be detected.

Study design, size, duration: Continuous collection of UK data is part of the regulatory functions of the HFEA statutory body, and clinics have to submit their annual data as part of their licence to operate. The data is available freely on their website and the various sub categories were analysed from 1995 to 2016.

Participants/materials, setting, methods: The data is derived from the HFEA website from figures available in the public domain, involving 1223629 cycles of IVF/ICSI. The data forms part of the mandatory requirement for clinics to obtain a licence to practice IVF/ICSI procedures in the UK and is therefore a comprehensive survey of UK practice.

Main results and the role of chance: The percentage of IVF cycles with 'primary male infertility' has diminished hugely since the beginning of our data set: 36.5% in 1995 to 0.26% in 2016.The percentage of IVF cycles with 'male factor' recorded as a contributor to their subfertility has reduced over the years. However, the most obvious drops can be explained by the reissuing of WHO guidance on semen analysis in 1999 and again in 2010 (before 1998 it was >80%; from 2001-2009 it was 40-50%; from 2011-2016 it was 30-40%). The rate of ICSI cycles as a percentage of all IVF cycles increased rapidly from 1995-2000 (this is to be expected as the technique was first described in 1991). It continued to rise throughout the 2000s, peaking at 43% in 2010 and 2011. Since then it has reduced somewhat to 38% in 2016. However, since 2011 there is a persistent discrepancy in between the percentage of IVF cycles using ICSI and the percentage where male factor is recorded as a contributor. In each year, between 5.2 and 6.4% of IVF cycles used ICSI despite there being no recorded male factor subfertility. In 2016, this equated to >4000 cycles per year.

Limitations, reasons for caution: The HFEA data is comprehensive, therefore removing biases of data collection. However, it is equally difficult for researchers to validate the data entered and therefore some caution must be applied to interpretation. Multiple factor correlation would therefore be difficult to evaluate for contributors and confounders.

Wider implications of the findings: It is possible that the above findings represent under-reporting to the HFEA, but with such a consistent rate it is hard to put this down to random error only. It raises the possibility that ICSI is not universally being recommended in an evidence-based manner.

Trial registration number: Not applicable

P-106 Cryopreservation of extremely low quantities of spermatozoa retrieved with microdissection testicular sperm extraction (micro-TESE) from non-obstructive azoospermia (NOA) patients

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Study question: Is that possible both to keep the viability and to exclude quantity losses during cryopreservation of single microsurgical retrieved spermatozoa from NOA patients?

Summary answer: The utilized method allows to cryopreserve extremely low numbers of spermatozoa retrieved with micro-TESE from NOA patients with normal survival rate and without quantity losses.

What is known already: Cryopreservation of single spermatozoa(CSS) is needed when only very limited numbers of viable spermatozoa are available as in cases of NOA. Conventional cryopreservation in relatively large volume is not optimal for those samples because associated with the quantity losses of spermatozoa and also requires prolonged post-thawing search-and-find step prior to ICSI. There are different carriers proposed for CSS: hamster zona, ICSI pipettes, alginate spheres, cryoloop, high security straw, cell slipper. However, there is no standard technique for CSS.

Study design, size, duration: For this case series twenty-three men with NOA who had micro-TESE in the Academician V.I. Gryshchenko Clinic for Reproductive Medicine were selected from November 2017 till December 2018. CSS was performed only in cases where an extremely limited number of motile spermatozoa was observed. Samples with a high number of motile spermatozoa were cryopreserved by a conventional method.
Participants/materials, setting, methods: Micro-TESE samples were prepared and evaluated for presence or absence of motile spermatozoa. For CSS from 4 up to 12 (24 in total) motile spermatozoa were selected and moved with micromanipulator to the microdrops of cryopreservation medium on the surface of polystyrene plate which was then frozen in LN_2vapor phase and subsequently immersed into liquid nitrogen. For thawing plates with microdrops containing cryopreserved spermatozoa were immersed in ICSI dishes with oil prewarmed to 37C.
Main results and the role of chance: Micro-TESE samples from twenty-three patients were evaluated for the presence of spermatozoa. In nine patients motile spermatozoa were found, in three patients only immotile spermatozoa were found, and in eleven patients spermatozoa were not found. CSS was performed for seven patients, in six of which homogenized testicular tissue was also cryopreserved by conventional method in parallel.

Five CSS samples were thawed to evaluate post-thawing motility and recovery rate. Out of 24 cryopreserved spermatozoa all 24 spermatozoa were found. Post-thawing motility of cryopreserved spermatozoa was 50±9% (12 of 24). In one case single frozen spermatozoa were used to inject the oocytes in ICSI cycle. Normal fertilization was achieved.
Limitations, reasons for caution: Based on the small number of participants in this study, a larger study is needed to evaluate the potential of this technique.
Wider implications of the findings: Based on the results obtained the utilized CSS technique can be potentially useful for severe male factor cases besides NOA and also for male fertility preservation.
Trial registration number: not applicable

P-107 Comparison of clinical pregnancy rate in donor insemination between women without a male partner and women with a partner at the insemination moment.

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Study question: Are there any differences between the D-IUI results performed on women without male partners at the moment of insemination, and women with male partners?
Summary answer: The pregnancy rate through D-IUI was higher in the group of women with male partner
What is known already: Most studies have not shown differences in the pregnancy rate through D-IUI between women with a male partner and women without a male partner, whether homosexual or heterosexual.

Published studies suggest a higher incidence of polycystic ovarian syndrome and some factor related with PCOS in homosexual women, these circumstances can increase even more the success percentages of reproduction techniques.

Giselle Crawford published a study in 2014 in which she proved that pregnancy rates through FIV were higher when women were exposed to seminal plasma at the previous moments before the realization of the technique, with a statistical significance
Study design, size, duration: Retrospective study in which donor's semen inseminations done in our Reproduction Unit have been analysed between January 2006 and December 2017, and the results of two different groups have been compared: one of them formed by women with a male partner at the time of the insemination and another group of women without a male partner, either homosexual or heterosexual.
Participants/materials, setting, methods: Participants were all women that underwent D-IUI cycles in this centre between January 2006 and December 2017.

188 patients participated, 81 of them not having a male partner and 107 of them with a male partner.

Inclusion criteria used for the D-IUI were: 1.Age (between 18 and 40); 2.Women without another previous healthy child; 3.Permeable tubes; 4. Ovarian function preserved. The maximum of cycles per woman was 6.
Main results and the role of chance: We analysed 501 D-IUI cycles, from which 223 cycles were realized on women without a partner, and 278 of them were done on women with a male partner. The clinical pregnancy rate per woman was lower in the group with women without a male partner than in the group of women with a male partner (35.8%) than in the group of women with a male partner 57.94% (p = 0.002).

The average number of D-IUI needed to obtain a clinic pregnancy in the group of women without a partner was 7.69, and in the group of women with partners was 4.48. When dividing each group by age:

- <35 : The pregnancy rate in the group of women without a partner was lower (41.9%) than in the group of women with a male partner (66.2%) (p =0.02).

The number of cycles needed to achieve a pregnancy in the group of women without a partner was 6, and in the case of women with a male partner was 3.9.

- >35 years: the pregnancy rate was 32.6% in women without a partner and 42% in women with a partner (p= 0.26). The average number of cycles per pregnancy was 8.9 and 6.4, respectively.

Limitations, reasons for caution: Some patients of the group without a male partner at the time of insemination may be maintaining relationships with men but do not recognize them as a couple at the time of therapy, which could equalize the conditions with the other group of patients and skew the results.
Wider implications of the findings: In opposition to what has been suggested in previous studies, D-IUI success rates are higher in women with a male partner at the time of the insemination. The immunoregulation created by the uterus's exposure to the seminal plasma might be one of the processes that influence such success
Trial registration number: Not applicable

P-108 Dynamic H2AX-phosphorylation reveals the sperm capacity to label DNA damage in protection of paternal genome

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Study question: Are mature sperm capable to identify and label DNA double strand breaks?
Summary answer: Dynamic H2AX-phosphorylation revealed a time-dependent increase of double strand breaks, and the capacity of mature sperm to identify and label this type of DNA damage.
What is known already: The phosphorylation of histone-2AX (gamma-H2AX) is an epigenetic hallmark which occurs in response to DNA double strand breaks (DSBs) in any cell type, including sperm and oocytes. Gamma-H2AX stimulates oocyte's DNA repair machinery to overcome DSBs lesions via NHEJ-pathway at pronuclear stage. Moreover, mouse zygotes expressing high levels of gamma-H2AX into male pronuclei showed poor embryo development or total arrest. These observations support the importance of DNA repair signaling, and the negative impact of paternal DSBs on embryo development. Sperm gamma-H2AX is associated with chromatin remodeling and physiological DNA fragmentation. However, variation of gamma-H2AX levels in laboratory conditions remains unknown.
Study design, size, duration: A total of 10 sperm samples were evaluated at three time points: t-0h, t-2h, t-24h. t-0h defines the time for sample fixation immediately after density gradients. Sperm samples were left at room

temperature for 2 and 24 hours. Two replicates were prepared per sperm sample, with a total of 200 sperm analyzed per replicate. Mean proportion of positive gamma-H2AX per sample was reported. Negative control was included in each sample preparation.

Participants/materials, setting, methods: Participants in our IVF program underwent sperm gamma-H2AX analysis and DNA fragmentation test (TUNEL). Gamma-H2AX sites were detected by immunofluorescence following standard fixation and permeabilization procedures. An additional DNA digestion procedure was used to expose target epitopes to primary antibody. Sperm samples were prepared by density gradients before performing DNA fragmentation and gamma-H2AX assays.

Main results and the role of chance: We observe that H2AX-phosphorylation increases in a time-dependent manner. Basal levels of gamma-H2AX were 2,99%±1,38%. After 2 hours, H2AX-phosphorylation showed in average a higher proportion of positive sperm (4,80%±5,08%). Similarly, an increase in the percentage of gamma-H2AX sperm after 24 hours was observed (7,31%±6,93%; Test-t p=0,025). To normalize the variation of gamma-H2AX levels over time, results were expressed as the proportion of positive sperm detected after 2 hours (t2-t0/2) and 24 hours (t24-t0/24). We observed that gamma-H2AX levels increase particularly during the first 2 hours after the sample preparation (t2-t0/2=1,10; t24-t0/24=0,11). Interestingly, in those samples with abnormal TUNEL dynamics (t2-t0/2) ≥ 2,5 %), gamma-H2AX levels at both, 2 and 24 hours, were higher than in samples in which TUNEL dynamics were normal (t2-t0/2= 1,96 vs t2-t0/2= 0,04 and t24-t0/24= 0,12 vs t24-t0/24= 0,10).

Limitations, reasons for caution: Detection of gamma-H2AX is an added-value to infer DNA fragmentation results. Dynamics of gamma-H2AX allows evaluating the efficiency of DNA damage signaling machinery in mature sperm.

Wider implications of the findings: The current data has been obtained in a limited number of samples. Further investigations are required estimate the impact of gamma-H2AX further human embryonic development.

Trial registration number: n/a

P-109 Temporal trend in semen quality among candidate sperm donors over 23 years: fertility potential over time.

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Study question: Is there a temporal trend in semen parameters and fertility potential of unselected candidate sperm donors over the last 23 years?

Summary answer: There has been no substantial clinical change in semen quality and fertility potential of candidate donors over a period of 23 years.

What is known already: A recent meta-regression analysis by Levine et al.2017 reported a temporal trend in sperm count showing a significant decline in sperm count between 1973 and 2011 in Western countries. Studies have shown geographical variation of semen quality. Semen quality has been shown to have declined in many areas of the world, plausibly associated with multiple environmental influences and lifestyle factors. Significant decline in male reproductive health has serious implications. Nonetheless, the issue remains controversial due to methodological errors in semen analysis.

Study design, size, duration: A retrospective cohort study of semen analysis of 439 men presenting as candidate sperm donor, regardless of proven fertility capacity, at the University Hospital of Antwerp between January 1995 and December 2017.

Participants/materials, setting, methods: The recruitment process was mostly conducted by national media campaigns and oral publicity. The reported sperm parameters were analyzed on the fresh ejaculate within 1 hour after production. Semen analysis was performed in accordance with the recommendations of the WHO manuals. Technicians received specific training via semen courses before carrying out the analysis over the entirety of the study period. Internal and external quality controls were performed regularly.

Main results and the role of chance: The mean (± standard deviation) age of candidate donors was 29.5 (± 7.3 years). The mean ejaculate volume (3.6 ± 1.7 ml), sperm concentration (71.1 ± 60.6 M/ml), progressive motility (56.2 ± 15.6 %) and morphology (10.0 ± 5.7 %) of a candidate's first semen sample was markedly higher than the fifth percentile values published in the WHO 5th Edition.

The multiple linear regression model taking into account year of donation, abstinence duration and year of birth showed a discrete, though statistically significant, decline per year of donation for total motility (β= -0.03, p< 0.05) and morphology (β= -0.15, p< 0.05). Substitution of year of birth by age revealed similar results (respectively β= -0.02, p< 0.05 and β= -0.18, p< 0.05). Semen concentration, ejaculate volume and total sperm count did not change significantly over the study period. The mean (± standard deviation) clinical pregnancy rate per effective donor recruited (n=104), defined as the number of pregnant women per number of women who initiated treatment with a donor's sperm, was 68.8% ± 26.2%. This measure did not show a significant change in function of year of donation (β= -0.30, p= 0.52).

Limitations, reasons for caution: Retrospective study, no adjustment for potential confounders such as lifestyle of the candidate donor or factors related to the acceptor.

Wider implications of the findings: These findings concerning sperm quality/fertility potential of candidate donors are reassuring given reports of decline. However, sperm quality remains the cornerstone of male fertility diagnosis and is considered an indicator of overall health, as such vigilance is required and research focusing on the prevention of sperm deterioration should be ongoing.

Trial registration number: /

P-110 Nanofibrous scaffold as a promising substrate for differentiation of embryonic stem cells toward germ-line

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Study question: Can nanofibrous scaffold (NS) promote differentiation of embryonic stem cells (ESCs) to germ-line cells.

Summary answer: NS significantly increase the differentiation rate of ESCs toward to germ-line cells when co-cultured with sertoli cells.

What is known already: The increasing prevalence of male infertility due to disability in production of functional sperms has remained as a main problem worldwide. The development of a new and reliable procedure for guided differentiation of stem cells toward sperm-producing cells may be a new window of hope for such infertile males.

Study design, size, duration: In this study, we studied the effects of nanofibrous scaffold on differentiation of ESCs co-cultured with sertoli cells toward germ-line cells. Nanofibrous scaffold were fabricated by electrospinning. Sertoli cells and ESCs were isolated from NMRI mice. The cells were treated with bone morphogenetic protein 4 (BMP4) to stimulate differentiation of ESCs. The differentiation of ESCs were studied at days 3 and 7 of induction with BMP4.

Participants/materials, setting, methods: Mechanical and biological properties of the scaffold for sertoli cells and ESCs were then studied *in vitro*. ESCs were cultured on gelatin nanofibrous sheet and then transferred to cell culture plate seeded with sertoli cells. The cells were feed using differentiation media containing BMP4. The differentiation value of ESCs was then measured after 3 and 7 days by different cellular and molecular evaluations.

Main results and the role of chance: NS showed a uniform morphology with good biocompatibility for both sertoli cells and ESCs. The ESCs cultured on NS co-cultured with mitomycin C-treated sertoli cells showed higher level of differentiation toward germ-line specific gene markers such as MVH, DAZL and others. The results showed that more than 50% of the ESCs seeded on NS expressed MVH, significantly higher than those cells cultured on plastic surface of cell culture plate. The ESCs seeded on culture plate showed minimum differentiation. Based on our findings, co-culture of ESCs with sertoli cells on both 3D nanofibrous scaffold and culture plate showed higher level of guided

differentiation when compared to those ESCs cultured on NS and culture plate without sertoli cells. This data is consistent with other relevant studies.
Limitations, reasons for caution: Culture of ESCs on one side of NS is difficult due to low mechanical property of nanofibrous sheet. It is suggested to improve mechanical behavior of NS using cross-linking agents or some blends. The function of the differentiated cells also need to be determined.
Wider implications of the findings: Our results are in agreement with other studies showing the positive effects of 3D scaffolds for improving proliferation and guided differentiation of stem cells. Many studies are underway to develop a new strategy or factors to increase differentiation of stem cells to a specific cell line.
Trial registration number: NA

P-111 Sperm freezing in 0.25 ml straws. Protocol optimization

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Study question: What is the most appropriate protocol for the sperm freezing in 0.25 ml straws?
Summary answer: Cooling time of 20 minutes at 4°C followed by 20 minutes nitrogen vapors recover higher proportion of live sperm unaffecting their motility and mitochondrial activity.
What is known already: Despite the successive improvements in the techniques of human sperm cryopreservation, today there are no optimized protocols that allows to recover samples with similar quality to that observed in fresh. Find the freezing and thawing rates and the most appropriate hardware are some of the most important aspects to consider for the optimization of this process.
Study design, size, duration: 48 sperm samples from 12 healthy young donors were collected (abstinence 48-72h) and cryopreserved following the WHO manual (2010) guidelines.Each sample underwent four cooling protocols: 40 minutes at 4°C plus 40 minutes nitrogen vapor, 20 minutes at 4°C plus 40 minutes nitrogen vapor, 40 minutes at 4°C plus 20 minutes nitrogen vapor and 20 minutes at 4°C plus 20 minutes nitrogen vapor.Evaluation after thawing: Motility (CASA), viability, mitochondrial membrane potential and membrane integrity (flow cytometry).
Participants/materials, setting, methods: Sperm donors were selected according to ESHRE guidelines for gamete donation. After liquefaction, we washed the sperm samples with PBS and diluted them in freezing medium (Irvine Scientific). Extended spermatozoa were loaded into 0.25-mL plastic straws and cooled down following the experimental design. The straws were thawed at 37 °C for 7 min. Data were analyzed by linear mixed-effects models using *SPSS Statistics* (IBM, versión 23).

Subsequently, motility, viability and membrane integrity studies were carried out.
Main results and the role of chance: The treatment of 20 minutes at 4°C followed by 20 minutes in nitrogen vapors, is the most suitable for freezing in straws of 0.25ml since it is the one that allows recovering a greater proportion of live sperm ($p = 0.036$) not affecting motility, viability or membrane integrity.
Limitations, reasons for caution: It would be interesting to compare the results obtained against those obtained with the protocol in straws of 0.5 ml. The number of samples is limited and more studies are needed to contrast these results.
Wider implications of the findings: The routine use of this protocol would mean a better management of the procedures for freezing, preparing and using of sperm, avoiding unnecessary losses of specimens.
Trial registration number:.

P-112 Evaluation of the ProAKAP4 Detection kits as functional tests of sperm quality under stress conditions

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Study question: Is proAKAP4 sperm concentration as evaluated by 4MID® tests, a pertinent new parameter to evaluate sperm quality in environmental stress conditions?
Summary answer: The 4MID® tests can be easily used to quantify proAKAP4 concentrations and concentration modulations under environmental and oxidative stress conditions in various settings.
What is known already: ProAKAP4 is the precursor of the AKAP4 protein that is required for motility, capacitation and fertilization. ProAKAP4 was recently described as a reservoir of sperm motility and a pertinent read out of sperm quality in mammals. As sperm quality assessment is still challenging, there is a real need for functional test of spermatozoa quality to improve fecundation success rate.
Study design, size, duration: In this study, both clinical data from a cohort of patients in assisted reproduction center and preclinical data from mice models design to study oxidative effects on fertility were collected.
Participants/materials, setting, methods: ProAKAP4 modulations were measured in patients and animals using dedicated 4MID® kits and by proteomic methods. 100 patients including smokers and no-smokers as well as normozoospermic patients and men with abnormal sperm parameters (WHO 2010 criteria) were included. In the experimental setting, mice were under low or high fat diet and placed in a cigarette smoke atmosphere for 5 months. Mice testes and epididymis spermatozoa were collected and analyzed for proAKAP4 and oxidative marker expression.
Main results and the role of chance: Using 4MID® kits and proteomic methods, our results showed that proAKAP4 in both human and mice sperm is significantly correlated with sperm motility and inversely correlated with DNA fragmentation. Cigarette smoke had clearly a negative impact on proAKAP4 expression in both human and mice spermatozoa. Indeed, in our different mice models, we showed that proAKAP4 concentrations were diminished by up to 2.5-fold in male under cigarette smoking. Furthermore, antioxidants as added in drinking water improved significantly the proAKAP4 levels in smoking mice as well as in overweight and obese mice. Furthermore, in a preliminary series of ART patients, we showed that high level proAKAP4 were significantly correlated with a lower proportion of abortions in intrauterine insemination settings. The marker proAKAP4 can be considered then as a pertinent new sperm parameter that could be assessed under any environmental conditions impacting male fertility or any antioxidant therapy. The detection of proAKAP4 using the 4MID® kit is a robust method to evaluate sperm quality.
Limitations, reasons for caution: The limited patient cohort might not be fully representative of the general population. Larger cohorts of mens from infertile couples and from volunteers are needed to substantiate the clinical application of the 4MID® test. With animal models to evaluate oxidative stress, we evaluate proAKAP4 present in epididymis spermatozoa.
Wider implications of the findings: The 4MID® tests could represent a pertinent sperm parameter to be evaluated routinely, that then may highlight the quality of spermatogenesis and consequently the sperm quality. This new parameter may be assessed during investigations for male infertility or to evaluate impact of antioxidative therapeutic approaches.
Trial registration number: not applicable

P-113 Novel association between apoptotic sperm biomarkers with seminal biochemical parameters and acetylcholinesterase activity in patients with teratozoospermia

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Study question: In order to determine whether the dysfunction of physiological apoptosis and specific seminal biochemical parameters could be associated with infertility and sperm morphology defects.
Summary answer: the present results emphasize the impact of apoptosis in the physiopathology of teratozoospermia and suggest that seminal biochemical disturbance might arise such damage.
What is known already: sperm morphological defects are associated with apoptosis
Study design, size, duration: Ejaculated sperm samples of seventy patients with isolated teratozoospermia and twenty-one fertile donors were analyzed.
Participants/materials, setting, methods: The proportion of both viable and dead spermatozoa expressing activated caspases was detected by Fluorescence microscopy through the use of different specific carboxyfluorescein-labeled caspase inhibitors FLICA. The different stages of apoptosis in human were qualitatively and quantitatively determined using the AO/EB Fluorescent staining method. The levels of the seminal biochemical parameters (Acetylcholinesterase (AChE), Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CK), Iron (Fe), Calcium (Ca), and Phosphorus (P)) were evaluated spectrophotometrically
Main results and the role of chance: Patients with teratozoospermia showed significantly high proportions of Dead and live spermatozoa with activated caspases and spermatozoa in the late stage of apoptosis when compared to controls. Among the different studied biochemical seminal parameters, the rates of acetylcholinesterase activity,creatine phosphokinase, iron, Calcium were significantly increased the patient group. However, the rate of phosphorus was significantly decreased. Interestingly, significant relationships were found between the studied biochemical and apoptotic biomarkers and the rates of atypical sperm forms. Furthermore, positive correlations were found between phosphorus acetylcholinesterase, iron, CPK, and LDH with apoptotic markers
Limitations, reasons for caution: we suggest further comparative studies connecting the spermatozoa morphology and the DNA integrity with more apoptotic markers.
Wider implications of the findings: the use of both AO/BET staining and the polycaspases assay data provide clear evidence that the apoptotic alterations are closely correlated to the morphological features of sperm. The defiance or overload of seminal trace elements or enzymes may cause functional and qualitative defects on spermatozoon.
Trial registration number: not applicable

P-114 Microfluidic sperm selection enhances ICSI outcomes by selecting spermatozoa with the highest chromatin integrity

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Study question: Does selecting spermatozoa with superior chromatin integrity lead to higher implantation and clinical pregnancy rates with ICSI?
Summary answer: Microfluidic sperm selection (MFSS) provides spermatozoa with optimal chromatin integrity and yields higher implantation and clinical pregnancy rates with ICSI.
What is known already: Sperm preparation methods aim at providing specimen for insemination with the highest progressive motility independent of phenotypic and genomic integrity. Both single-strand (ss) and double-strand (ds) DNA nicks and breaks inhibit the ability of the male genome to support embryonic development. While different mechanisms are in place to prevent this phenomenon, they may be hindered by a defective epididymal function or a suboptimal or aged oocyte.
Study design, size, duration: From October 2016 to January 2019, consenting men (N=32) known to have higher DNA fragmentation in their ejaculate and prior ART failure had their ejaculates simultaneously processed by density gradient centrifugation (DGC) and MFSS. TUNEL was carried out on the raw specimens and on the differently selected aliquots. In men (N=13) treated by ICSI with their female partners, clinical outcomes were recorded. Semen parameters, chromatin integrity, embryo implantation, and pregnancy characteristics were compared.
Participants/materials, setting, methods: Fresh ejaculated specimens from consenting men were collected for standard semen analysis in accordance with WHO 2010 criteria. DGC and MFSS were used to isolate motile spermatozoa based on cell motility and fluid dynamics. Sperm chromatin fragmentation (SCF) was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of ≥15%. ICSI was performed in the standard fashion.
Main results and the role of chance: A total of 32 men with an average age of 41±9 years had the following average semen parameters: concentration of 48.1±37 x 10^6/mL, motility of 31.5±14.6, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 33.8±25 and 11.6±12 x10^6/mL, with 59.4±33% and 97.6±9% motility, respectively ($P<0.0001$).

The morphology of the raw sperm sample improved from 2.3±1% to 4.0±1% after MFSS, while it remained at 2.6±1% after DGC. The average SCF decreased from 24% in raw samples to 15% following DGC and became 1.7% after MFSS processing ($P<0.0001$).

Couples (n=13) who underwent ICSI had an SCF in their raw sample of 30.3%, which reached 22% after DCG selection and was only 1.5% after MFSS ($P < 0.0001$). These couples (female age, 36.5±3 years; male age, 42±9 years) underwent 28 cycles with DGS sperm selection, achieving a fertilization rate of 67%. The implantation rate was only 3.4% (1/29) with a clinical pregnancy rate of 6.6% (1/15) that ended in pregnancy loss. Subsequently, these couples underwent ICSI cycles with MFSS and achieved a fertilization rate of 61%. The implantation rate rose to 31% (7/23) *($P<0.05$)* with a clinical pregnancy rate of 54% (7/13) *($P<0.05$)*. The pregnancy loss was 15.3% (2/13).
Limitations, reasons for caution: This study represents a preliminary experiment on a small number of subjects. If confirmed, MFSS yields a male gamete with the highest chromatin integrity, progressive motility, and improved morphology. MFSS should be used in couples afflicted by high levels of SCF in their raw ejaculate.
Wider implications of the findings: According to our study, SCF appears to be linked to the kinetic characteristics of the sperm cell. MFSS yielded the highest portion of progressive motility with the highest DNA integrity. This novel microfluidic system may serve to identify spermatozoa with the highest functional and genomic integrity.
Trial registration number: N/A

P-115 Exploring the interaction of seminal fluid exosomes within the male reproductive tract

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Study question: To identify, quantify, and describe exosomes at different levels of the male reproductive tract in order to understand exosome function within seminal fluid.
Summary answer: Exosomes are differentially produced at various levels of the male reproductive tract. Exosome composition differs amongst men with infertility and those with proven fertility.
What is known already: Exosomes are small lipid-membrane bound vesicles containing functional biomolecules, such as proteins, lipids, micro-RNA, and DNA. They are released from most cells in the body and are also present in many bodily fluids. Seminal fluid exosomes have been previously isolated, however, little is known about their source and their role in the male reproductive tract. Exosomes in other tissues have been shown to mediate immunomodulatory functions and even to direct tumor metastasis. Seminal fluid exosomes have been postulated to act via a similar mechanism during fertilization and implantation, as well as to possibly participate in directing spermatogenesis.
Study design, size, duration: A total of 30 ejaculated specimens from 30 different subjects with normal semen analysis parameters (5 ejaculated specimens from men with normal semen analyses and proven fertility served as controls) and 17 surgically retrieved testicular specimens from 17 different subjects (13 non-obstructive azoospermia, 4 obstructive azoospermia) were obtained from consenting men from March 2018 to August 2018.

Participants/materials, setting, methods: Specimens were centrifuged at 500xg for 10 mins followed by 3,000xg for 20 mins, and then 12,000xg for 20 mins. The resulting supernatant was centrifuged at 100,000xg for 70 mins for exosomal isolation. Protein concentration was measure by BCA. The NanoSight LM10 nanoparticle analysis system was used for exosome characterization. Isolated exosomes were labeled with PKH-26 and cultured with spermatozoa labeled with PKH-67 for 24 hours, then observed with confocal microscopy at various time points.

Main results and the role of chance: The total number of isolated exosomes was significantly higher in ejaculated specimens compared to testicular specimens (2075x109/µL vs 71x109/µL, respectively, $p<0.01$). This likely reflects the known proportional contribution of the male genital tract organs to the ejaculate (2-3% testicular contribution to the ejaculate). Additionally, there was significantly higher overall exosomal protein concentrations within ejaculated specimens compared to testicular specimens. However, when analyzed on a protein concentration per exosome basis, there was significantly more protein per exosome in testicular specimens compared to ejaculated specimens (0.6 µg/exosome vs 0.08 µg/exosome, respectively, $p<0.01$). There was no significant difference in exosomal number in testicular specimens from men with non-obstructive azoospermia compared to those with obstructive azoospermia. There was no significant difference in mean exosome size between ejaculated and testicular specimens. Data were analyzed using Welch's t-test and the Mann-Whitney U test for non-parametric data. After incubation, seminal fluid exosomes were visualized to be taken up in the head of spermatozoa at 3 hours and at 24 hours.

Limitations, reasons for caution: All specimens were obtained from different subjects, and therefore there is not an ejaculated specimen and a testicular specimen from the same subject available for comparison in the study cohort.

Wider implications of the findings: The unique packaging pattern of exosomes indicates a specialized population of exosomes within the testicle, which may be involved in the communication between Sertoli cells and in ordaining waves of spermatogenesis within the seminiferous tubule network.

Trial registration number: None.

P-116 Artificial intelligence as a tool in predicting sperm motility and morphology

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Study question: Can artificial intelligence (AI) algorithms predict sperm motility and sperm morphology following the WHO criteria, from videos of wet semen preparation.

Summary answer: AI methods have the potential to predict both sperm motility and sperm morphology with high reliability from live sperm videos.

What is known already: Although computer-aided sperm analysis (CASA) has been available for several decades, manual semen analysis according to WHO guidelines is still regarded as the gold standard. The assessment of sperm motility by CASA systems is rapidly performed, however, the tracking for spermatozoa in fresh semen is prone to error, and results may differ from manual analysis. Assessment of sperm morphology is performed on stained cells for both manual and CASA and is time-consuming. AI methods may have a large potential in classification and interpretation of sperm imaging and thereby replace the subjective and time-consuming methods.

Study design, size, duration: Semen characteristics and videos from semen samples from 85 participants recruited from the general population and clinics. One video was captured for each semen sample. We used three-fold cross-validation to compensate for the relatively limited dataset.

Participants/materials, setting, methods: Men aged 18 years and above. Sperm motility and morphology was manually assessed according to WHO criteria. Sperm videos were recorded at 400x magnification and analysed using the 250 most representative frames per video as input for a 2D convolutional neural network (CNN) InceptionV3 architecture. For training the CNN regression, Nadam as optimizer and mean absolute error (MAE) for loss function were used. For baseline, ZeroR (pseudo regression) was performed. Results are reported as MAE.

Main results and the role of chance: Prediction for sperm motility based on videos led to an additive MAE of 20.92 compared to the ZeroR baseline which had an additive MAE of 25.07. This indicates an overall good performance. The MAE of the AI method for the progressive motile spermatozoa was 12.85 and for the non-progressive motile 8.07, compared to 17.21 and 7.86 from the baseline. This shows that the AI method is better in predicting the progressive motile spermatozoa than the non-progressive. For the sperm morphology, the additive MAE for the AI method is 21.61 compared to 21.80 from the ZeroR baseline. Although smaller than with motility, this is an improvement. The individual MAE is considerably smaller for morphology prediction than for the motility. The AI method has an MAE of 1.91 for normal spermatozoa, 2.63 for head defects, 8.32 for midpiece defects, 6.39 for tail defects and 2.35 for cytoplasmic droplets.

Limitations, reasons for caution: For this algorithm, the optical flow between frames was not taken into account. The performance of the model should be tested in further studies by comparison with human performance, including inter-observer variation.

Wider implications of the findings: 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. The AI model created may have a potential in semen analysis, especially in predicting sperm motility from videos of wet sperm preparation.

Trial registration number: not applicable

P-117 RNA aptamers for the recognition of the human SRY protein

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Study question: This research project aimed to produce RNA aptamers for the sex-determining region Y (SRY) protein by the systematic evolution of ligands by exponential enrichment (SELEX) technique.

Summary answer: The SELEX technique was successfully implemented, and we identified seven RNA-based synthetic molecules. The best of them showed a *Kd*of 314 nM.

What is known already: To date, sexual sorting is achieved with the fluorescent dye Hoechst 33342 and sorting of the X- and Y- spermatozoa by the fluorescent intensity they emit. However, the Hoechst stain binds to the spermatozoa's DNA, which leads to viability reduction and membrane status changes. The SRY is a gene that produces the testis-determining factor, also known as SRY protein; exclusively in Y- spermatozoa. This nuclear and cytosolic protein could be a good target for sperm identification and sex-sorting.

Study design, size, duration: Does not apply. This was a basic science study with *in vitro* biochemical and biophysical experiments.

Participants/materials, setting, methods: A recombinant human SRY protein was produced and used for the SELEX technique to identify RNA-based synthetic molecules. Five SELEX cycles were performed and final products were identified by Next Generation Sequencing. The bioinformatic toolkit FASTAptamer highlighted top-ranked RNA molecules. These were chemically synthetized and their affinities to the SRY protein were measured using Micro Scale Thermophoresis.

Main results and the role of chance: Seven RNA aptamers were identified and were tested to determine their affinities using a Micro Scale Thermophoresis apparatus. The best of them showed a Dissociation constant of 314±48 nM.

Limitations, reasons for caution: The pool of RNA molecules for SELEX was incubated using a recombinant human SRY protein produced in a*E. coli* system. The RNA aptamers identified were tested using the same protein as well.

Wider implications of the findings: RNA aptamers that bind the SRY protein could be further modified to produce RNA aptamer beacons to bind SRY and emit a fluorescent signal. This approach could lead us to develop new sperm sex-sorting techniques that do not compromise DNA integrity.

Trial registration number: Not applicable.

P-118 Prevalence of Ureaplasma urealyticum and Mycoplasma hominis in infertile couples

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Study question: To determine the prevalence of genital colonization by *Ureaplasma urealyticum* (*Uu*) and *Mycoplasma hominis* (*Mh*) in infertile couples

Summary answer: Genital colonization by *Uu* and *Mh* seems to be widespread among infertile couples, especially in women and with a higher prevalence in both sexual partners.

What is known already: *Uu* and *Mh* colonize the genital tract of sexually active men and women. It is still unclear whether they should be considered as commensals or associated with pathological conditions. They are suspected to be the causative agents of urethritis, pregnancy complications, prenatal infections, bacterial vaginosis and pelvic inflammatory disease.Their impact on fertility has been widely studied but still remains controversial. Considering the impact that these microorganisms can have on fertility, and the high number of unrecognized colonized individuals, a better knowledge of the prevalence of *Uu* and *Mh* is crucial to detect potential reservoirs.

Study design, size, duration: We conducted a retrospective study over the period of six months (from January 1st to June 30th 2018), including 443 couples attending the gynecology and obstetrics clinic. All patients were undergoing investigations for infertility and the samples collected were obtained as part of the diagnostic work-up.

Participants/materials, setting, methods: Cervicovaginals swabs from women and semen samples from their male partners were collected on the same day. Samples were obtained after 3 days of sexual abstinence. Patients were not taking antibiotics for at least 2 weeks prior to the analysis. Gram staining and bacteriological and yeast cultures were performed on all samples. *Uu* and *Mh* DNA was detected using *C.trachomatis/Ureaplasma/M.hominis* Real-TM PCR kit (Sacace biotechnologies Srl®, Italy) according to the manufacturer's instructions.

Main results and the role of chance: *Uu* was detected inc ervicovaginal samples in 34.7% of cases, *Mh* in 6.3% and the association *Uu+Mh* in 3.8%.Female colonization by *Uu* and *Mh* was significantly associated withbacterial vaginosis ($p<0.001$). Semen samples were positive in 20.8% of cases for Uu and in 2.3% for *Mh*. The association of both species was observed in 1.4%of cases.All men were asymptomatic; 49.1% of the women presented genital symptoms including abnormal discharge (38.8%), itchiness (26.7%), dyspareunia (28.5%) and urinary symptoms (11.5%). However, these symptoms were not associated with *Uu/Mh* colonization. Women whose male partners tested positive for *Uu* were five times more likely to be colonized by *Uu* ($p<0.001$; odds ratio: 5.73 [3.49-9.42]).Female partners of *Mh*-colonized men were 18 times more likely to be infected with *Mh* ($p<0.001$; odds ratio: 17.82 [4.82-65.99]).

Limitations, reasons for caution: We found that *Uu* and to a lesser extent *Mh* were frequently present in infertile patients.However, further research is needed to determine whether colonization by these pathogens is more frequent in infertile couples than in general population.

Wider implications of the findings: Taking in consideration the high prevalence of *Uu* and *Mh* in infertile couples, further research is warranted to evaluate the actual impact of these pathogens on male and female fertility.

Trial registration number:

P-119 High level of Oxidative stress does not predict normal sperm parameters

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Study question: The evaluation of seminal oxidation–reduction potential can predict normal sperm parameters ?

Summary answer: Seminal oxidation–reduction potential, DNA fragmentation and chromatin decondensation were inversely associated with normal sperm parameters.

What is known already: Oxidative stress (OS) is one of the major mediators in etiologies of male infertility; it has deleterious effects on spermatozoa, including DNA damage. The evaluation of seminal oxidative stress have a crucial role in the identification of patients who may benefit from treatments. Various tests including total antioxidant capacity assay, reactive oxygen species assay or malondialdehyde assay used to evaluate OS. Seminal Oxidation-reduction potential (sORP) is a measure of overall balance between oxidants and antioxidants, providing a comprehensive measure of oxidative stress. The MiOXSYS System has been introduced recently as a measure of OS with high specificity.

Study design, size, duration: This is a prospective comparative study includes patients with primary or secondary infertility($\geq$ 3 years). Human semen samples were obtained from 134 patients performing a complete exploration of semen parameters at a private ART clinic. Sperm parameters were evaluated according to World Health Organization (WHO) 2010 guidelines. Exclusion criteria included azoospermia and samples with a concentration $<3 \times 10^6$ sperm/mL.

Participants/materials, setting, methods: In each semen sample, which was collected after sexual abstinence of 2–5 days in addition to conventional sperm parameters the following parameters were measured : (i)Spermatozoa with DNA fragmentation using the TUNEL assay, (ii)Abnormal chromatin condensation using Aniline Blue assays, (iii) Oxidative stress was measured by MioXSYS System.

Main results and the role of chance: The study subjects were grouped into two groups refering to a cut-off value of 1,48 mV/10^6 sperm/mL. There was 84 patients in group 1 with low level of sORP (<1,48 mV/10^6 sperm/mL) and 52 patients in group 2 with high level of sORP ($\geq$1,48 mV/10^6 sperm/mL). Comparing to patients of group 1, patients of group 2, had a significantly lower mean sperm count (25.33 vs 39.41 x 10^6 sperm/mL), progressive motility (23.5% vs 30.5%), and vitality (58% vs 63%). Conversely patients of this group had significantly higher levels of DNA fragmentation and chromatin decondensation. This results confirm that sORP, DNA fragmentation and chromatin decondensation were inversely associated with normal sperm parameters. When subgroups of patients were investigated according to normal or abnormal semen parameters we identified 2 subgroups in each group : a subgroup containning 37% of patients (n=31) of group 1 failed to meet one or more criteria for sperm quality and a second subgroup contain 88% (n=48) of group 2. For these two subgroups we identified a negative correlation between sperm parameters and levels of these two parameters : DNA fragmentation and chromatin hypocondensation ($p<0.001$).

Limitations, reasons for caution: We must continue our study with a greater cases number to determinate a new cut-off value and for a stronger conclusion statement.

Wider implications of the findings: Sperm DNA fragmentation, chromatin hypocondensation and sORP should be included in assessment of male infertility because they can have prognostic implications for couples experiencing male infertility and undergoing ART.

Trial registration number: Not applicable

P-120 Artificial intelligence predicts sperm motility from sperm fatty acids

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Study question: Can artificial intelligence (AI) predict sperm motility from sperm fatty acids (FAs) and estimate sperm FA levels from videos of live sperm?

Summary answer: Sperm fatty acid data can be used to predict sperm motility, and sperm videos can be used to estimate sperm fatty acid levels.

What is known already: Omega-3 FAs are abundant in the sperm and are positively associated with sperm motility, especially progressive motility. Other sperm FAs present in lower levels may also be associated with sperm characteristics. AI may have the potential to predict sperm motility based on FA composition and thereby provide more insight into FAs impact on sperm function.

Study design, size, duration: Sperm videos, sperm motility evaluations and fatty acid data from 85 men 18 years old and above. Participants were recruited from the general population and clinics. One video was captured for each semen sample. Three-fold cross-validation was used to compensate for the low sample size.

Participants/materials, setting, methods: Sperm motility was classified according to WHO guidelines (2010), and sperm videos were recorded at 400x magnification. Sperm FAs were measured by gas chromatography. Prediction of FA composition from videos was performed using 250 representative frames per video as input for a convolutional neural network (CNN) InceptionV3 architecture. Motility prediction from FAs was performed using random forest, linear regression and ZeroR (baseline). Results are reported as mean absolute error (MAE) and by Pearson's correlation coefficient.

Main results and the role of chance: Random forest was the best predictor of progressive motility (correlation = 0.53, MAE = 14.06) and of non-progressive motility (correlation = 0.24, MAE = 7.31) based on sperm fatty acid values. It outperformed the average baseline (ZeroR) and standard linear regression. Progressive motile spermatozoa had the lowest individual MAE whereas non-progressive had the highest. Random forest could predict total sperm motility with an additive MAE of 21.37 and performed better than the average baseline (ZeroR) and standard linear regression. Progressive motile spermatozoa had the lowest individual MAE whereas non-progressive had the highest. Linear regression also outperformed the average baseline but not for every single MAE, whereas random forest did. The MAE for predicting the observed FA levels from the videos using CNN was 19.62. This is good taking into account that only sperm videos were used to predict the FAs. Using the predicted FAs from the videos to perform motility prediction lead to an additive MAE of 22.71 which is only 1.34 higher than the MAE from the measured FAs. This is a good indicator that FAs predicted from the videos have the same predictive power as the measured one.

Limitations, reasons for caution: The present study was performed on a relatively limited data set. A follow-up study including a larger sample size should be conducted.

Wider implications of the findings: AI-models applied on sperm FA data can predict sperm motility, and sperm videos can be used to predict sperm FA composition with similar predictive power as the measured ones. This indicates a strong relation between sperm motility and sperm FA composition.

Trial registration number: Not applicable

P-121 Improvement of sperm quality in hyperviscous semen following DNase I treatment

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Study question: TTo evaluate the ability of exogenous DNase I to release entrapped human sperm cells, with a view to improving spermatozoa quality

Summary answer: DNase I treatment results in a spectacular improvement of spermatozoa motility and morphology

What is known already: Studies have documented that semen hyperviscosity (HSV) occurs in 12–29% of ejaculate. SHV is a condition that can result in male infertility, and it has been associated with reduced sperm motility, a poor outcome in intrauterine insemination (IUI), and increased production of Reactive Oxygen Species. Several therapeutic approaches have been proposed in the settings of reducing the viscosity of SHV semen. Over hydration, prostate massage and semen gentle aspiration and expulsion through a 5-ml syringe, were not effective. Proteolysis through the use of chymothrypsin improves the handling of HSV semen although some alterations occur in sperm proteins.

Study design, size, duration: A prospective study was conducted in patients with a history of infertility at the "Locus Medicus" Medical Clinic, for three years. Semen samples with HSV and with normal viscosity (NSV) were obtained as study and control group, respectively. A set of NSV(ten) and HSV(thirty two) were treated with DNase-I. Another set (twenty six and fifty two, respectively), were processed by Density Gradient Centrifugation, and nineteen with HSV were processed by a combination of the methods.

Participants/materials, setting, methods: Semen samples after liquefaction underwent conventional analysis, motility (types a, b, c, d), vitality and morphology. Taking into consideration that postwashed total progressively motile sperm count (TPMSC) could be useful for predicting the efficacy of IUI the yield of the each method was also evaluated. Moreover, the outcome of yield (i.e. % final PR/ total spermatozoa before) was compared to the % initial PR/total spermatozoa before any treatment. TZI was also reevaluated after each treatment.

Main results and the role of chance: The use of DNase I increases the motility of PR spermatozoa in samples from HSV men in a statistically significant manner But had no effect in sampmles from NSV men.

We then compared the results from sperm that underwent density gradient centrifugation(DGC) following DNase I treatment, with these found after DGC treatment alone. The density gradient centrifugation treatment was employed for IntraUterine Insemination (IUI) as well. The percentage of (a) movement after DGC following DNase I treatment, increased 10.27-fold compared to 4.242-fold improvement in the case of DGC alone. Furthermore, PR movement in the first group increased 2.375-fold in comparison to a 1.776-fold improvement in the second group.

The yield in DGC treatment between individuals with NSV and HSV is statistically significant, i.e. $p=0.0179$. The yield of DGC treatment in the case of HSV individuals was 18.519% while under DNase treatment the corresponding yield was 42.47% ($p<0.0001$). The combination of DNase treatment which is followed by DGC outcomes to yield 29.782% ($p=0.0029$ with DGC alone).

The appraisal of spermatozoa morphology following incubation with DNase-I, results in a statistically significant increase in the percentage of normal spermatozoa ($p=0.0076$). As a result TZI decreased from 1.205 to 1.084 ($p<0.0001$).

Limitations, reasons for caution: In this study we hypothesized that SHV is caused by neutrophil extracellular traps (NETs) and that they are sensitive to DNA degradation via DNase-I. The data presented show that the digestion of NETs is feasible, leading to spermatozoa motility improvement making them suitable for use in assisted reproductive technologies

Wider implications of the findings: The concept which attributes hyperviscosity to inflammation has almost been established. The inflammatory reactions encompass NETs formation from neutrophils for microorganism trapping. Our findings suggest that a main cause of SHV is the formation of NETs and we thus propose the therapeutic potential and utility of this approach

Trial registration number: not applicable

P-122 Shedding light on the origin of seminal microbiome

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Study question: Do maturing sperm cells contain its own microorganisms in the testis?

Summary answer: Using 16S rRNA gene sequencing, our preliminary data suggest that immature spermatozoa in the testis are not sterile and carry its own bacteria.

What is known already: Semen has its own microbiome, harbouring polymicrobial communities, where *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria* are the most widespread phyla. Comparing microorganisms in semen

samples to urine samples in men, it has been identified that these microbial communities shared only 30% of species, indicating that not all the seminal microorganisms originate from the urinary tract. Novel studies are beginning to demonstrate that male reproductive organs, such as prostate, seminal vesicle and testes contain its own microbiome. However, it is not known in which step microorganisms contribute to the seminal microbiome and whether the maturing spermatozoa are sterile or not.

Study design, size, duration: Microbiome analysis using 16S rRNA gene sequencing was carried out on testicular spermatozoa samples. 2 infertile men with azoospermia donated in total 35 single spermatozoa cells for research.

Participants/materials, setting, methods: In total 35 single testicular spermatozoa cells were picked out from fresh testicular biopsies that were beforehand cultivated for 6-24h. The testicular spermatozoa were pooled into 5 samples, followed by genomic DNA extraction and PCR amplification using primers targeting V3-4 hypervariable regions of 16S rRNA gene. The resultant amplicons were sequenced using paired-end Illumina MiSeq system. The quality of raw reads was assessed with Mothur. MG-RAST was used for analysis of all sequences.

Main results and the role of chance: The bacterial 16S rRNA gene was analysed in the testicular spermatozoa and additionally in the cell storage buffer as negative control samples. There were few bacteria present in the storage buffer samples, which were taken into account when analyzing spermatozoa samples. *Actinobacteria* (especially *Bifidobacteriales*), *Bacteroidetes* and *Verrucomicrobia* comprise the highest proportion of detected bacteria.

Limitations, reasons for caution: This is a preliminary study on limited sample size, which will be confirmed in a bigger sample set.

Wider implications of the findings: This is the first study demonstrating presence of microorganisms on testicular spermatozoa. Our findings provide new insight into the origin of seminal microbiome, indicating that some accompanying bacteria originate already from the testicular maturation phase.

Trial registration number: NA

P-123 CRISP1 (Cysteine-rich secretory protein-1) as an epididymis-specific protein in seminal plasma has a prognostic role for the distinction among 3 different subgroups of non-obstructive azoospermia

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Study question: Are there protein markers in seminal plasma that can predict the presence of testicular sperm before an invasive surgery in men suffering from non-obstructive azoospermia?

Summary answer: CRISP1 (Cysteine-rich secretory protein 1) in seminal plasma has the potential role to predict the success of micro-dissection sperm retrieval in non-obstructive azoospermia men (NOA)

What is known already: Men with NOA have no sperm in the semen. Testicular sperm suitable for Intracytoplasmic sperm injection (ICSI) and other assisted reproductive technologies (ART) could be present in NOA patients.

Sperm is collected by micro-testicular sperm extraction (M-TESE). However, there is not a reliable diagnostic test to predict the presence of sperm in the testis biopsy before surgery. About half of azoospermic men are unable to have sperm retrieved at M-TESE and therefore have surgery unnecessarily. The ability to predict the presence of sperm in the testis prior to invasive TESE would improve sperm retrieval rates and avoid harmful surgery.

Study design, size, duration: Specific proteins related to spermatogenesis in seminal plasma(SP) could provide newfound proteomics assay for sperm presence prior to biopsy. This study designed to compare the SP proteomes of 3 different subgroups of NOA. Men with mixed-testicular atrophy(MA) and men with Spermatogenesis arrest(SA) with a positive sperm retrieval at M-TESE. Sertoli-cell-only(SCO) patients with a negative sperm retrieval at M-TESE. Results also compared with men suffering obstructive azoospermia(OA), Klinefelter syndrome and at the end with the control-group.

Participants/materials, setting, methods: SP was collected from men before and after vasectomy (n=10/group). All samples were compared by two-dimensional gel electrophoresis proteomics. Differentially expressed proteins were defined as those with a fold-change >1.5 and a significant difference between the groups ($p<0.05$). Among the significant proteins identified, CRISP1 with more than 3 fold down-regulation (after vasectomy) was selected for further validation by 2 different antibodies, which recognizing 2 different epitopes of protein in the method of Western blotting (n=15/group).

Main results and the role of chance: The significant difference was seen among 3 different subgroups of nonobstructive azoospermia (MA, SCO, and SA). CRISP1 was significantly higher in the seminal plasma of SA patients in compare of SCO ($p<0.01$) and MA ($p<0.001$). On the other hand, CRISP1 was significantly higher than MA patients ($p<0.05$) in patients with SCO and as mentioned in the above less than SA. In the end, MA patients had a significantly less amount of CRISP1 in compare to two other groups.

In addition, the significant difference was found between OA and NOA. This subject had been proved and reported already by *Legare et al. 2013* and our results proved it again.

Interestingly, the achieved results from both antibodies which had different epitopes affinity showed the same pattern of comparison for all different groups of patients. Regarding the achieved results in the compare of controls with other groups of patients, the results were more or less similar for both antibodies. However, it was not 100%. For example, the one antibody showed a significant difference between control and SA, but another antibody didn't show that.

Limitations, reasons for caution: To get a higher impact of achieved significant differences, it is required to increase the number of samples. To find the cut off of protein rate for each subgroup of NOA should be examined.

Wider implications of the findings: CRISP1 was significantly different in SP among 3 different subgroups of NOA patients(namely spermatogenesis arrest, mixed atrophy and Sertoli-cells-only syndrome) by confirmation of at least 2 different antibodies for proteomics-method of Western blotting, and thus has the potential to predict the presence of sperm in the testis prior to biopsy.

Trial registration number: The trial registration number for this project is IRTG-62280541.

POSTER VIEWING
EMBRYOLOGY

P-124 Specific alternative splicing events associated with human oocyte meiotic maturation

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Study question: Is alternative splicing involved in the developmental competence acquisition in *in vivo* mature oocytes?

Summary answer: There is a pattern of alternative spliced genes involved in the regulation of transcription and mitochondrial translation processes present in *in vivo* matured oocytes.

What is known already: During growth and maturation, the oocyte transitions from a transcriptionally active state (oogonia) to a transcriptionally inactive stage, the GV, which subsequently matures to the MII without generally reactivating transcription. Therefore, regulation of expression at the MII stage is mostly driven by post-transcriptional regulatory mechanisms. Alternative splicing (AS) is an important regulatory mechanism throughout development, but little is known regarding its role in the acquisition of oocyte competence. The purpose of this study is to characterize and compare the splicing patterns of fully grown GV oocytes and *in vivo* MII oocytes.

Study design, size, duration: The AS signature of GVs and MII oocytes were compared. Oocytes from 16 women were grouped according to age (young or old) and antral follicle count (high AFC or low AFC) resulting in 4 groups: GVs from young women with high AFC (GV); MII from young women with high AFC (H-MII); MII from women with low AFC (L-MII) and MII from old women with high AFC (O-MII).

Participants/materials, setting, methods: Total RNA from each oocyte was isolated, amplified, labeled and hybridized on HTA-2.0 microarrays. Microarray data analysis was performed in groups: GV group (4; 26.3±4.1 y.o and 27±13 follicles), H-MII group (4; 26±4.6 y.o and 24±3 follicles), L-MII group (4; 27.5±5.4 y.o and 7±1 follicles) and O-MII group (4; 32.8±1.5 y.o and 27±6 follicles). AltAnalyze (FIRMA and ASPIRE algorithms) were used for statistical analysis using default parameters and results validated by qPCR.

Main results and the role of chance: Genes regulated by AS in three different comparisons (H-MII vs GV, L-MII vs GV and O-MII vs GV) were identified using the FIRMA algorithm. The 1253 AS events detected were involved in regulation of transcription and mitochondrial translation. More than 50% of AS events were related to cassette-exon and alternative C' terminus events, suggesting that our results might reflect the physiological control of pre-mRNA processing during oocyte maturation to achieve proper oocyte developmental competence. To determine the inclusion or exclusion of a given exon, we subjected the AS events detected with FIRMA to additional analysis with the ASPIRE algorithm, which takes into consideration the exon-junction values. A total of 36 differential AS events in 35 genes were confirmed to occur between in vivo MII and GV oocytes. Hierarchical clustering of differential AS events showed that the 4 GVs clustered together, and the 12 MII oocytes clustered in a single group despite their differences in age and ovarian reserve. Ten of these genes were differentially spliced in all three comparisons and two of them, *PIK3CD* and *DIAPH2*, have been previously associated with ovarian dysfunction suggesting a possible role in acquisition of developmental competence.

Limitations, reasons for caution: GVs oocytes were collected after ovarian stimulation and thus might not represent the transcriptome of the immature GVs in the ovary.

Wider implications of the findings: The differences observed in the transcribed splice variants in MII and GV oocytes could provide biomarkers of oocyte quality, since we found a profile of confirmed AS events that could determine acquisition of oocyte developmental competence.

Trial registration number: not applicable

P-125 Zygotic cytoplasm size pattern (ZCP) as an early predictor of blastocyst formation

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Study question: A robust early embryo predictor is lacking, can the dynamic change of zygote cytoplasm serve as an early predictor for embryo development?

Summary answer: The dynamic change of two zygotic cytoplasm size patterns (ZCPs) can serve as an early predictor for blastulation.

What is known already: After fertilization, a series of dynamic and complex events are triggered in a zygote. The size of a zygote cytoplasm is changing over time before its first cleavage. Zygotes with changing of cytoplasmic size behavior, such as too active or inactive, tend to have low grade embryos.

Study design, size, duration: This was a prospective study and involved 184 zygotes cultured in the time-lapse incubator. Their images were captured every ten minutes from 18 hours after fertilization to the first cleavage. The size of cytoplasmic cross-section at the focal plane was measured by our previous developed automated recognition system (Poster presentation P-348, ASRM 2017). The dynamic size of zygote cytoplasm size changes was considered as time series.

Participants/materials, setting, methods: We used dynamic time warping (DTW) for calculating the similarity among each zygote and partition around medoids (PAM) to cluster the zygotes into different patterns. For each pattern, DTW barycenter averaging method was used to figure out the dynamic patterns. We used the pattern value to predict blastocyst formation by logistic regression. The receiver operating characteristic curve (ROC) was used for evaluating the predicting value of zygote cytoplasmic size pattern.

Main results and the role of chance: We measured the size of 184 zygotes from 9397 images. Two ZCPs were clustered based on the similarity of cytoplasmic changes. Zygotes of ZCP 1 follow the change that their cytoplasm size decreases all over time. However, Zygotes of ZCP 2 follow the change that their cytoplasm decreases at first but gradually recovers to its original size and then further increases. The logistic regression result showed that for a given zygote, the closer the development pattern to ZCP 2 the higher probability of the it to develop into a blastocyst. For blastulation prediction, the area under the ROC is 0.65 comparable to the Alpha/Eshre morphology assessment method. It should be noted that the ZCPs only use the information of Day 1 development. It has a high potential to combine with existent time-lapse algorithm.

Limitations, reasons for caution: The zygote is spherical, but we can only get its size of cross-section under the time-lapse incubator.

Wider implications of the findings: We first exploited zygotic cytoplasm change dynamics, for predicting the blastulation. We found two main patterns during zygote development and the information is useful as an early predictor for blastocyst formation. This morphology-based predictor has a potential to combine with other embryo selection algorithms for choosing better quality embryos.

Trial registration number: not applicable

P-126 Impact of different time intervals from ovulation trigger on implantation and treatment outcome in IVF and ICSI procedures.

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Study question: Does the variation in time intervals from hCG triggering in IVF and ICSI procedures affect reproductive outcome?

Summary answer: In ICSI cycles we observed better outcome with shorter time intervals to injection; in IVF cycles, a longer gap prior to insemination had better outcomes.

What is known already: Previous reports have observed a greater number of available embryos and a significant increase in fertilization and clinical pregnancy rates when oocyte collection occurred at or even after 36 h from the hCG trigger. Other authors have also proposed delaying oocyte retrieval to optimize oocyte maturation.

Modifying oocyte incubation time could also affect reproductive outcomes and synchronise nuclear and cytoplasmic maturation. Some authors concluded that the incubation of oocytes for approximately 1.5-2 hours before denudation significantly influences the implantation rate.

Study design, size, duration: An initial set of 1305 patients performing ART treatment using their own oocytes over an 18 month period was reviewed. After exclusion of patients with less than 3 oocytes retrieved (226), a cohort of 1079 patients was analysed. Among them, 567 underwent ICSI and 512 conventional IVF insemination. We analysed the number of oocytes retrieved, the maturity rate, fertilisation rate, blastulation rate, as well as the implantation and clinical pregnancy rates at different time intervals.

Participants/materials, setting, methods: Patients were grouped into the following time-intervals:

(1) hCG trigger to oocyte retrieval (<35 and >35 h; early and late retrieval)
(2) oocyte retrieval to denudation (<2.30 and >2.30 h)
(3) denudation to ICSI (<10 and >10 min)
(4) oocyte retrieval to IVF/ICSI (<3 and >3 h)
(5) administration of hCG to IVF or ICSI (<38 and >38 h).

Continuous variables were compared by T-test; categorical variables were compared by X2 test.

Main results and the role of chance: We compared 295 patients (early retrieval group) and 784 (late retrieval group) showing similar baseline characteristics. In ICSI cycles, we observed a higher fertilization rate (69.7% vs. 75.6% p=0.002) and a higher number of good quality embryos on day 3 (55.5% vs. 63.9% p=0.0001) in the late retrieval group. Also in ICSI cycles, there was an increase in clinical pregnancy (44.7% vs. 28.6%, p=0.003), ongoing pregnancy (36.8% vs. 22.6%, p=0.006), implantation rates (31.4% vs. 19.7%, p=0.002) and live birth rate (41.1% vs. 27.3%, p=0.01) when time between oocyte collection and denudation was less than 2h 30min. Furthermore, these improved outcomes were also verified when the time interval between oocyte collection and ICSI was less than 3h and when oocytes were injected at least 10 minutes after denudation. Similarly, differences between groups were also observed in IVF cycles, which showed an increased fertilization rate (67.4% vs. 72.5%, p=0.004) and clinical pregnancy rate (17.65% vs. 28.52%, p=0.03) in the late retrieval group.

Limitations, reasons for caution: This is an analysis of time intervals in an unselected population, where most retrievals were done in a narrow window between 35 and 36 hours of hCG trigger. Subtle differences between patients or undetected premature luteinization could affect these results.

Wider implications of the findings: Even a slight alteration in time intervals from hCG trigger can have a substantial impact on treatment outcome because it could influence oocyte nuclear and cytoplasmic maturation and quality. Modifying these intervals in different laboratory procedures could optimize reproductive outcomes.

Trial registration number: Not applicable.

P-127 Artificial collapse improves the efficiency of a closed blastocyst vitrification system: retrospective analysis of 1459 warming FET cycles

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Study question: The effect of artificial collapse by laser on a closed blastocyst vitrification system.

Summary answer: Artificial collapse (AC) by laser on Day 5 or Day 6 embryos significantly increases the survival rate in closed blastocyst vitrification compared with non-collapsed.

What is known already: Blastocysts contain a large amount of fluid in the blastocoel, which poses a risk of ice crystal formation in in the course of cryopreservation. This risk may be higher for closed vitrification compared to open vitrification because the former theoretically suboptimal as it is bound to inhibit rapid cooling. Studies have shown that artificial shrinkage of the blastocyst prior to vitrification can have a positive effect on blastocyst survival after warming and can improve clinical outcomes. However, the evidence is scarce when it comes to assessing closed embryo vitrification systems.

Study design, size, duration: This retrospective study was conducted to review vitrified-warmed embryo transfers in the period between Feb 2012 and Oct 2018. A total of 1459 vitrified-warmed cycles were included in this study.

Participants/materials, setting, methods: Supernumerary expanded blastocysts on day 5 or 6 were vitrified using a closed system following AC or without AC. AC was achieved by creating a breach in the blastocoel with a laser pulse. After warming, viable blastocysts were transferred to patients in hormone replacement cycles. Blastocyst survival, clinical pregnancy (foetal cardiac activity at 8 weeks), implantation and ongoing/ live birth rates were compared between the AC and Non-AC patients.

Main results and the role of chance: A total of 1459 vitrified-warmed cycles were included in this study, of which 1268 embryos in 1001 cycles had AC performed prior to vitrification and 652 embryos in 458 cycles did not have AC. 4 cycles in AC group and 25 cycles in non-AC group were cancelled due to embryos not surviving. A higher number of embryos warmed per cycle was noted in the non-AC group (1.42 vs 1.27). The survival rate was significantly higher in the AC group (97.71%) than the non-AC group (87.88 %), p<0.001.

There were no differences in patient age (35.8 vs. 35.3) or number of embryos transferred per cycle (1.24 vs. 1.32) between the AC and Non-AC groups. The positive, clinical pregnancy, implantation, and ongoing/ live birth rates of the AC group were not significantly different to the non-AC group (52.36% vs. 55.43%, 36.58% vs. 37.88%, 32.28% vs. 32.64%, and 34.0% vs. 35.6%, P>0.05). On average, an implantation could be achieved from every 3.17 vitrified-warmed embryos in the AC group, as opposed to every 3.49 embryos in the non-AC group (p<0.05).

Limitations, reasons for caution: The confirmation of these findings requires further prospective studies to adequately judge the efficiency of AC as a routine intervention prior to vitrification.

Wider implications of the findings: Artificial collapse by laser pulse before blastocyst vitrification improves the efficiency of a closed vitrification system.

Trial registration number: not applicable

P-128 Piezo ICSI has clinical advantage to conventional ICSI by remarkably simple and stable technique that enables minimal damage on oocyte.

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Study question: Does Piezo ICSI (PI) has an effect on the fertilization rate, degeneration rate and embryo development compared to conventional ICSI (CI) in IVF cycles?

Summary answer: The degeneration rate of PI oocytes with fragile and normal oolemma was significantly lower than those of CI oocytes.

What is known already: Clinically, CI is a common method and widely used, while there are few reports with respect to PI. There are several animal studies reporting that PI is effective in degeneration rate and fertilization rate. It is known that the survival rates of mice oocytes are low after CI; however, degeneration rate improved markedly using PI. PI is an effective technique for cases with fragile oocytes. However, with PI, it is recognized that mercury is necessary in the microinjection pipette and there is concern about toxicity (Ricky Li et al., 2017).

Study design, size, duration: This is a retrospective single-center study conducted from April 2018 to November 2018. Of the 295 cycles, CI was performed in 142 cycles in134 patients, while PI was performed in 153 cycles in 132 patients. 16 cycles in 16 cases were sibling studies. Written informed consent was obtained from all the patients involved in this study.

Participants/materials, setting, methods: CI consists of mechanical penetration of the zona pellucida, breaking the oocyte membrane by aspiration of cytoplasm. CI was performed in 896 mature oocytes obtained from 142 cycles. PI consists of breaking the oocyte membrane and zona pellucida by Piezo pulse. PI was performed in 689 mature oocytes obtained from 153 cycles. In consideration of toxicity, fluorocarbon was used for all PI. P-value of 0.05 or less was considered to be statistically significant.

Main results and the role of chance: The average age of women at PI was significantly higher (39.5±4.7 years old) than that at CI (37.5±4.2 years old, P<0.001). There was no significant difference in terms of men's age between PI and CI (41.5±7.4 vs. 40.4±6.6 years old, P=0.235).

The degeneration rate after PI was significantly lower (2.8%) than that after CI (5.1%) (P=0.018). The degeneration rate of fragile oocytes (membrane breakage without using aspiration or pulsing) after PI was significantly lower (8.1%) than that after CI (20.5%) (P=0.011). On the other hand, there was no statistically significant difference in the fertilization rates, cleavage rates, blastocyst rates or good quality blastocyst rates between CI and PI (77.9% vs. 81.4% (P=0.085), 98.3% vs. 97.0% (P=0.165), 60.3% vs. 57.3% (P=0.301) and 43.1% vs. 41.3% (P=0.542), respectively).

Limitations, reasons for caution: The clinical results using vitrified oocytes or artificial oocyte activation were not included in this study. The pregnancy outcome has not been confirmed in our study.

Wider implications of the findings: Compared to CI, PI yielded more surviving oocytes. This study showed that PI could minimize damage to those oocytes with fragile oolemma. This study demonstrates that stable clinical results can be easily obtained with PI while reducing risk of toxicity by using fluorocarbon as a substitute for mercury.

Trial registration number: Not applicable.

P-129 impact of direct unequal cleavage on embryo developmental potential and clinical outcome

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Study question: Does the timing of direct unequal cleavage (DUC) influence embryo developmental potential and clinical outcome? What factor does influence the incidence of DUC?
Summary answer: Embryo developmental potential and pregnancy rate are related with timing of DUC. ICSI with testicular sperm significantly increased DUC compared with ICSI with ejaculated sperm.
What is known already: Time-lapse (TL) imaging has revealed cleavage anomalies in embryos, such as DUC. Recent studies suggested that DUC was closely related with lower embryo developmental potential compared with normal embryos. Although embryos with DUC show lower blastocyst formation rate and implantation rate compared with normal embryos, there is still controversy regarding the increase in aneuploidy in embryos with DUC. In addition, the influence of the timing of DUC on embryo development and the risk factor related with incidence of DUC are not well understood.
Study design, size, duration: This retrospective study was conducted at Kyono ART Clinic from August 2013 to April 2018. This study includes a total of 6691 two-pronuclear embryos after IVF/ICSI with ejaculate sperm or testicular sperm. All embryos were cultured in a TL system. DUC was defined as the abrupt cleavage of one blastomere into three daughter blastomeres at the same time.
Participants/materials, setting, methods: Depending on the timing of DUC, DUC embryos were classified as follows: DUC1, at the first cleavage; DUC2, at the second cleavage; DUC3, at the third cleavage. We examined the correlation between embryo development with each kind of DUC and maternal age, and between DUC incidence and fertilization method (ICSI or IVF). Furthermore, we compared the incidence rate of DUC in ICSI cycles between ejaculated spermatozoa and testicular sperm.
Main results and the role of chance: The incidence of DUC1, DUC2, and DUC3 was 2.2% (138/6300), 2.9% (185/6300), 0.6% (40/6300), respectively. Good quality embryo rates in DUC1 (3.6%, 5/137), DUC2 (3.3%, 6/183), and DUC3 (15.0%, 6/40) embryos were significantly lower than that of non-DUC embryos (36.3%, 2054/5659) ($p<0.05$). Blastocyst formation rate in DUC1 (11.5%, 15/130) embryos was significantly lower than those of DUC2 (49.1%, 85/173), DUC3 (65.7%, 23/35), and non-DUC embryos (55.5%, 2894/5212) ($p<0.05$). There was no significant difference in the incidence of DUC between each age groups, <34 (6.2%, 93/1508), 35-39 (7.0%, 148/2257), 40< (5.3%, 127/2540). Interestingly, there was no significant difference in the pregnancy rate of frozen-thawed blastocyst transfer between DUC1 (25.0%, 1/4), DUC2 (40.0%, 8/20), DUC3 (50.0%, 3/6), and non-DUC embryos (45.8%, 11/24). None of the infants from DUC1 embryos (n=1) and DUC2 embryos (n=3) showed congenital malformation. There was no significant difference in the incidence of DUC between ICSI and IVF: 3.5% (158/4539) vs. 2.9% (46/1603). However, DUCs and DUC1 incidence were significantly higher in testicular sperm compared with ejaculated sperm, [DUC: 11.8% (46/391) vs. 3.5% (158/4539), $p<0.05$; DUC1: 7.8% (29/374) vs. 2.4% (106/4487), $p<0.05$].
Limitations, reasons for caution: We did not analysis the correlation between the incidence of DUC and chromosomal abnormalities. Because the number of infants derived from DUC embryos was small, further investigation is necessary to confirm the safety of DUC embryos. As of now, DUC, especially DUC1 embryos, should not be selected for day3 transfers.
Wider implications of the findings: The incidence of DUC, particularly DUC1, had a negative effect on embryo development outcome. Our data suggests that blastocysts derived from DUC embryos might be suitable for selection for transfer when non-DUC embryos cannot develop to blastocyst.
Trial registration number: Not applicable.

P-130 the cohesin release factor Wapl interacts with Bub3 to govern SAC activity in female meiosis I

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Study question: What is the function of the cohesin release factor Wapl in female meiosis I?
Summary answer: The cohesin release factor Wapl stabilizes Bub3 to promote SAC(spindle assembly checkpoint) activation in female meiosis I
What is known already: Cohesin complex mediates sister chromatid cohesion to guarantee faithful chromosome segregation. During mitotic prophase, a bulk of cohesins is removed from chromosome arms by Wapl, a pivotal cohesin release factor, to ensure sister chromatid separation. However, during female meiosis I, homologs separate from each other and the resolution of chiasmata requires proteolytic cleavage along chromosome arms of cohesin's Rec8 subunit by Separase.
Study design, size, duration: Mouse oocytes were randomly assigned to control and Wapl-depleted groups. In Wapl-depleted groups, gene-targeting morpholino microinjection were performed to deplete Wapl, non-targeting morpholino oligo concurrently was injected as a control. The meiotic progression, spindle assembly,kinetochore-microtubule (K-MT) attachment and euploidy were assessed in control and Wapl-depleted oocytes.
Participants/materials, setting, methods: 3-4 week-old female ICR mice were used in all experiments. Fully-grown oocytes were collected from ovaries in M2 medium and cultured further in M16 medium under liquid paraffin oil at 37°C in an atmosphere of 5% CO2 incubator for *in vitro* maturation. At different time points after culture, oocytes were collected for subsequent analysis. Immunofluorescence, immunoblotting, LC-MS/MS and immunoprecipitation were performed to assess oocyte meiotic competence and explore molecular mechanisms.
Main results and the role of chance: Depletion of Wapl in oocytes accelerates meiotic progression and inactivates SAC($p<0.001$). Depletion of Wapl also causes meiotic defects such as aberrant spindle assembly($p<0.01$), improper chromosome alignment ($p<0.001$)and incorrect kinetochore-microtubule (K-MT) attachment($p<0.001$), consequently leading to aneuploid eggs($p<0.01$). The involvement of Wapl in SAC control is mediated by its role in maintenance of Bub3 stability. Expression of exogenous Bub3 could restore the meiotic defects in oocytes depleted of Wapl($p<0.01$).
Limitations, reasons for caution: We investigated the involvement of Wapl in SAC control on the oocyte meiotic maturation in vitro, but not in vivo.
Wider implications of the findings: Our findings not only discover a novel function and a new downstream effector of Wapl during meiosis, but also extend our understanding of the molecular basis underlying the occurrence of aneuploid eggs in human clinics.
Trial registration number: not applicable

P-131 Oocyte size and morphology are predictive of embryo quality.

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Study question: Do oocyte size and morphology serve as predictive factors for embryo quality?
Summary answer: Oocytes with larger total and oolemma diameter were more likely to develop into high-quality cleavage stage embryos.
What is known already: Though oocyte quality is crucial for embryo quality and has been demonstrated to affect embryo development, most in vitro fertilization (IVF) laboratories do not assess oocyte size and morphology. Oocyte cryopreservation and legally mandated limits on the number of oocytes to be fertilized demand more thorough oocyte quality assessment.
Study design, size, duration: This observational study prospectively performed morphokinetic evaluation of 351 mature (MII) oocytes from 80 women who consecutively underwent controlled ovarian hyperstimulation and intracytoplasmatic sperm injection (ICSI).

Participants/materials, setting, methods: Women undergoing ICSI at an academically-affiliated private IVF center were eligible for enrollment. Total oocyte diameter was measured as the maximum diameter of zona pellucida enclosed oocytes. Oolemmal diameter was measured as the maximum diameter of the oolemma.
Main results and the role of chance: Patients produced 19.0±17.3 oocytes. Oocyte measurements did not relate to oocyte yield. Oocytes with larger oolemma diameter fertilized more frequently than those with smaller oolemma diameter (110.4±4.3 vs. 109.1±4.9 µm, P=0.016), the relationship persisted after adjustment for age. Ooplasm granulation was inversely related to fertilization (P=0.009). Oocytes with larger total oocyte (164.4±6.6 µm) and oolemma diameter (110.8±4.4 µm) were more likely to become cleavage stage embryos of high-grade (P=0.011 and P=0.016, respectively). This effect was again independent of female age. Consequently, embryos from oocytes with larger total diameter were more likely to be transferred or cryopreserved (P=0.023).
Limitations, reasons for caution: Observational study.
Wider implications of the findings: Detailed oocyte assessments may improve embryo selection.
Trial registration number: Not applicable

P-132 Oocyte size and morphology are related to female age, ovarian reserve and body weight in non-polycystic ovary syndrome patients.

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Study question: Do age, body weight and ovarian function affect oocyte size and measurements in non-PCOS patients?
Summary answer: Advancing age and increasing BMI are associated with smaller total oocyte diameter. Ooplasmic granulation appears reflective of advancing age, lower AMH and fewer oocytes.
What is known already: Most in vitro fertilization programs select embryos suitable for transfer based on embryo morphology, though currently utilized embryo grading has limited potential to identify embryos with maximum implantation potential. Morphokinetic studies show embryo quality to primarily relate to oocyte, rather than to sperm parameters. Indeed, oocyte sizes were previously reported to be smaller in obese women with polycystic ovary syndrome.
Study design, size, duration: This observational study prospectively performed morphokinetic evaluation of 308 mature (MII) oocytes from 77 women who consecutively underwent controlled ovarian hyperstimulation and intracytoplasmatic sperm injection (ICSI) between April and October 2017.
Participants/materials, setting, methods: Women undergoing ICSI were eligible for enrollment in the absence of polycystic ovary syndrome. Total oocyte diameter was measured as the maximum diameter of zona pellucida enclosed oocytes. Oolemmal diameter was measured as the maximum diameter of the oolemma. Perivitelline space (PVS) and ooplasmic granulation were assessed.
Main results and the role of chance: Patients presented with a mean age of 40.3±5.0 years, had a BMI of 25.1±6.1 kg/m^2, AMH levels of 1.2±1.7 ng/ml and produced 6.9±8.9 oocytes. Mean total oocyte diameter was 163.2±7.4 µm (range 145.8-182.1 µm), while oolemmal diameter was 109.4±4.1 µm (range 98.5-122.3 µm). BMI was inversely related to total oocyte diameter (P=0.019), this effect persisted after controlling for female age and oocyte yield (P=0.047 and P=0.016, respectively). Total oocyte diameter was also inversely associated with AMH levels and oocyte yield. In contrast to total oocyte diameter, oolemmal diameter was not related to patient characteristics. Ooplasmic granulation was more frequently observed in oocytes from older women, those with lower AMH and lower BMI (P=0.001 for all). Conversely women with large oocyte yields demonstrated fewer oocytes with ooplasmic granulation (P=0.004). These relationships persisted after adjustment for age. Fertilization was more likely in oocytes with larger oolemmal diameter (p=0.008). Embryos from oocytes with larger total and ooplasmic diameters were more likely to be transferred or frozen (p=0.004 and p=0.011).
Limitations, reasons for caution: Results may not be applicable to younger women with excellent pregnancy potential.
Wider implications of the findings: These findings indicate the importance of detailed oocyte assessments, which may aid the currently used criteria for embryo selection.
Trial registration number: Not applicable

P-133 MicroRNA miR-294 in spent culture medium is correlated to embryo apoptosis and can serve as a biomarker for non-invasive embryo assessment.

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Study question: To study whether microRNA analysis of spent culture medium (SCM) from individually cultured embryos can be used as a non-invasive approach to assess embryo apoptosis.
Summary answer: MiR-294 is detectable in SCM of singly cultured blastocysts and its levels are strongly correlated to the extent of embryonic apoptosis.
What is known already: MicroRNAs have important regulatory actions on cell processes such as stress signaling, cell cycle progression, differentiation, and cell death. It is known that extensive apoptosis in pre-implantation embryos affects implantation and pregnancy rates. Mammalian embryos release microRNAs and cell-free microRNAs are linked to aneuploidy and implantation in humans. However, information is lacking about the potential use of SCM microRNA analysis for assessing the apoptotic status of pre-implantation embryos and the application of this method for elective single embryo transfer cycles.
Study design, size, duration: Cryopreserved mouse zygotes (N=70) were cultured individually for 80 hours until they reached the blastocyst stage. SCM were collected and further analysed for the presence of miR-294, a key anti-apoptotic mediator in cells. Blastocysts were stained at Day 5 of development to assess the apoptotic extent. MicroRNA levels were compared to the apoptotic percentage to identify possible correlations. The study was carried out in two consecutive repetitions.
Participants/materials, setting, methods: Frozen mouse zygotes (B6C3F-1 x B6D2F-1) were individually cultured at 37°C, 5% CO_2 to the Expanded Blastocyst (ExB) stage. Blastocysts were graded using a standard morphology grading system based on the quality of the trophectoderm and inner cell mass. Media samples (N=59) were collected (20µl) and analysed for miR-294 with the polymerase chain reaction method (PCR). The blastocysts were stained for apoptosis using the TdT-mediated dUTP-X nick end labelling method (TUNEL).
Main results and the role of chance: Blastocyst formation reached 84% (59/70 embryos). Using the scoring data the blastocysts were further categorised in Good, Fair, and Poor morphology groups. For the majority of the blastocysts morphology was Fair (N=37), whereas 6 blastocysts were Poor and 4 were of excellent quality and graded as Good. The apoptotic percentage ranged from 0 to 55% with an average of 15% (N=52). Mean comparisons between the Good, Fair, and Poor groups showed that apoptosis was comparable between the groups ($P>0.05$), meaning that morphological scoring is not valuable for assessing the extent of cellular death in pre-implantation embryos. MiR-294 was detected in 42 SCM samples and was strongly correlated to the apoptotic status of the blastocysts ($P<0.05$). The direction of this association is positive ($rho=0.394$), with higher levels of miR-294 linking to increased apoptotic percentage. According to our findings, it is speculated that embryos with abnormally high number of apoptotic cells express abundant levels of miR-294 in an effort to limit cell death and survive.
Limitations, reasons for caution: The use of cryopreserved embryos is a limitation of this study. These results are limited to mouse embryos and further validation is needed for applications in humans. Consideration should be given when interpreting the results for other species because of the differences in microRNA sequences.
Wider implications of the findings: Mouse embryos release microRNAs in the surrounding microenvironment, similarly to bovine and human embryos. DNA fragmentation in the embryo possibly leads to increased miR-294 in SCM that can be ultimately used as a non-invasive indicator of impaired embryo quality.

Trial registration number: not applicable

P-134 Protein profile of euploid single embryo transfer reveals differential patterns among them.

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Study question: Can we differentiate the implantation potential of euploid single blastocyst transfers using a proteomic profile?

Summary answer: A differential proteomic profile can be observed among all single blastocyst transfered depending on their implantation (yes/no), quality and culture media used.

What is known already: There have been initial attempts to select the best embryo for transfer according to proteomic patterns of developing embryos. Nevertheless, euploid single embryo transfers have never been assessed before. In this study a well-defined population of euploid and good quality blastocyst were singled transferred, and their culture media analyzed in order to determine the secretomic profile and its relation with implantation potential.

Study design, size, duration: 81 euploid single blastocyst transfer (SET) conditioned media (CM) and 8 controls were recruited for the study from September 2017 to March 2018 in our clinic. Morphokinetic and Morphology parameters were also recorded from all embryos using a Time-Lapse monitoring incubator (Embryoscope, Vitrolife).

Participants/materials, setting, methods: Eighty-one patients included in our PGT program were enrolled in this study from which 81 euploid blastocyst were analyzed. A Proximity Extension Assay (PEA) technology was used for analyzing 25 different secreted proteins in all media, including IL-6, IL-8, VEGFA, MCP-1, IL-1, CSF-1, SCF and others. Classical Morphology of blastocyst were evaluated including grade of expansion, inner cell mass (ICM) and trophoectoderm (TE). Additionally morphokinetcis and morphology dynamics were evaluated by using EmbryoVIewer (Vitrolife, Denmark).

Main results and the role of chance: First, we observed a clear protein pattern of consumption and secretion of the blastocyst when we compared with controls in all proteins analyzed. We confirmed a significantly high secretion of IL-6 and IL-8 of growing embryos, highlighting the potential of these molecules during the embryo development.Concerning the differences between implanted and non-implanted embryos only IL-8 seem to have a significant difference between groups. Furthermore, most of the protein concentrations presented a pattern of higher values in full hatched blastocyst and were directly related with ICM and inversely with TE quality.

Limitations, reasons for caution: Although we use the most sensitive system available in the market to measure proteins, the very low amount of some of them make us difficult to asses their implication in the implantation potential of these blastocyst.

Wider implications of the findings: Using a combined biochemical/morphology/morphokinetic approach we may be able to distinguish an embryo with higher implantation potential, compared to those that will have very low chances of implantation.

Trial registration number: Not applicable.

P-135 Distinct roles of cohesin acetyltransferases Esco1 and Esco2 during porcine oocyte maturation

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Study question: What are the roles of cohesin acetyltransferases Esco1 and Esco2 during porcine oocyte meiosis?

Summary answer: Here, we document that Esco1 and Esco2 exert distinct functions during oocyte meiosis beyond their canonical roles in chromosome cohesion during mitosis.

What is known already: In mammalian cells, the cohesin acetyltransferase Eco1 evolves into two orthologues Esco1 and Esco2 that acetylate cohesin subunit Smc3 to establish chromosome cohesion. We have previously demonstrated that Esco1 and Esco2 have unique functions during mouse oocyte meiotic maturation beyond their conventional role in cohesion establishment during mitosis.

Study design, size, duration: Porcine oocytes were randomly assigned to control group and knockdown group.Depletion of Esco1 or Esco2 by microinjection of RNAi-mediated gene silencing respectively. The *in vitro* cultured oocytes from each group after treatment were applied to the subsequent analysis.

Participants/materials, setting, methods: Acquisition of oocyte meiotic competence was assessed by the immunostaining, fluorescence intensity quantification and/or immunoblotting analysis to analyze the localization, protein expression at various stages, spindle assembly, chromosome alignment, acetylated α-tubulin levels, acetylated H4K16 levels, spindle assembly checkpoint activity. Interactions between proteins were examined by co-immunoprecipitation assay.

Main results and the role of chance: We find that Esco1 localizes to the spindle apparatus while Esco2 localizes to the chromosomes during porcine oocyte meiotic maturation. Depletion of Esco1 by RNAi-mediated gene silencing causes the meiotic progression arrest($P < 0.001$) and defective spindle/chromosome structure($P < 0.001$). Depletion of Esco2 by RNAi leads to the precocious polar body extrusion($P < 0.001$) and activation of spindle assembly checkpoint($P < 0.001$). Furthermore, we show that Esco1 binds to α-tubulin and is required for its acetylation to ensure the microtubule stability, and that Esco2 binds to histone H4 and acetylates it at lysine 16 to maintain the SAC activity.

Limitations, reasons for caution: We investigated the functions of Esco1 and Esco2 on the oocyte meiotic maturation *in vitro*, but not *in vivo*.

Wider implications of the findings: Our findings not only provide evidence that the meiotic functions to Esco1 and Esco2 beyond their roles in the cohesion establishment are conserved during oocyte meiotic maturation, but also extend to provide an indicator of oocyte quality.

Trial registration number: Not applicable.

P-136 From triggering to ICSI: Optimal timing between triggering, OPU, denudation and ICSI

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Study question: Which is the optimal time range between triggering, OPU, denudation and ICSI for optimal reproductive outcomes?

Summary answer: This study suggests that 38.5h between triggering and ICSI increase maturation rates and 2h between OPU and denudation increase fertilization rates without increasing pregnancy rates.

What is known already: In order to achieve successful fertilization, both nuclear and cytoplasmic maturity are required. Some studies support that pre-incubation time before ICSI can be beneficial when it comes to fertilization and pregnancy rates while late fertilization may have negative results due to oocyte ageing. There are also studies supporting that there is no statistically significant difference in fertilization and pregnancy rates when ICSI is performed between 2-5 hours post OPU. When it comes to triptorelin/hcg administration in IVF cycles, it is widely accepted that the best timing for OPU is 34-39 hours after triggering.

Study design, size, duration: This retrospective cohort study included ICSI treatments performed at Embryolab Fertility Clinic, in Thessaloniki, Greece between September 2017 and May 2018. The study group consisted of stimulated cycles with own oocytes fertilized with partner or donor sperm. The embryos transferred were either fresh or frozen, but were always derived from the same stimulation.

Participants/materials, setting, methods: 617 stimulated cycles with similar ovarian stimulation protocols were analyzed as per time range between triggering, OPU, denudation and ICSI. The exact time between the procedures was recorded manually. The results were categorized in classes containing the same number of cycles and were analyzed using Kruskal-Wallis test and scatter plots for maturation and fertilization rates and Mann-WhitneyU test and logistic regression for pregnancy/clinical pregnancy/live birth rates using IBM Statistical Package for the Social Sciences (SPSS).

Main results and the role of chance: The women included in the study were 37.23 ($\pm$4.867) years old with BMI 23.22 ($\pm$9.4). The number of oocytes collected at OPU was 9 ($\pm$7.15) and the mean number of MII was 6.82 ($\pm$5.65).

The mean fertilization rate was 72.24 (±28.13), pregnancy rate was 61.55% and clinical pregnancy rate was 56%.

The 6 categories studied were triggering-OPU (DT1), OPU-denudation (DT2), denudation-ICSI (DT3), triggering-denudation (DT4), triggering-ICSI (DT5) and OPU-ICSI (DT6) and were measured in minutes. Kruskal-Wallis test showed statistically significant differences in DT3 and DT5 categories regarding to maturation rate (31-45 minutes, 85.27% and 2371-2385 minutes, 83.12% respectively) and DT2 with fertilization rate (106-120 minutes, 74.57%). Mann-Whitney U test showed no statistically significant difference in any DT between positive and negative pregnancies or live births.

Ideal maturation rates were observed when OPU was performed 36.5h after triggering (p=0.089), denudation 2-2.25h after OPU (p=0.393) and 38.5-38.75h after triggering (p=0.836) and ICSI 30-45min after denudation (p=0.017), 2.5-2.75h after OPU (p=0.145) and 38.5-38.75h after triggering (p=0.008). Optimal fertilization rates were observed when OPU was performed 36.5h after triggering (p=0.464), denudation 1.5-2h after OPU (p=0.023) and 38h after triggering (p=0.138) and ICSI 2.7-3.5h after denudation (p=0366), 5-5.5h after OPU (p=0.592) and 39.5h after triggering (p=0.114).

Limitations, reasons for caution: Results presented in the abstract are based in existing cycles and time ranges. As a result, some classes were few in some categories due to similar time ranges between procedures. Additionally, as the cases were recent enough not all the results for pregnancy/clinical pregnancy/live birth are available.

Wider implications of the findings: Our results indicate that if all the procedures occur within a specific time range, the number of mature and fertilized oocytes can be higher. A positive outcome, can also be expected higher due to more available embryos for embryotransfer.

Trial registration number: N/A

P-137 Blastulation timing is significantly associated with clinical pregnancy outcomes in day 6 frozen blastocysts

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Study question: Is the blastulation timing of day 6 blastocysts associated with clinical outcomes in single frozen-thawed embryo transfer (sFET)?

Summary answer: The morphologically good-quality day 6 blastocysts which have not intiaited blastulation on the morning of day 5 have high clinical pregnancy rate in sFET.

What is known already: Many studies have indicated that day 6 blastocysts (D6BL) includes normal ploidy status with pregnancy potential. Based on morphokinetic data from time-lapse monitoring (TLM) in D6BL, our previous study showed that clinical pregnancy groups demonstrated significantly shorter time intervals between the start of blastulation and the expanded blastocyst stage (tEB-tSB) than non-clinical pregnancy groups. This result suggested that we may be able to predict for clinically pregnant D6BL from tEB-tSB analysis. However, morphokinetic analysis is time-consuming and high workload task in the busy IVF laboratory, therefore, in D6BL culture, simple and effective method is needed for clinically potential embryo selection.

Study design, size, duration: This retrospective observational study included 412 expanded blastocysts among 321 couples who underwent IVF/ICSI treatment and sFET cycles between January 2017 and October 2018. Cycles with advanced maternal age (>43 years), not expanded blastocysts (internal diameter of <170 μm) and blastocysts that underwent rescue-ICSI were excluded.

Participants/materials, setting, methods: Embryos were cultured in EmbryoScope+ (Vitrolife, Sweden), and conventional morphological parameters were observed at each fixed time point (on the morning of days 1-6) without morphokinetic analysis. D6BL divided into the two groups according to whether initiated blastulation on the morning of day 5 (D5BL) or not (Non-D5BL) and evaluated morphological grade by Gardner-Schoolcraft method. Then, clinical pregnancy rate (CPR) and pregnancy loss rate (PLR) were analyzed.

Main results and the role of chance: The mean patient age was similar between the two groups (D5BL: 37.4 ± 3.4 vs. Non-D5BL: 37.9 ± 3.3 years). Overall CPR was significantly lower in the D5BL group than Non-D5BL group [D5BL vs. Non-D5BL: 27.3% (95/348) vs. 42.2% (27/64), P=0.0362], whereas PLR was similar between both groups [D5BL vs. Non-D5BL: 24.7% (21/85) vs. 32.0% (8/25)]. In blastocysts with AA, BA or AB, CPR was significantly lower in the D5BL group than Non-D5BL [D5BL vs. Non-D5BL: 30.8% (77/250) vs. 48.8% (20/41), P=0.01], whereas PLR was similar between both groups [D5BL vs. Non-D5BL: 26.5% (18/68) vs. 21.1% (4/19)]. In blastocysts with BB, CPR was significantly lower in the D5BL group than Non-D5BL [D5BL vs. Non-D5BL: 20.3% (16/79) vs. 46.7% (7/15), P=0.049], whereas PLR was higher in the Non-D5BL groups but no significant difference [D5BL vs. Non-D5BL: 20.0% (3/15) vs. 66.7% (4/6)]. In blastocysts with AC, BC, CA or CB, Non-D5BL group had no clinical pregnant cycles [D5BL vs. Non-D5BL: 10.5% (2/19) vs. 0.0% (0/8)].

Limitations, reasons for caution: The main limitation is only the blastocysts that targeted on blastocysts of day 6 with small sample size and CPR and PLR were only analyzed in sFET cycle. Further studies are required to clarify whether the blastulation timing associates with live birth rates in D6BL.

Wider implications of the findings: This study revealed that the fixed time point observation is useful to predict high pregnancy potential in D6BL without using TLM. Combination of blastulation timing and morphological grading may offer a new direction in D6BL selection for pre-implantation genetic testing for aneuploidy or sFET.

Trial registration number: None.

P-138 Embryonic morphokinetics is potentially associated with clinical outcomes of single embryo transfers in preimplantation genetic testing for aneuploidy cycles

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Study question: Is morphokinetics of euploid blastocysts evaluated by a generally applicable algorithm associated with clinical outcomes of single embryo transfers (SETs) in patients undergoing preimplantation genetic testing for aneuploidy (PGT-A) cycles?

Summary answer: Morphokinetic grades evaluated by the KIDScore™ D5 algorithm are associated with the clinical outcomes in PGT-A cycles using high-resolution next-generation sequencing (hr-NGS).

What is known already: Using hr-NGS for PGT-A can enable the exclusion of mosaic embryos before embryo transfer and thus improve pregnancy outcomes as compared with PGT-A using array comparative genomic hybridization. To select euploid blastocysts with high implantation potential in advance, several groups have proposed the implementation of morphokinetic algorithms for implantation prediction. However, most of the available morphokinetic algorithms were developed based on clinic-specific settings, raising concerns about their general applicability. Therefore, appropriate external validation is essential before routine clinical use of a specific morphokinetic algorithm in varied clinical settings.

Study design, size, duration: The current study enrolled a total of 108 consecutive patients < 38 years and with more than four mature oocytes undergoing 119 PGT-A cycles in Lee Women's Hospital from January 2017 to August 2018. Morphokinetic data derived from 694 biopsied blastocysts cultured in a time-lapse incubator were retrospectively assessed. Clinical outcomes from 106 euploid blastocyst SETs were included in this study.

Participants/materials, setting, methods: Time-lapse microscopy was used to compare morphokinetic variables between expanded blastocysts derived from PGT-A cycles using hr-NGS. The clinical efficacy of the morphokinetic algorithm KIDScore™ D5 was evaluated after SETs of euploid blastocysts. Statistical analysis was conducted using the Mann–Whitney–Wilcoxon test for morphokinetic parameters and the Fisher's exact test for morphological changes, developmental paces, and clinical outcomes.

Main results and the role of chance: Compared with euploid blastocysts, low-level mosaic blastocysts presented comparable morphokinetic and morphological features. However, high-level mosaic blastocysts exhibited significant delays in t5 (median, 51.9 h post insemination (hpi), P = 0.034) and t8 (median, 58.6 hpi, P = 0.032) accompanied by prolonged CC3 (median, 14.7 h, P = 0.012). A significantly higher incidence of multinucleation indicated the

susceptibility of the high-level mosaic blastocysts to mitotic errors. By contrast, only a delay in tB (median, 106.0 hpi, P = 0.039) was revealed in aneuploid blastocysts, reflecting a reduced capability to form good-quality blastocysts (42.6 vs. 65.7%, P < 0.001). Euploid blastocysts with specific morphokinetic characteristics were graded by the KIDScore™ D5 algorithm. Notably, the grade C euploid embryos not only exhibited a consistent delay during preimplantation stages, but also achieved significantly lower rates of clinical pregnancy, implantation and ongoing pregnancy (25%, 25% and 10%) compared with the grade A (76.2%, 79.4% and 68.3%) or grade B (62.5%, 66.7% and 62.5%) euploid embryos.

Limitations, reasons for caution: Because of the retrospective nature of the study, its major limitation was the lack of randomization, which may have presented a risk of selection bias. In addition, this study included only patients aged < 38 years; hence, the results may not represent all the patients undergoing infertility treatment.

Wider implications of the findings: Although morphokinetic features appear dissimilar in embryos with different diploid–aneuploid mosaic levels, predicting chromosomal abnormalities using morphokinetics alone is insufficient. In combination with hr-NGS, the use of generally applicable morphokinetic algorithms may be practicable in independent clinics to support embryo selection for the purpose of improving clinical outcomes of SETs.

Trial registration number: The study is retrospective hence registration was not required. The study was approved by the local IRB committee.

P-139 Human giant oocytes; - cytoplasmic diameter and chromosomal abnormalities -

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Study question: Do Giant Oocytes with large cytoplasmic diameters have chromosomal abnormalities?

Summary answer: Giant oocytes with cytoplasmic diameters ≥ 130 μm have chromosomal abnormalities.

What is known already: The diameter of a human oocyte is approximately 110μm, but larger diameter oocytes have been documented in ART and referred to as Giant Oocytes (GO). GO appear to be a product of the cytoplasmic fusion of two primary oocytes, and there are examples of both ova in which some mitotic spindles may or may not be fused. Accordingly, they have a higher risk of aneuploidy than normal oocytes. However, the identification criteria of GO are not well defined as the classification is often dependent on the experience of the embryologist and it is not definitively known whether they are abnormal.

Study design, size, duration: We examined 49,612 oocytes collected from 6,193 ART cycles which underwent controlled ovarian stimulation. Cytoplasm diameters and centromere numbers of oocytes determined to be larger than normal were measured. 63 MII phase oocytes analyzed had a diameter of ≥130μm and were classified as GO. MII oocytes from conventional IVF cycles in which fertilization did not occur were used as a control group.

Participants/materials, setting, methods: Cytoplasmic diameters of oocytes defined as GO were measured and subjected to fluorescent immunostaining using α-Tubulin antibody and DAPI (4'-6-diamidino-2-phenylindole). Spindles were classified into three groups GO 1 (1 spindle present), GO 2 (2 spindles present) and control oocytes. The size of the spindle was measured. Furthermore, we counted the centromere number of GO using anti - centromere antibody recognizing the part where two homologous chromosomes join.

Main results and the role of chance: GO (n=63) had a mean cytoplasmic diameter of 144.3±10.7μm. After staining of 56 GO, it was possible to analyze 47. Of these, 25 were classified as GO1 and 22 oocytes were GO2. The average equatorial plane diameter of the spindle was 18.0±3.3μm in GO1, 11.7±2.2μm in GO2 and 12.3±1.3μm in control oocytes. The average diameter between the two spindle poles was 19.1±2.2μm in GO1, 14.5±3.2μm in GO2 and 13.2±2.0μm in control oocytes. GO1 were significantly different ($p<0.05$) in all these parameters compared to the other groups. There were 16 GO in which the number of centromeres were analyzed, among which 11 GO had one spindle and 5 GO with two observed. The mean number of centromeres in GO where one spindle was observed was 82.2 ± 9.9. GO, with two spindle bodies, 41.3 ± 3.1 on each spindle.

Limitations, reasons for caution: Whilst the incidence of GO is very low (0.1% of oocytes), a larger sample size would be desirable in order to further validate the association between GO and chromosomal abnormality.

Wider implications of the findings: Normally, the number of centromeres in the normal human oocyte is considered to be 46, so there seems to be some evidence from this study that GO are chromosomally abnormal and should be avoided for use in an ART cycle.

Trial registration number: None.

P-140 New insights into the nature of embryo's aneuploidy, arisen from preimplantation genetic testing for monogenic disorders (PGT-M)?

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Study question: What are the frequencies of early chromosomal errors and the parental origin according to haplotype analysis during PGT-M?

Summary answer: Results demonstrate 12.5 % trisomy versus 87.5% monosomy, and semi-equivalent parental origin of aneuploidy. This counters the dogma of major maternal contribution to total aberrations.

What is known already: It is accepted that the majority of aneuploidies in the early developing embryos are derived from errors occurring during meiosis .These anomalies, as deduced from analyses of miscarriage products, primarily involve trisomies of chromosomes 16,22,21,18,15 and XO, majorly attributed to maternal origin. Haplotype analyses at the earliest stages of embryo development, 3 days after fertilization, can be utilized to identify chromosomes' aneuploidity and to confirm or refute the assumptions concluded from prenatal testing.

Study design, size, duration: This is a retrospective study of PGT-M cycles performed between the years 2008-2017. Data were collected from the genetic analysis of embryos, based on haplotypes of at least 5 informative markers. This study includes 2084 embryos, derived from 210 PGT-M cycles of 113 patients. Patients were already diagnosed as carriers of severe monogenic mutations in various genes located on 9 different chromosomes.

Participants/materials, setting, methods: Prior to PGT-M, polymorphic markers were designed and haplotypes characterized. Diagnosis by mutation testing paralleled to haplotype analysis, was validated in single leucocytes from affected family members. Diagnosis of single blastomeres, biopsied from embryos on their day 3 of development, was performed using multiplex nested single cell PCR technique. While analyzing the embryos for the familial genetic disorder, the numerical chromosomal aberrations and parental attribution of monosomy and trisomy, were determined by informative haplotypes.

Main results and the role of chance: In our study we reviewed and analyzed haplotypes results of 2084 embryos from 210 cycles of 113 different patients. We inspected data of 40 genes located on the following chromosomes: 6, 16, 2, 7, 17, 19, 1, 20, X.

The analyzed embryos were chromosomally distributed as follows: on chromosome 6 203 embryos were tested for 5 genes (locations); 151 embryos for 4 loci on chromosome 16; 118 embryos for 6 loci on chromosome 2; 723 embryos for 5 loci on chromosome 7; 132 embryos for 3 loci on chromosome 17; 331 embryos for 5 loci on chromosome 19; 321 embryos for 8 loci on chromosome 1; 41 embryos for 1 locus on chromosome 20; and 84 embryos for 3 loci on chromosome X.

Out of the 2084 embryos analyzed in 210 PGT-M cycles, 152 (7.3%) embryos presented numerical aberrations in the tested chromosomes. Out of them, 133 (87.5%) displayed monosomy and 19 (12.5%) trisomy. Unexpectedly, 71 (47 %) of all aberrations were of paternal origin and 81 (53%) of maternal origin.

Limitations, reasons for caution: Chromosomes' numbers were determined by haplotypes localized at specific points. Misidentification of trisomy was due to limited ability to identity copies that originated from same allele, and failure to distinguish between trisomy and parental contamination. There is no

way to determine whether aberrancy represents whole embryo or biopsied cell only.

Wider implications of the findings: We add new insights concerning chromosomal aberrations in early developing embryos. We demonstrated higher frequency of monosomy compared to trisomy and a significant paternal contribution. This is in contrast of maternal dominancy described in studies of early miscarriage. The paternal role in IVF failure and chemical pregnancies should be re-evaluated.

Trial registration number: not applicable

P-141 Comparison of outcomes between Piezo-assisted versus conventional intracytoplasmic sperm injection for human oocyte: A prospective RCT study

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Study question: Does piezo-assisted intracytoplasmic sperm injection (ICSI) for human oocyte lead to a different outcome from conventional ICSI?

Summary answer: When compared to conventional ICSI, the blastocyst and good-quality blastocyst development rate was positively affected by Piezo-ICSI in patients with age older than 35.

What is known already: In conventional-ICSI, an injection needle penetrates the oolemma through the zona pellucida, which can cause harmful effect to the zona. Piezo-actuated micromanipulation makes the constant vibration to the tip, which makes the smooth puncture of the zona without need for suction of ooplasm, minimizing the damage of oocyte during procedure. Piezo-ICSI is now widespread among the animal laboratory especially mice, because it's plasma membrane has high extendibility and ooplasm has low viscosity. However there are few evidences among human oocyte and no prospective randomized controlled trail (RCT) is reported at this time.

Study design, size, duration: In this study, we performed prospective paired RCT with sibling oocyte retrieved more than two oocytes per women at our institution, between October 2017 and July 2018. Oocytes were randomly allocated to Piezo-ICSI and conventional ICSI for fertilization subsequently to oocyte retrieval, all ICSI procedures were performed by single expert technician.

Participants/materials, setting, methods: Retrieved oocytes were cultured after 3-5 hours of culture and pipetting was conducted to remove cumulus cells. For conventional ICSI, microinjector of NARISHIGE was used, to which gauge of outside diameter 6.0 um and inside diameter 4.7 um needle was attached. A injection tube was connected through a needle holder. For piezo-ICSI, microinjector of outside diameter 6.7 um and inside diameter 4.5 um was connected to the drive unit of piezo-micromanupulator.

Main results and the role of chance: We randomized 957 matured oocytes retrieved from 137 women to conventional-ICSI (472 oocytes) and piezo-ICSI (475 oocytes). The overall outcomes were similar among conventional-ICSI vs piezo-ICSI; fertilization rate 82.0% vs 86.5% p=NS, blastocyst development rate 50.2% vs 54.9% p=NS and good quality blastocyst development rate 51.2% vs 54.9% p=NS respectively. However, subgroup analysis among patient's age > 35 revealed that the oocytes underwent piezo-ICSI showed significantly better outcomes (conventional vs Piezo-ICSI: fertilization rate 79.7% vs 86.3% p<.05, blastocyst development rate 39.6% vs 52.4% p<.05, good-quality blastocyst development rate;50.7% vs 56.0% p<.05). There were no differences among the patients age 35 or younger between two groups.

Limitations, reasons for caution: This study only evaluated fertilization and blastocyst development and there is no data about the pregnancy rate or live birth rate.

Wider implications of the findings: Piezo-ICSI showed more favorable outcomes than conventional-ICSI in case with patient's age was older than 35 years, indicating that flat tip and vibration of the piezo can be more protective to oocyte and cause less damage to the aging oocyte.

Trial registration number: not applicable

P-142 Survivability after vitrification-warming of human embryos following use of different polymeric substances in warming media

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Study question: What is the optimal composition of vitrification-warming medium for human embryos?

Summary answer: Only blastocysts not enclosed by the zona pellucida should be warmed in medium containing serum substitute rather than Hydroxypropylcellulose(HPC).

What is known already: The advancement of vitrification technology including the Cryotop method has facilitated cryopreservation of human embryos. Media including serum substitute is thought to have some disadvantages, such as qualitative fluctuations and risk of contamination by unidentified viruses, so that recently there has been the introduction of artificial serum substitutes. As a candidate for a non-human supplement to vitrification-warming media, HPC maybe a potential replacement for serum substitute. However, information on whether serum substitute or HPC is suitable for vitrification-warming of human embryos is limited.

Study design, size, duration: This was a retrospective study in an experienced ART laboratory. The time period was 60 months (January 2013 to December 2017). Results from vitrified-warmed pronuclear embryos (n=40483), 13255 blastocysts with intact zona pellucida and 45 blastocysts without a zona pellucida were assessed. Statistical tests were applied to determine an effect at the level of $p<0.05$ using the chi-square test.

Participants/materials, setting, methods: Patients were seeking infertility treatment in an IVF clinic. All vitrification-warming cycles were performed using the Cryotop method (Kuwayama et al. 2005). Vitrifified human embryos were warmed in two types of warming media containing serum substitute or HPC. The survival rate of each medium was compared.

Main results and the role of chance: The survival rate of vitrified-warmed pronuclear embryos was 99.0% (27742/28031) with the use of serum substitute and 99.0%(12329/12452) for HPC. For blastocysts with an intact zona pellucida, the survival rate was 99.9% (7678/7684) for the use of serum substitute and 99.8% (5560/5571) for HPC. There was no significant difference in survival rates after vitrification-warming between serum substitute and HPC in these two stages of embryo development. However, in blastocysts without a zona pellucida, the survival rate in the warming medium containing the serum substitute was 100% (21/21), which was significantly higher than that using a warming medium containing HPC (62.5% (15/24); $p<0.01$).

Limitations, reasons for caution: This study only compared elements of the warming medium, the composition of the vitrification medium was not taken into account.

Wider implications of the findings: It's thought that the surfactant action of HPC does not tolerate fragile blastocysts which are free of the zona pellucida as it was observed on such occasions that the blastocyst came to the surface as soon as the Cryotop was immersed in the warming medium.

Trial registration number: None.

P-143 Failure of complete hatching of intracytoplasmic sperm injection-derived vitrified–thawed human blastocyst by cell herniation via small slit and insufficient expansion despite ongoing cell proliferation

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Study question: Does intracytoplasmic sperm injection (ICSI) affect the complete hatching of vitrified–thawed human blastocysts *in vitro*?

Summary answer: ICSI-derived blastocyst displayed cell proliferation, intermittently herniating trophectoderm cell and/or inner cell mass via small slit and insufficient expansion, leading to failure of complete hatching.

What is known already: The hatching pattern is associated with the fertilisation method, and the complete hatching rate of ICSI-derived blastocysts is lower than that of *in vitro* fertilisation (IVF)-derived blastocysts until day 6; however, whether incompletely hatched blastocysts can escape after day 6 remains unclear. Several studies have reported on the effect of zona pellucida thinning by using the assisted hatching (AH) method, but the results of studies on zona pellucida thinning in ICSI-derived blastocyst and pregnancy outcomes are ambiguous. Moreover, few reports have discussed the effects of ICSI and zona pellucida thinning procedures on the embryo hatching process.

Study design, size, duration: Donated cryopreserved blastocysts from patients who had a baby were used for all analyses. Differences in the hatching process between IVF- (n=56) and ICSI-derived (n=56) human blastocysts were observed, and the effect of laser-assisted zona pellucida thinning (IVF and ICSI, n=58 and n=54, respectively) on their hatching was evaluated under time-lapse microscopy (CO_2, 6%; O_2, 5% at 37°C and 80%±20% humidity).

Participants/materials, setting, methods: Vitrified–thawed blastocysts were individually cultured in a time-lapse incubator until degeneration. The spontaneous hatching pattern was categorised (type A: initial penetration of small trophectoderm projections and type B: sudden large rupture of the zona pellucida followed by fast extrusion of the blastocyst). The rates of hatching commencement and completed hatchings were determined. The blastocyst diameter and estimated number of trophectoderm cells were measured at hatching commencement and at maximum expansion.

Main results and the role of chance: No significant difference was found in the hatching commencement rate between the IVF and ICSI groups. However, hatching patterns differed between the IVF and ICSI groups (type A: 25.0% vs. 92.5% and type B: 75.0% vs. 7.5%, P<0.01). The completed hatching rate in the IVF group was significantly higher than that in the ICSI group (67.9% vs. 23.2%, P<0.01). Diameters at hatching commencement and maximum expansion during hatching in the IVF group were greater than that in the ICSI group (P<0.01). The maximum expansion diameters in completely hatched blastocysts were greater than those in incompletely hatched blastocysts of the IVF and ICSI groups (P<0.05). The completed hatching rate significantly increased with AH in both groups (IVF-AH vs. IVF, 89.7% vs. 67.9%, P<0.01 and ICSI-AH vs. ICSI, 77.8% vs. 23.2%, P<0.01). The maximum expansion diameters of the completely hatched blastocysts in the AH groups were significantly smaller than that in the non-AH groups (P<0.01). Moreover, the estimated trophectoderm cell numbers significantly increased in ICSI-derived blastocysts with type A hatching patterns from hatching commencement to their maximum expansion points (P<0.01). The trophectoderm cells and/or inner cell masses were intermittently herniated via small slits until degeneration in the incompletely hatched ICSI-derived blastocysts.

Limitations, reasons for caution: First, the zona pellucida of blastocyst–uterus interactions could not be evaluated during implantation. Second, the contribution of male factors to blastocyst development/hatching process remains unclear. Although our results do not completely explain the hatching process, they should be helpful in elucidating the process.

Wider implications of the findings: ICSI-derived blastocysts do not adequately increase their internal hydrostatic pressure despite ongoing cell proliferation because of presence of a small slit; consequently, blastocysts insufficiently expand, leading to failure of hatching. Laser-assisted zona pellucida thinning leads to complete hatching in the blastocyst stage of less expansion and lower internal hydrostatic pressures.

Trial registration number: Not applicable

P-144 Human oocytes are ready for ultra fast vitrification after two minutes of exposure to cryoprotectant solutions

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Study question: Can the duration of the exposure of human oocytes to cryoprotectant solutions, that is necessary to create the adequate intracellular environment for vitrification, be reduced?

Summary answer: The critical intracellular solute concentration necessary for successful vitrification of human oocytes can be achieved in just two minutes with standard cryoprotectant solutions

What is known already: Despite the cooling and warming of the oocytes is ultra-fast, the procedure of vitrification as a whole is time consuming. The standard preparation protocol to ready the oocytes for vitrification takes from 8 to 15 minutes, most of which consists on a long exposure to a non-vitrifying solution (n-VS) to allow for osmotic equilibration. A reduction in the duration of the protocol is desirable to improve the workflow in the IVF setting and reduce the time of exposure of the oocytes to suboptimal conditions: solutions with high molarity at a low temperature.

Study design, size, duration: We employed biologic material from patients of a private IVF unit, that was donated for research by a signed informed consent form. Human metaphase-II oocytes that did not show fertilization signs 48 hours after being subjected to ICSI were employed for in vivo osmotic behavior assessment and for viability tests. Human zygotes with 3 pronuclei at 17 hours after ICSI were employed for viability tests.

Participants/materials, setting, methods: We developed in MatLab a simulation of the flux of water and solutes through the plasma membrane of the oocyte during its exposure to hypertonic EG, Me2SO and sucrose solutions, using the 2Parameter formalism. The conditions of the simulation were reproduced in vivo using microvolumes of the solutions, and the volumetric excursions of the oocyte were recorded in an inverted microscope. Ultimately, oocytes were subjected to vitrification with a closed carrier after different preparation protocols.

Main results and the role of chance: We compared a standard equilibration protocol (EP) of 10 minutes of exposure to n-VS (7.5% EG, 7.5% Me2SO) followed by 1 minute of exposure to vitrification solution (VS; 15%EG, 15% Me2SO, 0.5M sucrose) with a short dehydration protocol (DP) consisting of 1 minute of exposure to each solution. The intracellular molarity of the oocyte at the end of both protocols was similar (5.40M in EP vs. 5.37M in DP), even though oocytes prepared for vitrification with DP showed a lower normalized water content (26.6% EP vs. 17.7% DP). The final relative volume of oocytes prepared for vitrification with EP (49.9% in silico, 62.5% in vivo) was higher than with DP (39.9% in silico, XXX% in vivo). Unfertilized oocytes survived vitrification in high rates after short dehydration protocols (30/30 DP). And 27/27 threepronuclear zygotes prepared for vitrification with DP survived the process and 24/27 resumed mitosis after 24 hours of culture. A similar proportion (25/27) of mitosis resumption was observed in a fresh control population of human threepronuclear zygotes. These results show that the critical intracellular solute concentration necessary for succesful vitrification of human oocytes and zygotes at currently attainable cooling and warming rates can be achieved in just two minutes.

Limitations, reasons for caution: Potentially deleterious effects of this short, dehydration based protocol should be studied in terms of oocyte ultrastructure, integrity of organelles, functional protein and RNA content, and ultimately and assessment of its effect on embryo development and clinical use in a controlled setting.

Wider implications of the findings: The duration of the phase of preparation of human oocytes to vitrification can be dramatically shortened even using a standard combination of penetrant and non penetrant cryoprotectants. The exposure to suboptimal, potentially deleterious conditions it entails could be minimized and the laboratory workflow improved.

Trial registration number: C.P. VITCOR - C.I. 1123-M1-17

P-145 D3 embryo quality predicts live birth of single vitrified non-top quality rather than top-quality blastocyst transfers

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Study question: Whether embryo quality on Day-3 (D3) has predictive values on clinical outcomes after single vitrified-warmed blastocyst transfer (SVBT)?

Summary answer: D3 embryo quality was an independent factor for predicting live birth of single vitrified non-top quality (NTQ) rather than top-quality (TQ) blastocyst transfers.
What is known already: Transferring TQ blastocysts originated from D3 NTQ embryos had lower implantation rate compared with those from D3 TQ embryos, while it had comparable clinical outcomes after transferring TQ blastocysts developed from either D3 TQ or NTQ embryos. Besides, there were still fewer studies about the predictive values of D3 embryo quality on clinical outcomes of neither single TQ nor NTQ blastocyst transfers.
Study design, size, duration: This study retrospectively analyzed the data of 1 121 SVBT cycles from 1 015 patients in single fertility center of Shenzhen zhongshan Urology hospital between April 2015 and December 2017.
Participants/materials, setting, methods: The cycles were divided into four groups according to blastocyst quality and D3 embryo quality: Group A1 (TQ blastocyst from D3 TQ embryos, n=577), Group A2 (TQ blastocyst from D3 NTQ embryos, n=193), Group B1 (NTQ blastocyst from D3 TQ embryos, n=152)and Group B2 (NTQ blastocyst from D3 NTQ embryos, n=199). The clinical outcomes were compared between A1 and A2, B1 and B2. Logistic regression analysis was performed.
Main results and the role of chance: The HCG positive rate (A1 vs. A2: 67.2% vs. 58.5%; B1 vs. B2: 57.9% vs. 37.2%, $P<0.05$) and clinical pregnancy rate (CPR) (A1 vs. A2: 66.3% vs. 54.3%; B1 vs. B2: 46.7% vs. 22.6%, $P<0.05$) of A1 and B1 group was significantly higher than that of A2 and B2 group, respectively. Ongoing pregnancy rate (OPR), early abortion rate (EAR) and live birth rate (LBR) were not statistically different between A1 and A2 group ($P>0.05$). B1 group had higher LBR than B2 group (30.9% vs. 14.6%), while no significant difference was found in OPR and EAR between the two groups. Multivariable analysis demonstrated that D3 embryo quality was not predictive for the CPR [crude OR=0.69 (95% IC 0.50-0.96), adjusted OR=0.79 (95% IC 0.55-1.14), $P>0.05$] and LBR [crude OR=0.75 (95% IC 0.53-1.05), adjusted OR=0.88 (95% IC 0.61-1.28), $P>0.05$] of single TQ blastocyst transfer, however, it was an independent factor for predicting CRP [crude OR=0.33 (95% IC 0.21-0.53), adjusted OR=0.43 (95% IC 0.26-0.70), $P<0.05$] and LBR of single NTQ blastocyst transfer [crude OR=0.38 (95% IC 0.23-0.64), adjusted OR=0.47 (95% IC 0.26-0.84), $P<0.05$].
Limitations, reasons for caution: Retrospective nature, small number sample and the selection bias caused by strategy of blastocyst culture applied in the fertility center were the main limitations.
Wider implications of the findings: D3 embryo quality should be taken into consideration when one NTQ blastocyst rather than TQ blastocyst was selected to transfer.
Trial registration number: Not applicable

P-146 Using the GnRH agonist leuprolide for luteal phase support (LPS) in, in vitro fertilisation (IVF) / intracytoplasmic sperm injection (ICSI) procedures - a randomised trial

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Study question: Does the addition of leuprolide (GnRH agonist) to standard LPS (vaginal progesterone) improve pregnancy outcomes in patients undergoing IVF/ICSI when studied in a randomised manner?
Summary answer: Addition of leuprolide to standard LPS improves ongoing pregnancy rate at 12 weeks in patients undergoing IVF/ICSI, when compared with patients receiving standard LPS alone.
What is known already: LPS is important for improving pregnancy outcomes. Multiple agents like progesterone, GnRH agonists and hCG have been studied for their roles in LPS. Evidence is divided as to which is the ideal agent of choice. Vaginal progesterone appears to be the most commonly used and the current standard of practice. There is conflicting data about the use of GnRH agonists for LPS. In 2015, a Cochrane meta-analysis (Linden et al) had shown a probable beneficial trend with GnRH agonists in terms of pregnancy outcomes, when used for LPS, albeit with evidence that had been classified as low quality.
Study design, size, duration: This is a single centre randomised open labelled trial with a view to recruit 106 patients each, in the intervention and control arms, initiated in the year 2018. The on-going study is being performed in the Department of Reproductive Medicine, Bangalore Baptist Hospital, which is a tertiary care teaching centre at Bangalore, India. The study was approved by the institutional review board and the ethics committee of the institution.
Participants/materials, setting, methods: Patients undergoing IVF/ICSI, 21-40 years of age, with antagonist protocol, hCG trigger and fresh embryo transfer (day 3) were randomised to standard LPS (vaginal progesterone - control) or standard LPS plus leuprolide (0.5 milligrams subcutaneously single dose, 6th day after oocyte retrieval - intervention). The primary outcome was live birth rate (LBR) and the secondary outcomes were implantation rate (IR), clinical pregnancy rate at 8 weeks (CPR), and ongoing pregnancy beyond 12 weeks (OPR).
Main results and the role of chance: This is an interim analysis of the first 50 patients enrolled in the trial (24 in the intervention and 26 in the control arm). Baseline characteristics were well balanced. Primary infertility was more common in both arms. There were no differences between the arms in the number of oocytes available after stimulation, the number of embryos available or the number of embryos transferred. The LBR was higher at 29.16% in the intervention arm compared to 7.69% in the control arm with a trend towards significance ($p=0.069$). The OPR was statistically higher in the intervention group as compared to the control group (33.33% versus 7.69%, $p=0.035$). CPR (37.5% in intervention, 23.7% in control) and IR (33.33% in intervention, 23.07% in control) were not statistically different between the two arms.

The initial results look promising in that the OPR appears to be better in the intervention arm, and there seems to be a trend towards benefit of the intervention with respect to the primary outcome (LBR). This may achieve statistical significance with higher population numbers on completion of the trial. The overall role of chance is small as the trial is randomised and the baseline characteristics are well balanced.
Limitations, reasons for caution: This is an exploration of the first 50 patients of this trial. The sample analysed is small with limited outcome events. Hence, though the results encourage continuation of the trial and reporting of the data, the final analysis at the end of the trial would be worth looking forward to.
Wider implications of the findings: These results will encourage other centres to test this agent in similar manners in their population to determine whether the effects are reproducible and generalisable. It would also be worth considering the use of other GnRH agonists for LPS and check for class effects of this group of drugs.
Trial registration number: The trial has been registered with the Central Trials Registry - India (CTRI), with the following number: CTRI/2018/07/014958.

P-147 Sibling oocyte study to determine effects of media refreshing on clinical outcomes with the use of a single step media

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Study question: Does using Sage Single Step (SS) media, without refreshing media on day 3, give the same Key Performance Indicators as refreshing?
Summary answer: Refreshing SS media shows a significant increase in top quality blastocyst formation and number of blastocysts vitrified, indicating a potential increased cumulative chance of pregnancy.
What is known already: With the increasing use of time-lapse embryo culture systems there has been renewed interest in single step culture media. There is debate around whether to refresh this media on day 3 of culture to optimise blastocyst formation and pregnancy rates.
Study design, size, duration: This prospective sibling oocyte study analysed 280 patients that had IVF/ICSI treatment between August 2017 and December 2018. A 2 staged study was used to examine Sage Single Step Media when refreshed (SSr) (day 3), Sage Single Step non refreshed (SSnr) and Sage Sequential media (SQ). The key performance indicators were analysed. The choice of which blastocyst to transfer would be based only on embryo quality and development not on the media in which cultured.
Participants/materials, setting, methods: Patients who had 4> 2PN's from sibling oocytes were included. Stage 1 study divided 2PN's between SSr and SQ. Stage 2 divided 2PN's between SSnr and SQ. Number of blastocysts, top quality blastocyst rate, number vitrified, positive HCG and clinical pregnancy

were the key indicators. To assess a cumulative rate of embryo utilisation any vitrified embryo transfers resulting from the study were analysed and included.
Main results and the role of chance: Blastocyst formation rate in SSr vs SQ was 67.7%, top quality blastocyst rate of 42.5% with 38.4% blastocysts being vitrified for SSr. The SQ group resulted in a 61.2% blastocyst formation rate, 43.1% top quality blastocysts and 39.3% blastocysts being vitrified.

Blastocyst formation rate in SSnr vs SQ group was 67.3% vs 60.6%, top quality blastocyst rate 38.6% vs 55.1% ($p < 0.05$) and 34.5% of blastocysts vitrified for SSnr vs 47.4% ($p < 0.05$) SQ.

SSr vs SQ group; SQ transfers resulted in 63% positive HCG and 52.9% clinical pregnancy rate per embryos transferred. SSr transfers resulted in 57.1% positive HCG and 45.9% clinical pregnancy rate per embryos transferred.

SSnr vs SQ group 50% positive HCG and 40.5% clinical pregnancy per embryos transferred for SQ transfer patients. Patients where SSnr embryos were selected a positive HCG rate of 57.9% and a clinical rate of 41.9% per embryos transferred.

Cumulative utilisation rates (embryos transferred and vitrified); averaged across both stages gave an embryo utilisation rate of 35.7% for SQ cultured embryos, 38% utilisation rate for SSr and 32.3% for SSnr.

Cumulative pregnancy rates per embryos transferred; SQ 42.2%, SSr 44.7% and SSnr 40.8%.
Limitations, reasons for caution: Results for SSnr vitrified embryo transfer date are based on lower numbers compared to SSr vitrified embryo transfers. The SQ media, acting as a control in the sibling oocyte groups, has provided varying results over the 2 groups.
Wider implications of the findings: Sage Single Step media provides comparative pregnancy results to Sequential media that can be of benefit in a time lapse system. By not refreshing we have seen a reduction in top quality blastocyst formation and embryo vitrification. Therefore cumulative pregnancy rates could be reduced from a single treatment cycle.
Trial registration number: not applicable

P-148 Oolemma rupture outside the intracytoplasmic sperm injection needle significantly improves the embryo quality.

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Study question: To compare the effect on fertilization, oocyte damage and embryo quality of two different techniques for rupturing the oolemma during ICSI.
Summary answer: Fertilization and degeneration rates were similar irrespective of the technique of oolemma rupture. However, top-quality embryo at days-2/3 and top-quality blastocyst rates were significantly different.
What is known already: Two procedures for rupturing oolemma during ICSI have already been described. The most commonly used in routine practice implies the application of a negative pressure. In this case, the breakage of the membrane is observed inside the injection needle. This leads to different patterns of breakage depending on the degree of suction. In the second technique, the rupture is obtained outside the needle by its stabbing action until the oolemma surrounding the needle recoiled. At present, no study had compared ICSI outcomes following these two techniques of oolemma rupture.
Study design, size, duration: This prospective, interventional, monocentric, auto-controlled study on sibling oocytes was performed from 11/2018 to 01/2019. Thirty-one patients, candidates for ICSI cycles were included when >2 metaphase-2 (M2) oocytes were available. Each M2 oocytes cohort was split into groups-1 and -2 according to the ICSI technique used. All ICSI included in the study were performed by the same senior embryologist.
Participants/materials, setting, methods: Oocytes in group-1 were injected by pushing the needle into the oocyte until the oolemma was observed to break outside the needle. In group-2 the oolemma was aspirated into the needle applying a negative pressure until it ruptured inside the needle. Fertilization (FR), degeneration (DR), top-quality embryo (TQE) at days-2/3 (D2/D3) and top-quality day-5 (D5) blastocyst (TQB) rates were compared, using paired-sample analysis with appropriate statistical test with a $p<0.05$ level of significance.
Main results and the role of chance: Briefly, 31 patients were included during the study period. Patients' mean age was 34.3 years, their mean serum AMH levels and antral follicle count were 2.9 and 19, respectively, and they underwent their 1.4th ICSI cycle. In total, 299 oocytes were retrieved, and 248 of them were M2 oocytes. Twenty-two fresh embryo transfers were performed (and 9 freeze-all due to risk of ovarian hyperstimulation syndrome), resulting in a clinical pregnancy rate of 50.0% (11/22).

When comparing groups-1 and -2 respectively, FR (76.4 *vs.* 78.1%; p=0.79) and DR (0.7 *vs.* 2.3%: p=0.29) were similar. Regarding embryo quality, TQE at D2 (defined as 4 blastomeres and <20% cytoplasmic fragmentation) and D3 (8 blastomeres and <20% fragmentation) were significantly improved in group-1 than those tabulated in group-2 (56.2 *vs.* 36.1% at D2; p=0.02, and 37.6 *vs.* 13.8% at D3; p=0.05, respectively). Among these cycles, 13 extended cultures were performed, resulting in comparable blastulation rates (69.8 *vs.* 57.4%; p=0.20). However, TQB rate at D5 (defined as blastocyst ≥B4BB according to Gardner and Schoolcraft's classification) was significantly greater in group-1 (49.4 *vs.* 22.8%; p=0.05). Finally, the rate of embryos suitable for transfer and/or cryopreservation was also higher in group-1 (69.2 *vs.* 48.4%; p=0.005).
Limitations, reasons for caution: The main weakness of this preliminary work is the low number of analysed cycles. A further prospective randomized study enrolling a larger number of patients is required to confirm these data. Moreover, the analysis of live birth rates among both groups would be relevant.
Wider implications of the findings: Taking those results into account, techniques used to rupture the oolemma during microinjection impact ICSI outcome with a deleterious effect of negative pressure applied onto the oocyte.
Trial registration number: No need.

P-149 Genes for Anti-Müllerian Hormone and Androgen Receptor are under-expressed in human cumulus cells surrounding morphologically highly graded oocytes

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Study question: Can expression profiles of AMH gene and its respective receptors in cumulus cells be biological markers for the oocyte, zygote or embryo quality?
Summary answer: Morphologically suboptimal oocytes had statistically significantly higher levels of AMH and AR gene expression in their associated cumulus cells.
What is known already: Morphological assessment of the oocyte, commonly used during ART, is not always a good predictor of successful fertilization and the developmental capacity of the ensuing zygote. Identification of specific genes or other biological components of the microenvironment surrounding each oocyte such as cumulus cells (CCs), could potentially enable more accurate distinguishing between high and low quality oocytes. Previous studies have investigated AMH gene expression levels in CCs, along with its correlation with the oocyte quality, but the obtained results were inconsistent and contradictory. A few studies have found connection between gene expression of AR, AMH and FSHR in CCs.
Study design, size, duration: Study design: cross sectional -morphologically highly (N=107) versus poorly (N=22) rated oocytes.

Size: 129 CCs and 35 follicular fluid samples from 58 patients undergoing ART.

Duration: one year.

Sampling procedure: Each follicle from the patients included was aspirated separately and the cumulus-oocyte complex (COC) was isolated. CCs were removed from the oocyte, oocytes were cultivated separately. Follicular fluids were included in the study if the COC was identified.
Participants/materials, setting, methods: Study included 129 CCs and 35 FF samples from 58 patients undergoing ICSI procedure. AMH, AMHR2, FSHR and AR gene expression levels were analysed on a real-time PCR device using TaqMan technology. Concentrations of AMH in FFs were measured by enzyme-linked immunosorbent assay. Morphological assessment of oocytes was conducted immediately before and during the ICSI procedure, Zygotes

and embryos were assessed 16-18 hours and 64-66 hours after fertilization, respectively.

Main results and the role of chance: The results yielded suggest a relationship between AMH, AR and oocyte morphology: AMH and AR gene expression levels in CCs surrounding morphologically optimal oocytes were significantly lower than in CCs surrounding oocytes with suboptimal morphology ($2^{-\Delta\Delta Ct}$(AMH) = 1.703; p = 0.011 and $2^{-\Delta\Delta Ct}$(AR) = 1.530; p = 0.008 respectively).

Statistically significant positive correlation was found between mRNA expression levels of AMH and FSHR ($p < 0.001$), AMH and AR (p = 0.001), AMHR2 and FSHR ($p < 0.001$), AMHR2 and AR ($p < 0.001$), as well as between FSHR and AR ($p < 0.001$).

No significant differences were found in AMHR2 or FSHR mRNA expression levels among different morphological groups of oocytes, zygotes and embryos.

The concentration of FF AMH did not differ significantly between different morphological groups of oocytes, zygotes and embryos (p = 0.082, p = 0.230 and p = 0.486, respectively). There are no correlations between FF AMH concentration and mRNA expression levels of the AMH, AMHR2, FSHR or AR genes in associated CCs (p = 0.195, p = 0.809, p = 0.461 and p = 0.240, respectively).

Limitations, reasons for caution: Due to heterogeneity of the study population regarding age and controlled ovarian stimulation protocol it is possible that some of gene expression differences could be the result of individual patient's characteristics and not of oocyte/zygote/embryo quality itself.

Wider implications of the findings: Research reinforces the importance of the COC and communication between an oocyte and its microenvironment for oocyte development, embryo formation and successful fertilisation. Obtained results suggest that the pursuit for the oocyte quality markers should be focused on its microenvironment.

Trial registration number: Not applicable

P-150 Implementation of a new single-step culture media: a randomized control trial (RCT) on sibling oocytes.

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Study question: Is the blastulation rate in Geri Medium comparable to well-established culture conditions?

Summary answer: Geri Medium (Gems, Genea-Biomedx) and Continuous Single Culture Medium (CSCM, Irvine) in an undisturbed incubator resulted in different timings of development but similar blastulation rates.

What is known already: It well-known that culture conditions play a key-role in IVF. Currently, the ideal framework is represented by embryo culture conducted in a time-lapse incubator and in single-step media to limit the exposure to sub-optimal environment and unnecessary handling-stress. Several studies to date compared single-step and sequential media, but few authors investigated the performance of different single-step media in the same ideal conditions. A new commercially-available medium has been recently introduced which contains anti-oxidant to limit the damage that might derive from reactive oxygen species. Aiming at a clinical implementation of such tool, we investigated its performance compared to well-established conditions.

Study design, size, duration: Interim analysis of a registered-RCT (April-December2018; 912 oocytes from 129 patient; mean maternal age: 36.7±3.6yr). Inclusion criteria: maternal age≤42yr, 4-8 MII oocytes, ICSI with ejaculated sperm, motile sperm-count>500,000/ml after swim-up, no carriers of single-gene diseases or chromosomal imbalances. Following denudation, the oocytes were randomized in two arms (Geri-Medium or CSCM). The primary outcome was mean blastulation rate per cohort. A sample size of 1182 oocytes (160-200 patients) is required to achieve a 80% statistical power.

Participants/materials, setting, methods: Controlled ovarian stimulation in an antagonist protocol, ICSI, culture to blastocyst in a time-lapse incubator (GERI, Genea Biomedx) at 6%CO_2 and 5%O_2, standard blastocyst morphological assessment and morphodynamic evaluation of preimplantation development (from time of second polar body extrusion to time of blastulation) were performed. In case of developmental arrest, the relative stage was annotated. T-test or Mann-Whitney U tests were performed to assess statistically-significant differences.

Main results and the role of chance: The mean number of oocytes per arm for each patient was 3.5±0.7 (2-4) in Geri-Medium versus 3.6±0.7 (2-4) in CSCM (p=0.5). The mean number of zygotes was 2.6±1.0 (0-4) versus 2.8±0.9 (0-4) (p=0.2). The mean fertilization rate per cohort per patient was 75%±26% versus 78%±22% (p=0.3). The mean number of blastocysts obtained was 1.5±1.1 (0-4) versus 1.4±1.0 (0-4) (p=0.6). The mean blastulation rate per inseminated oocyte per cohort per patient (i.e. primary outcome) was 42.4%±30% versus 40.6%±29% (p=0.56). No difference was reported also if the blastulation was calculated per zygote (54.9%±33.9% versus 52.2%±34.3%, p=0.48). The mean quality of the blastocysts obtained from each cohort was comparable in the two groups (rate of blastocysts ≥4BB according to Gardner and Schoolcraft's classification: 72%±40% versus 81%±34%, p=0.08). Of note, the embryos cultured in Geri-Medium were significantly 2-3 hr slower (p≤0.02) from the 5-cell stage (t5) up to full-blastulation (tB). The developmental arrest rates were similar in both media, but the embryo cultured in CSCM were more prone to arrest in the transition from starting-compaction (tSC) to morula (tM) formation (arrest at tSC n=20/339 zygotes, 5.9% in Geri-Medium versus n=36/357, 10.1% in CSCM, p=0.05; arrest at tM n=8/339, 2.4% versus n=22/357, 6.2%, p=0.01).

Limitations, reasons for caution: This is an interim analysis (77% of the sample size), therefore we did not reach yet the required statistical power. The use of ideal low-tension oxygen conditions might have hindered the benefit of the anti-oxidant compounds contained in Geri Medium .

Wider implications of the findings: Ideal culture conditions are required to define benchmark outcomes expected from the implementation of novel tools in IVF. If our data will be confirmed, Geri-Medium and CSCM might be considered equally-efficient in an undisturbed culture-system. When standard conditions are adopted, a difference cannot yet be excluded and should be investigated.

Trial registration number: ClinicalTrials.gov ID: NCT03497052

P-151 The supraphysiological levels of Oxidation Reduction Potential (ORP) present in the human embryo culture media affects blastocysts formation and ongoing pregnancies in IVF cycles

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Study question: Does the adjustment of the supraphysiological ORP levels in embryo culture system improves the blastocyst formation, pregnancy and ongoing pregnancy in in vitro fertilization cycles?

Summary answer: The adjustment of the supraphysiological ORP levels in embryo culture to physiological ORP levels found in follicular fluid of oocyte donors improves the clinical outcomes.

What is known already: The oxidative stress in embryos can be induced by means of atmospheric oxygen concentration, culture media, cell manipulation, cryopreservation etc. This results in cell membrane damage, DNA damage, delayed development and reduced their viability. The supraphysiological levels of ORP measured using MiOXSYS system in culture media affects the development of 3PN human embryos. The overall levels of ORP found in follicular fluid from oocyte donor is 250% lower than Hepes media and 175% lower than embryo culture media. Hence ORP is the best indicator of oxidative stress in embryo culture media compared to other parameters.

Study design, size, duration: This prospective cohort study was performed from May to December 2018. 104 patients less than 37 years old with at least one transferred blastocysts or without embryo transfer due to all embryos of the cohort were arrested in embryo development (no blastocysts formation) were selected for this study, 52 cases used adjusted ORP media, Group 1, and other 52 used non adjusted ORP media, Group 2. Each patient was asked to sign an informed consent.

Participants/materials, setting, methods: The ORP levels were determined in culture media (Global total, Life Global Group, USA), and in the Hepes

media. Further, the ORP levels were adjusted to 60 -80 mV using EmbryORPâ (combination of antioxidants). The addition of the EmbryORPâ to both media was done 3 hours before either oocyte collection or the observation of the oocyte fertilization. After fertilization, normal fertilized zygotes were transferred to the ORP adjusted culture media up to blastocyst stage.

Main results and the role of chance: The total blastocyst formation and ongoing pregnancy per each cycle with at least one oocyte with normal fertilization were significantly higher in the group 1 vs group 2. [(63% vs 42%) P (<0.005) and (67% vs 39%) P (0.021)], respectively. The pregnancy rate was comparable between two groups (75% vs. 54%) P (0.0863). In addition no differences were found between groups in terms of mean patient's age (31.96 ±5.7 vs. 33.17±5.76) (p=0.4285), blastocysts formation at day 5 (79% vs. 83%), and at day 6 (21% vs 16%) respectively. The significant difference in the ongoing pregnancy between the two groups suggest that the adjustment of ORP levels in embryo culture media helps in reduced miscarriages in these patients.

Limitations, reasons for caution: Small cohort of patients is a limitation of the study. More studies are necessary to corroborate our findings.

Wider implications of the findings: The result from the current study indicate that the EmbyORP® can be used to scavenge the supraphysiological levels of ORP found in the embryo culture media. Adjustment of ORP levels in human embryo culture media increased the blastocyst formation and ongoing pregnancy rate in IVF cycles.

Trial registration number: Not Applicable

P-152 Clinical outcome of vitrified-warmed blastocyst transfer performed on day 5 and day 6 after LH surge detected by urine tests

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Study question: Is clinical outcome of vitrified-warmed blastocyst transfers comparable if they are performed on day 5 or day 6 after LH surge detected by urine tests?

Summary answer: There is no difference in the clinical outcome between vitrified-warmed blastocyst transfers performed on day 5 and days 6 after positive LH urine tests.

What is known already: In true natural cycle (tNC) the timing for vitrified-warmed blastocyst transfers (FET) is determined by investigating the spontaneous LH surge. It is estimated that windows of implantation are opened 7 days after LH surge and last 2–4 days. To achieve synchronization between embryonic and endometrial development, blastocyst transfer on day 6 after LH surge was proposed. Urine LH increases 21 hours after the detection of the blood LH surge and synchronization could also be achieved if FET is performed on day 5 after positive LH urine tests. However, there are currently no studies in the literature covering this topic.

Study design, size, duration: All vitrified-warmed blastocyst transfers performed in tNC between 2013 and 2017, at the Department of Reproductive Medicine, University Medical Centre Maribor, were included in this retrospective study. In women with the regular menstrual cycle (24-35 days), after observing the selection of the leading follicle and thickening of the endometrium on ultrasound, urine LH tests were used twice daily to monitor the LH surge onset. FET was performed on day 5 or 6 after LH surge.

Participants/materials, setting, methods: On day 5, FET was scheduled according to doctor-patient agreement to avoid FET on some days during weekends. [UA1] Progesterone supplementation (400 mg of micronized vaginal progesterone per day) was started after FET. Patients' characteristics and clinical data were collected from our software database. Reproductive outcomes, cycles and patients characteristics between FET on day 5 and on day 6 after LH surge were compared using the Pearson's Chi-squared test, Student's t-test, and Mann–Whitney U-test.

Main results and the role of chance: Among 2041 vitrified-warmed blastocyst transfers, 1631 (79.91%) were performed on day 6 and 410 (20.09%) on day 5 after positive LH urine tests. There were no statistically significant differences between these two groups in the clinical pregnancy rate (37.80 vs. 39.05%), implantation rate (35.08 vs. 33.86%), miscarriage rate (15.13 vs. 19.62%) and delivery rate (29.95 vs. 30.35%). There were also no differences in women's age (33.75 ± 4.18 vs. 34.13 ± 4.13 years), number of previous IVF attempts (1.69 ± 1.30 vs. 1.85 ± 1.99), cause of infertility, proportion of cycles with births after fresh embryo-transfer (19.51 vs. 19.80), proportion of cycles with FET after freeze-all cycle (12.19 vs. 12.38%), average menstrual cycle length (28.23±2.85 vs. 28.17±1.76 days), menstrual cycle variability (3.61±3.55 vs. 3.44±3.08 days), day of LH surge (13.61±2.31 vs. 13.36±2.22), endometrium thickness on the day of FET (10.02±2.11 vs. 9.96±227 mm), endometrial morphology, number of blastocysts transferred (1.21±0.41 vs. 1.25±0.50), proportion of difficult embryo-transfers (7.36 vs. 6.38%), proportion of transferred blastocysts vitrified on day 5 (69.51 vs. 66.09%) and proportion of transfer of morphologically optimal blastocysts (23.17 vs. 25.44%) between cycles with FET on day 5 and FET on day 6 after LH surge detected by urine tests.

Limitations, reasons for caution: This is a single-centre retrospective analysis and despite an acceptable number of included cycles and robust methodological approach, the presence of potential bias cannot be excluded. There is a need for a properly designed prospective randomized study to confirm our findings.

Wider implications of the findings: The main disadvantage of tNC in that there is no flexibility in timing for FET. However, the results of our study suggest that vitrified-warmed blastocyst transfer could be scheduled on day 5 or day 6 after positive LH urine tests without having an impact on the clinical outcome.

Trial registration number: Not applicable.

P-153 Ranking of vitrified blastocysts for frozen transfer based on kinetics and absence of dysmorphisms increases likelihood of implantation- a time lapse (TL) study

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Study question: Determine if time lapse imaging provides morphokinetic data that can be used to rank vitrified blastocysts according to implantation potential.

Summary answer: Embryos with delayed blastulation should be ranked last for FET, regardless of morphology. Further selection by absence of dysmorphisms can increment viable pregnancies.

What is known already: Ranking and selection of vitrified blastocysts according to implantation potential is critical to shortening time to pregnancy , cost to patients and still transferring only a single blastocyst in the frozen cycle. Morphologic grading alone does not allow sufficient discrimination between blastocysts of similar grades. TL offers a unique set of morphokinetic c markers to potentially aid the selection process. Little is known about the implantation potential of blastocysts arising from embryos with multinucleation or anomalies such as direct uneven cleavage (DUC), reverse cleavage (RC) and irregular chaotic division (ICD) as so often such embryos are deselected in fresh transfers.

Study design, size, duration: Retrospective examination of KID data (Known implantation data) from 427 consecutive frozen embryo transfers (FET) with vitrified warmed blastocysts (n=558). The blastocysts were derived from women undergoing IVF/ICSI (without PGS) between 2014 and 2017. All embryos had been cultured to blastocyst in the EmbryoScope time lapse (TL) incubator. Kinetics of cell division and morphologic features were annotated daily. Good quality blastocysts were vitrified on day 5/6. FET cycles were performed after endometrial priming.

Participants/materials, setting, methods: The primary outcome parameter was embryo implantation (sac). Secondary outcomes monitored were fetal heart, viable pregnancy and live birth rate. Kinetic variables analyzed were vitrification day, time (t) to 2,3,4,5 cells, morula,blastulation, expansion and intervals between cell stages. Implantation potential was compared between embryos with different dysmorphisms and kinetic behaviors. Statistical analysis performed using Pearson's chi-square test and logistic regressions to calculate odds ratio (OR) and 95% confidence interval (95%CI) after controlling for patient age.

Main results and the role of chance: The clinical pregnancy rate was 56.4% with a mean of 1.32 embryos being transferred. Implantation potential of day 5 versus day 6 vitrified blastocysts (BL) was far higher (60.6%, 28.4%, respectively p<0.001). A significantly higher percentage of D6 BL (56%) vs D5 BL (44%) were derived from embryos displaying specific cleavage stage dysmorphisms : MU 47% vs 23%; DUC 8% vs 2%; or a combination of dysmorphism 13% vs 5%. The second cleavage interval cc2 (t3-t2) was observed to be <=5 hrs in 12% D6BL as compared to 6% of D5 blastocysts. This characteristic is often linked to poor developmental potential. After correcting for age, the odds ratio of a day 5 blastocyst with no dysmorphisms implanting with a FH was 1.54 (95% CI 1.042-2.280; p=0.03). OR for D5 MU BL was 0.674 (95% CI 0.432-1.051; p=0.08) and for D5 ICD BL 0.589 (95%CI 0.336-1.031;p=0.06). After adjusting for the presence of two or more dysmorphisms and age, we found that tSB significantly affected implantation potential. Embryos with longer tSB had a decreased probability of resulting in an implantation with a fetal heart. The probability decreases 4% per hour as the tSB prolongs (OR, 0.960; CI 0.940-0.980; P<.001).

Limitations, reasons for caution: The retrospective design of the study did not allow for control of patient-specific variables. Although regression analysis took age into consideration, the chromosomal status of the embryo(s) could not be controlled for. Validating the impact of specific dysmorphisms on implantation rate requires a larger data set with age matched controls.

Wider implications of the findings: In the optimized continuous culture environment of the EmbryoScope time lapse chamber, blastocysts with the highest implantation potential are formed and vitrified within 122 hours of ICSI. Early cell cycle kinetics and observed dysmorphisms provide additional criteria to discriminate among blastocysts and enhance pregnancy outcomes in frozen transfer cycles.

Trial registration number: Not applicable

P-154 Cytological analysis of sperm penetration and arrest of fertilization in mature oocytes that did not form pronuclei following conventional IVF

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Study question: Why does fertilization sometimes fail in conventional IVF (c-IVF)?

Summary answer: After c-IVF, most unfertilized oocytes showed no signs of sperm penetration, but some arrested during the fertilization process even after sperm penetration.

What is known already: Unfertilized oocytes after c-IVF may fail to form pronuclei due to lack of sperm penetration. For patients who do not obtain fertilized oocytes by c-IVF, intracytoplasmic sperm injection (ICSI) is the only hope for pregnancy. However, ICSI may not result in fertilization, even though sperm are definitely injected, with research showing: absence of calcium oscillation causes failure of second polar body extrusion; lack of decondensation of sperm nucleus causes unsuccessful pronuclei formation; and sperm centrosomal dysfunction causes developmental arrest at the pronuclear stage. Thus, ICSI has been investigated extensively, but few reports on unfertilized oocytes after c-IVF have been published.

Study design, size, duration: Unfertilized mature oocytes (n=50) without pronuclear formation within 44 hours after insemination by c-IVF were donated by 13 infertile couples. We conducted a cytological analysis of the donated oocytes, using immunofluorescent staining, utilizing anti-pericentrin antibody as a marker of centrosomes to examine sperm penetration. This was based on our previous study on abnormally fertilized oocytes, which revealed that the number of centrosomes matches the number of sperm that penetrate the ooplasm.

Participants/materials, setting, methods: Microtubules and centrosomes of the oocytes were identified using a mouse polyclonal anti-alpha tubulin antibody and a rabbit polyclonal anti-pericentrin antibody, respectively (1:100 dilution for both, Abcam). These primary antibodies were detected by Alexa Fluor 488 goat anti-mouse IgG antibody and Alexa Fluor 568 goat anti-rabbit IgG antibody, respectively (1:250 dilution for both, Life Technologies). DNA in the ooplasm was stained with Hoechst 33342 (Thermo Fisher). Images were obtained using an FV1000 confocal microscope (Olympus).

Main results and the role of chance: Out of the 50 unfertilized mature oocytes, 44 showed a double-positive signal for DNA and alpha tubulin in the ooplasm, which was thought to indicate the metaphase spindle of the second meiotic division (MII spindle). In addition, there was no pericentrin signal in these 44 oocytes, suggesting that sperm penetration did not occur. On the other hand, two oocytes showed pericentrin signals, suggesting that sperm had successfully penetrated these oocytes. One of these two oocytes showed a triple-positive signal for DNA, alpha tubulin and pericentrin, which was thought to be derived from a sperm and the MII spindle, indicating that the fertilization process of this oocyte had arrested during formation of the sperm aster. Interestingly, the other oocyte showed two pericentrin signals at the poles of the assembled spindle. The male and female chromatin in this oocyte may have fused without forming pronuclei, with development thus arrested before the first cleavage. The remaining four oocytes showed two spindles without pericentrin signals in the ooplasm, although the reason for this was unclear.

Limitations, reasons for caution: It is possible that the pericentrin signal was undetectable in some oocytes. In addition, since this was a preliminary study, more detailed investigations are needed to clarify the reasons for fertilization failure in c-IVF oocytes.

Wider implications of the findings: We found that most unfertilized c-IVF oocytes lacked signs of sperm penetration. However, fertilization had begun, but arrested, after sperm penetration in some oocytes. We identified several cytological patterns in the unfertilized oocytes, therefore more detailed analyses using larger numbers of c-IVF oocytes might help determine why fertilization sometimes fails.

Trial registration number: None.

P-155 Effect in sequential medium with or without renewal on day-2 and day-4 during culture for blastocyst transfer

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Study question: Are there any differences on clinical outcomes following fresh medium renewal on day-2 and day-4 for blastocyst transfer?

Summary answer: Pregnancy rate and implantation rate were statistically significantly higher than those of the non-renewal group, while differences of development were not present.

What is known already: Elective single blastocyst transfer (eSBT) begins to be widely used in assisted reproductive technology and eSBT is also required to avoid multiple gestation pregnancies. For eSBT to be widely used, culture systems should be more improved for recapitulate the in vivo environment. But, embryos are exposed to the risk of accumulating embryo-toxic volatile organic compounds and ammonium during in vitro culture treatments. Change of media would advocate to deplete embryo-toxic substances and to reduce naturally occurring inhibitors. However, some studies have not been detected differences in cultures, whether the culture medium renewed or not.

Study design, size, duration: A retrospective study was performed from January 2018 to December 2018. Total of 86 blastocyst transfer cycles, including 29 cycles with renewal medium and 57 cycles without renewal medium, were carried out. The cycles with genetic diagnosis, poor responder, and advanced maternal age (≥38 years) were excluded.

Participants/materials, setting, methods: The couples were randomly divided into two groups: medium renewed at day-2, day-3, and day-4, respectively and medium not renewed on day-2 and day-4. Three-hundred fifty-nine embryos for renewal medium were transferred into freshly prepared droplets covered with oil. Except for the day-3, 759 embryos were cultured intact without renewal according to our standard protocol with sequential media. All transfers were performed on day-5. Formed blastocysts from surplus embryos were cryopreserved on day-5 and day-6.

Main results and the role of chance: The proportion of good-quality cleavages obtained with the renewal medium protocol (54.0%) was statistically similar to those in the without renewal group (51.0%). Significant differences were not observed in the blastocyst formation (transferred plus frozen; 43.5 vs. 45.6%) and good-quality blastocyst (≥3BB, transferred plus frozen; 67.9 vs. 68.8%) rates. Blastocyst transfers of good quality (≥3BB) in the renewal group were 85.4%, and 86.7% in the without renewal media group. The rates of positive β-hCG tests (69.0 vs. 42.1%, p=0.018), clinical pregnancy (62.1 vs.

38.6%, p=0.039) and implantation (51.2 vs. 31.3%, p=0.025) were statistically significantly higher in the renewal group than the without renewal group.
Limitations, reasons for caution: Further studies will be required to accumulate sufficient data and prospective study with sibling oocytes. And it is the results of single and double blastocyst transfer. Therefore, further studies should be done by only eSBT to determine the accurately analysis on pregnancy.
Wider implications of the findings: Medium renewal on day-2 and day-4 has higher pregnancy outcomes in blastocyst transfer. Our observation suggests that advanced extended culture system could facilitate more successful eSBT and would contribute to reducing the risk of multiple births.
Trial registration number: Not applicable.

P-156 A comprehensive description of the clinical contribution of poor-quality blastocysts to live birth: analysis of 2757 oocyte retrievals with preimplantation genetic testing for aneuploidies.

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Study question: Which are the clinical benefits and risks of including poor-quality blastocysts (PQBs) in the cohort of biopsied embryos during an IVF cycle with preimplantation-genetic-testing-for-aneuploidies (PGT-A)?
Summary answer: Including PQBs involves higher implantation-failure and miscarriage rates after euploid transfer, but also an overall ~2.5%-increase in the number of live births (LBs) after PGT-A.
What is known already: PQBs (<BB according to Gardner and Schoolcraft's classification) are generally disregarded for clinical use and/or research purposes. Therefore limited data exist in literature to estimate the benefits and risks deriving from the transfer of a PQB. In Italy, the Law imposes the transfer or the vitrification of all embryos, unless proven not viable. This regulation involved a consistent amount of data produced regarding poor-quality embryos. Previous reports outlined a lower chance of euploidy and implantation for PQBs. Yet, a comprehensive picture of their real clinical contribution is missing.
Study design, size, duration: Observational cohort study including 2757 oocyte retrievals for PGT-A (mean maternal age: 39.6±3.3yr) conducted at a private IVF center between April-2013 and May-2018. 1497 PQBs were obtained and their embryological, chromosomal and clinical features were compared to 5250 non-PQBs (≥BB according to Gardner and Schoolcraft's classification). Once defined the overall increase in LBs due to PQBs, we outlined the population of patients that might benefit the most from their clinical use.
Participants/materials, setting, methods: IVF cycles after controlled-ovarian-stimulation (antagonist protocol), blastocyst culture, trophectoderm biopsy, vitrification, comprehensive chromosome testing by qPCR (until October-2017) or NGS (afterward) and warmed euploid single embryo transfers (SETs) were conducted. Overall analyses and sub-analyses in populations of patients clustered according to maternal age at retrieval and size of the cohort of sibling non-PQBs were performed. Lastly, the risk of miscarriage and the chance of LB per biopsied PQB and non-PQB were estimated.
Main results and the role of chance: PQBs involved a 12.4%-increase in the cycles where ≥1 blastocyst was biopsied (n=2217 versus n=1973 if accounting only non-PQBs). To date, we reported a concurrent 2.5%-increase in the cycles resulting in ≥1 LB (n=724 versus n=708) and a 13.8%-increase in the cycles where no LB was achieved but cryopreserved euploid blastocysts are left to transfer (n=206 versus n=181).

On average 0.7±0.9(range:0-9) PQBs were obtained per cycle to biopsy, and 0.2±0.4(range:0-5) euploid PQBs. No patient/cycle feature associated with higher/lower chance of producing them. However, the 18 women achieving their only LB thanks to PQBs clustered among patients with a limited cohort of sibling non-PQBs produced (1.1±1.1,range:0-3).

The 1497 PQBs compared to the 5250 non-PQBs showed slower developmental (day5:10.1% versus 43.9%, day6:60.5% versus 50.8%, day7:29.4% versus 5.2%;p<0.01) and lower euploidy rates (23.5% versus 51%;p<0.01). Among the 195 and 1697 transferred euploid PQBs and non-PQBs, the former involved a lower implantation (16.9% versus 52.3%;p<0.01), and higher miscarriage (36.4% versus 13.9%;p<0.01), therefore resulting in a lower LB-rate (LBR:10.8% versus 44.6%;p<0.01). Based on these rates, we estimated an overall 1.4% risk of miscarriage and 2.5% chance of LB after euploid vitrified-warmed SET per each biopsied PQB. The same estimates for non-PQBs were 3.7% and 22.8%.
Limitations, reasons for caution: The clinical benefit of PQBs is under-estimated since they are the last option for transfer and this analysis entailed only the first LB. Specifically, 42 women never pregnant and 90 that already delivered still have euploid PQBs left. Lastly, cost-benefit analysis is required in a prospective non-selection fashion.
Wider implications of the findings: PQBs show higher aneuploidy rates. If included, PGT-A is recommended. When selecting against aneuploid-PQBs, euploid ones could still involve higher implantation-failure and miscarriage rates; yet, their LBR is not negligible. Women should be acknowledged that implantation potential is not defined by a poor-morphology, but PQBs might entail more clinical risks.
Trial registration number: None.

P-157 The ART score: a score time independent able to predict frozen embryo transfer (FET) outcome.

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Study question: The ART score (Labrune et al, 2018) could be used as predictive score of FET outcome after a failed in vitro fertilization procedure.
Summary answer: The ART score issued from fresh ART procedure, from which are derived the frozen embryo, was able to predict FET outcome and was time independent.
What is known already: The factors linked to the FET outcome were multiple. The percentage of remaining blastomers after the thawing procedure, the number of ART procedure carried out previously were important factors to obtain childbirth. However, the appreciation of the anteriority of the ART procedure by their only rank, although interesting, does not apprehend the history of the ART procedure in its entirety. The ART score synthesizes an ART procedure into a single note, higher is the ART score, higher is the delivery chance. The ART score could be used to predict the FET outcome according its fresh ART procedure.
Study design, size, duration: A retrospective cohort study was conducted in the department of reproductive medicine at the Hôpital Femme Mere Enfant (Lyon, France). Couples performing an ART procedure during the study period were included according to the following exclusion criteria: delivery during the ART procedure from whom the freezed embryos originated, freeze all procedure, oocytes donation, none embryo freezed during the ART procedure. 1500 couples with 2508 FET procedures were included, from January 2010 to December 2016.
Participants/materials, setting, methods: Embryos were frozen at early stage (D2 or D3) with slow freezing process; or at blastocyst stage, in this case slow freezing process or vitrification process were used. Only embryos classified as "top embryos" were freezed or vitrified. The endometrial preparation was performed with substitution protocol or gonadotropin protocol. Monovariate and multivariate regression logistics analysis were performed to study the link between predictive parameters and FET outcome.
Main results and the role of chance: At the FET time, the women mean age was 33.4 ± 4.3 years. The mean number of transferred cryopreserved embryos was 1.52±0.62. The overall delivery rate was 18.0% (452/2508). The FET delivery rate varies significantly over time, starting at 5.9% (3/51) in the first semester of 2010 to 14.2% (26/183) in the fourth semester of the year 2016 (p<0.001). The following parameters were tested for FET outcome prediction: ART score, woman age, number of embryo transferred, number of performed FET (rank), stage of embryo transfer, the freezing procedure, and the endometrial preparation protocol. With the multivariate logistic analysis, the chance of FET delivery increases according to ART score (OR=1.43, p<0.05), and number of transferred embryo (OR=1.78 for two embryos transferred with p<0.001). The chance of delivery decreases according to FET rank (OR=0.22 after the first FET cycle, p<0.001), woman aged above 40 years (OR=0.51, p<0.05). However, among all these parameters, only the ART score

was independent of the time in which the FET was performed (p = 0.094). In conclusion, with this study conducted over a 7-year period, among the factors that influence the delivery rate, only the ART score was time independent and related to the birth rate.
Limitations, reasons for caution: The limitations of this study were: - the non-inclusion of the blastocyst grade at the time of cryopreservation; however, our cryopreserving policy would limited this variable effect, - the study was monocentric, however the ART score has been shown to be reproducible in other ART center (Labrune et al, 2018).
Wider implications of the findings: It seems even if the cryopreserved embryos were classified as good, the ART procedure from where they were originated have an in influence on their destiny. An embryo classification based on its morphology and its history should be elaborated.
Trial registration number: not applicable

P-158 A novel system of imaging mammalian zygotes to track pronuclear migration in 3D

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Study question: Establishing a method to track pronuclear migration using confocal live-cell imaging and 3D Image analysis in mouse zygotes fertilized through IVF.
Summary answer: Successfully established the tools to quantitively track maternal and paternal pronuclei in high spatiotemporal resolution, allowing first observations of the dynamics of their migration.
What is known already: Live cell imaging systems have been established for meiotic progression of mouse (Schuh and Ellenberg, 2008) and human (Holubcova *et al.*, 2015) oocytes, but not zygotes. Previous studies investigating mechanisms of pronuclear migration in mouse zygotes used either immunofluorescence imaging of fixed cells over time, or spinning-disk images of live cells with very low temporal resolution (i.e. images taken at start and end of pronuclear migration) (Schatten, 1994; Chaigne *et al.*, 2016). Therefore, no spatiotemporal analysis of pronuclear migration over the full time course from fertilization to the nuclear envelope breakdown had been established.
Study design, size, duration: Mouse zygotes were imaged on confocal microscopes every 3-15 minutes over the course of 4-17 hours. Experiments were of observational nature in order to quantify the dynamics of pronuclear migration and other proteins or structures of interest. Each type of experiment was repeated at least 3 times, and each repetition was done with at least three, and up to 20 zygotes per group, depending on the nature of the experiment.
Participants/materials, setting, methods: Experiments were conducted in a basic research laboratory setting. MII oocytes were collected from the oviducts of superovulated C57BL/J x CBA/J F1 mice. The oocytes were subsequently injected with mRNA encoding for fluorescently labelled proteins and structures of interest, such as DNA, cell membrane, or vesicles. The oocytes were fertilized with capacitated sperm from male C57BL/J x CBA/J F1 mice, imaged using confocal microscopy and analysed using IMARIS 3D imaging software.
Main results and the role of chance: We successfully established a method to culture and image mouse zygotes expressing fluorescently tagged proteins of interest from fertilization until nuclear envelope breakdown and subsequent first mitotic division. By injecting the zygotes with mRNA encoding for different fluorescently tagged proteins, we were able to conduct a series of experiments to further our understanding of pronuclear migration in mammals. First of all, by labelling DNA and cell membrane and imaging a z-stack of the zygotes every 15 minutes, IMARIS could subsequently be used to reconstruct pronuclei and zygote in 3D. Using this approach, we could quantify the distance of each pronucleus (maternal and paternal) from the zygote centre over time. Furthermore, we were able to image other structures of interest in high spatial and temporal resolution; for example, we were able to image actin vesicles with an interval of 3 minutes zoomed into smaller parts of the cell, allowing us to analyse the dynamics of these structures over time.
Limitations, reasons for caution: The zygotes were fertilized and cultured in vitro, which may affect observations. Also, we have not tested this method in zygotes from other species, and thus whether the method works or similar dynamics exist in e.g. human zygotes is not known.
Wider implications of the findings: Correct pronuclear migration is required for successful early embryogenesis. This method allows us to systematically test the function of various cytoskeletal components in mouse pronuclear migration, and may be applied to other model systems in the future, to help us understand the mechanisms of this process.
Trial registration number: not applicable

P-159 The impact of reproductive ageing on the human oocyte transcriptome during the GV to MII transition

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Study question: What are the differences in gene expression between human germinal vesicles (GV) to metaphase II (MII) stage transition during reproductive ageing?
Summary answer: A decrease in mitochondrial-related transcripts from GV to MII oocytes was observed, with a much greater reduction in the MII with advance age.
What is known already: Early embryonic development is governed by maternal transcripts stored within the oocyte during oogenesis. Transcriptional activity of the oocyte ultimately dictates its developmental potential and may be influenced by maternal age, resulting in reduced competence of older oocytes compared with young. Others performed gene expression studies in human and animal oocytes, using qRT-PCR and array-CGH based techniques. An RNA sequencing (RNA-Seq) study showed energy-related transcript reduction during the GV to MII transition in bovine oocytes. In the current study, RNA-Seq was used for transcriptome profiling of human GV and MII oocytes of young and advanced maternal age women.
Study design, size, duration: Fifteen patients treated for infertility in a single IVF unit agreed to participate in this study. Five GV and 6 MII oocytes from eight 21-26 yo women (young group) and 5 GV and 6 MII oocytes from seven 41-44yo women (old group) were donated for this research (Ref. No.: 19964/04-09-2014) by women undergoing IVF treatment. The samples were collected within 3 months. RNA was isolated and deep sequenced at the single cell level.
Participants/materials, setting, methods: Cumulus dissection from donated oocytes was performed 38 hours after hCG injection and oocytes were frozen at -80°C in lysis buffer supplemented with RNase inhibitor. RNA from GV and MII oocytes underwent deep RNA sequencing using the SMART-Seq v4 Ultra Low Input RNA protocol (Takara-Clontech, USA) and Nextera XT DNA library preparation kit (Illumina, USA). Data processing, quality assessment and bioinformatics analysis were performed using source-software, including FastQC, HISAT2, StringTie, edgeR and DAVID.
Main results and the role of chance: Following deep single-cell RNA-Seq of GV and MII oocytes, hundreds of transcripts were significantly differentially expressed between young and old groups, with the most significant biological processes relating to mitochondrial reserves. The GV to MII transition shares common biological processes between young and old groups, however, some genes involved in mitochondria function were altered during ageing. A decrease in mitochondrial-related transcripts was also observed in the GV to MII transition. However, there was a much greater reduction of mitochondrial-related transcripts in MII oocytes with advanced age. This observation was confirmed when young MII oocytes were compared with the old MII group with mitochondrial-related transcripts being significantly higher expressed in the young group, including biological processes such as mitochondrial electron transport and ATP biosynthetic process (FDR 7.7E-12 to 1.5E-3). These results indicate a higher energy potential in young MII oocytes that is decreased with age. Other biological processes that were significantly higher in the young MII group include transcripts involved in the regulation of ubiquitin-dependent degradation. Lack of these transcripts could lead to a non-appropriate removal of oogenesis remnants following fertilisation in the old MII group.
Limitations, reasons for caution: The different stimulation treatments among the recruited patients/donors could be a reason for caution. However, the RNA-Seq results cluster quite closely with low intra-group variation. Furthermore, 5-6 biological replicates were derived from at least 3 different donors per group, minimising the potential impact of chance on the findings.

Wider implications of the findings: Understanding reproductive ageing effects at the RNA level in human oocytes may reveal differences in the mechanisms regulating the GV to MII transition that impact on oocyte quality in young and old patients. Further investigations of the up-/down-regulated transcripts during ageing could guide and improved IVF outcomes for older patients.
Trial registration number: Not applicable

P-160 Three-dimensional biodynamic imaging: a novel approach to assessing oocyte maturity in an intact cumulus-oocyte complex to enhance in-vitro maturation yield

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Study question: Is it possible to assess oocyte maturity without removal of cumulus cells utilizing biodynamic imaging?
Summary answer: Oocyte maturity can be accurately assessed with non-invasive imaging techniques.
What is known already: Clinicians and patients share the common goals of increased success rates and reduced dependence on hormone support of the in-vitro fertilization ("IVF") workflow. Both goals are currently hindered by the absence of suitable means to perform non-destructive assessment of harvested oocyte maturity while preserving the prospect of in-vitro maturation ("IVM") for immature oocytes. Current practices accept a very high loss rate among harvested eggs given the requirement to denude the cumulus oocyte complex ("COC") to accommodate optical microscopy, rendering immature oocytes non-viable. An assessment method that circumvents denuding would greatly improve IVF outcomes.
Study design, size, duration: This study aimed to establish the feasibility of a novel interferometry technique combining near-infrared light, advanced optics, and proprietary bioinformatic analysis to produce a three-dimensional computer visualization of fresh oocytes within the intact COC. Safety and efficacy of polar body detection was assessed in two experimental models: murine oocytes (672), and porcine oocytes (147).
Participants/materials, setting, methods: This research was conducted in collaboration with animal facilities at Indiana University and Purdue University. Safety testing involved imaging 331 test COCs for approximately 30 seconds, while holding 341 matched controls under otherwise identical conditions prior to completing routine IVF protocols. Efficacy was established by subjectively scoring interferometry images of fresh porcine oocytes as either mature or immature, after which they were denuded and blindly re-scored by an experienced embryologist using conventional methods.
Main results and the role of chance: Our safety experiments demonstrated a difference in mean fertilization rates between test and control cohorts of 0.96% (90% CI: -5.19% to 7.11%). Day 6 blastocyst yield differed by 1.42% (90% CI: -4.50% to 7.35%). The data support statistical equivalence of test and control groups for both study endpoints (equivalence limit +/- 0.075; fertilization rate p=.0399; day 6 blastocyst yield p=.0456). These favorable results are not unexpected given that exposure of biological samples to near-infra red light is generally regarded as being safer than visible and ultra-violet light due to its far lower single photon energy. To our knowledge, however, this is the first study of near-infrared light in the IVF setting.

Among 147 COCs evaluated using the technique, 135 (91.8%) were correctly predicted to contain mature or immature oocytes as subsequently confirmed by conventional microscopy. Technician evaluations yielded only 7 (4.8%) false-negative and 5 (3.4%) false-positive reads (sensitivity=89.7%, specificity=93.7%, $p<0.001$). With practice, the imaging procedure and subsequent interpretation demonstrated high efficiency and repeatability among two independent technicians and were deemed generally amenable to a high throughput embryology laboratory environment. These findings imply that the proposed imaging technique holds promise for safe and effective use in clinical practice.
Limitations, reasons for caution: Results of these porcine and murine studies may not generalize to human subjects. Further research is warranted to independently reproduce safety and accuracy results in additional animal models, followed by human trials subject to satisfactory results of expanded animal studies.
Wider implications of the findings: These promising preliminary results offer the potential to dramatically improve the IVF process both by increasing viable oocyte yield per harvest and reducing dependence on hormone support. Continued research and development should be a high priority given the unmet need for technologies that enable expanded adoption of IVM.
Trial registration number: not applicable

P-161 the negative effect of bisphenol A on preimplantation embryos via the induction of oxidative stress

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Study question: we investigated whether BPA negative effects on early embryonic development is associated the oxidative stress
Summary answer: our results indicate that BPA displays an adverse effect on porcine early embryonic development because of the excessive accumulation of ROS
What is known already: BPA is an environmental contaminant widely used in the plastic industry, and has been detected in good and water consumed by animals and humans. BPA has been demonstrated to be an endocrine disruptor and has an adverse effect on the embryonic development of mammals. However, the mechanism of action of BPA is limited. Because of the porcine germ cells that has been shown to be more similar to human than others, the pig was used as the animal model in this study.
Study design, size, duration: Ovaries from prepubertal gilts were obtained from a local slaughterhouse. Follicles that were 3–6 mm in diameter were aspirated. The oocytes were maturated *iv vitro* and activated of parthenogenesis. For BPA treatment, the porcine parthenotes were randomly assigned to four groups. The adverse effects on the embryos were detected after treatment with BPA at a concentration of 100μM for 5 days
Participants/materials, setting, methods: The porcine parthenotes were randomly assigned to four groups: control, 50μM, 100μM, 200μM. The developmental rate was observed at different stage. 100μM was used in this study. The embryos were analyzed for ROS, and ROS related effects with immunofuorescence and RT-PCR
Main results and the role of chance: We found that blastocyst formation was impaired and the parthenotes were arrested at the 4-cell stage after treatment with 100μM BPA. Second, ROS increased following the addition of BPA, which further caused mitochondrial damage, and cytochrome c was released from the mitochondria to induce apoptosis. The adaptive response was demonstrated through LC3 immunofluorescence staining and by assessing autophagy-related gene expression. In addition, BPA caused DNA damage through the p53-p21 signaling pathway.
Limitations, reasons for caution: Because of the ethical limitations, the pig was used as animal model in this study. This justifies was adopted to human embryos need to be further investigated
Wider implications of the findings: These results will help us understand the reason that BPA cause the arrest of pre-implantation embryonic development. Therefore, the BPA toxicity observed in this study may contributed to understanding the reduced development potential in human oocytes.
Trial registration number: not applicable

P-162 Effect of Dynamic Oxygen Concentration on Mouse Preimplantation Embryos Development and Apoptosis with the Double Channel Gas Supply Incubator System

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Study question: Is a reduction of the oxygen concentration from 5% to 2% during in vitro culture of mouse preimplantation embryos beneficial for developmental competence?
Summary answer: A reduction of oxygen concentration after day-3 could improve blastocyst quality and peri-implantation embryonic developmental competence.
What is known already: The various oxygen concentrations have effects on embryonic development rates and quality of in vitro culture. The majority of modern IVF labs have accepted the 5% or 20% of oxygen concentration. Recently, a new debate has emerged regarding whether a further reduction of oxygen concentration on day-3 after fertilization on development represents more similar in vivo physiological condition. This new approach is based on the premise that oxygen concentration is actually lower in the uterus than in the oviduct. There were several studies that in vitro culture of pre-implantation embryos using less than 5% of oxygen concentration.
Study design, size, duration: We examined the effect of different oxygen conditions of 20% (high O_2), 5% (low O_2) and 5% to 2% (dynamic O_2). In dynamic O_2 group, mouse embryos were cultured from the 1-cell to morula stage under 5% O_2 (for 3 days), followed by cultured under 2% O_2 concentration to the blastocyst stage. The other high O_2 and low O_2 group were cultured 1-cell to blastocyst stage under 20% and 5% oxygen concentration, respectively.
Participants/materials, setting, methods: We used a novel double channel gas supply incubator (DCGS, CNC biotech, Korea) to provide dynamic oxygen concentration environment. Embryos were cultured in 5% O_2 until day-3 and automatically changed 2% O_2 concentration in the DCGS incubator. Cleavage, blastocyst formation rate and TUNEL assay were performed to assess the embryo quality. Outgrowth assay for in vitro model of implantation was performed to evaluate the developmental potential of peri-implantation embryos.
Main results and the role of chance: Cleavage rates to 2-cell stage were similar in high, low, and dynamic O_2 group as 86.9 ± 5.8%, 87.9 ± 3.5%, and 90.1 ± 2.0%, respectively. However, blastocyst formation rate was significantly increased in low O_2 (75.7 ± 2.2%) and dynamic O_2 (75.6 ± 4.6%) group compared with high O_2 (54.0 ± 2.3%) group. Total cell number was significantly increased in dynamic O_2 (128.9 ± 3.3) group compared with low O_2 (115.4 ± 3.6) and high O_2 (87.5 ± 2.5) group, also, apoptotic index was significantly reduced in low O_2 (4.47 ± 0.49%) and dynamic O_2 (4.15 ± 0.35%) group compared with high O_2 (8.67 ± 0.82%) group. Outgrowth rates of blastocyst on day-8 after fertilization were increased in low O_2 (58.32 ± 2.26%) and dynamic O_2 (66.67 ± 11.11) group compared with high O_2 (43.41 ± 5.23) group, but, there was no significant differences. Mean area of TE were significantly increased low O_2 (1.05 ± 0.05 mm^2) and dynamic O_2 (1.11 ± 0.07 mm^2) group compared with high O_2 (0.70 ± 0.07 mm^2) group.
Limitations, reasons for caution: We could not clearly demonstrate dynamic oxygen concentration was superior to 5% static culture condition in terms of embryo development. Also, extra oxidative stress caused by inappropriate culture condition is not fully investigated. Moreover, embryo transfer is also required to fully investigate the implantation potential of in vitro cultured embryos.
Wider implications of the findings: Our results showed that slight benefits of dynamic oxygen concentration from 5 to 2% on day-3. It may be related to the physiological condition for pre-implantation embryos in uterus. Dynamical optimization of oxygen concentration with the novel DCGS incubator could improve embryo developmental competence in human IVF-ET program.
Trial registration number: This work (Grants No. 17-05) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea SMBA in 2018.

P-163 The combination of embryo morphokinetics, blastocyst morphodynamics and classical morphology grading by using last generation time-lapse incubators improve embryo selection

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Study question: Can the blastocyst expanded diameter (BD) be useful to determine the embryo implantation potential?
Summary answer: A combination of BD with two well-known parameters: blastocyst quality (ASEBIR criteria, 2015) and s3 (t8-t5), can predict the implantation potential of the embryos.
What is known already: In recent years, several authors have found different morphokinetic variables that could be predictors of implantation potential. The relevance of s3 predicting blastocyst development have been shown (Motato et al., 2016) and the success of the ASEBIR criteria to assess blastocyst quality is checked in many IVF laboratories daily. However, although a correlation between BD and implantation rate was found by our research group, BD had never been described as embryo selection criterion. In this study, a precise morphokinetic evaluation was conducted to identify the appropriate variables to generate a predictive algorithm.
Study design, size, duration: A retrospective analysis including 578 women undergoing single embryo transfer from 03/2017 to 08/2018 was performed. Embryos were cultured to the blastocyst stage in a Time-lapse monitored incubator (Embryoscope Plus®, Vitrolife, Denmark) in groups up to 8 embryos per slide.
Participants/materials, setting, methods: 578-SET embryos from younger than 38-year-old women were evaluated with the Embryoviewer®. Timings of the main events of embryo development including the transition of 5 to 8-blastomere embryos and the blastocyst morphology dynamics including the expanded diameter were annotated. A ratio between BD and the timing of this measurement was conducted to normalize data. Blastocyst quality was evaluated with ASEBIR blastocyst assessment classification. ANOVA, Chi-square-analysis and logistic regression method were conducted in SPSS as appropriate.
Main results and the role of chance: A detailed analysis of morphokinetic parameters showed that many of them were significantly correlated with subsequent implantation**.** We identified three variables most relevant: blastocyst quality (ASEBIR), blastocyst expanded diameter and time between division to 5 cells and division to 8 cells (s3). Optimal ranges were established for each relevant parameter: ASEBIR - A (**65.70%** implantation rate for A vs. **53.27%** for B+C)*, BD ≥ 159 µm (**61.8%** vs. **44.50%** for BD < 159 µm)** and s3 ≤ 14.5 h after ICSI (**61.30%** vs.**43.80%** for s3 > 14.5)*. Based on these results we elaborated a good discriminatory algorithm that classified embryos from A to C according to implantation potential. When the embryo parameters were inside the optimal range (A quality, BD ≥ 159 µm and s3 ≤ 14.5 h) embryos were classified as A, according to the algorithm, (**66.40%** implantation rate); if some of three parameters were outside the optimal range embryos were classified as B (**58.9%**); if three parameters were outside the optimal range embryos were classified as C (**37.4%**)*.
Note: *(p=.000) **(p=.001)
Limitations, reasons for caution: This analysis is limited by its retrospective nature as well as the subjectivity of blastocyst morphology evaluation. A larger retrospective sample size maybe necessary and a prospective trial would be important to validate our algorithm.
Wider implications of the findings: The combination of morphology with morphology dynamics of the blastocyst and morphokinetics could be used as a powerful non-invasive tool to select embryos to transfer. The subjectivity associated with the blastocyst evaluation can be overcome by the measurement of blastocyst features in a time-dependent fashion.
Trial registration number: Not applicable

P-164 Assessment of embryo implantation potential with a cloud-based automatic software

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Study question: ¿Does the embryo selection made by the DANA system correlate with a good implantation rate in patients undergoing IVF treatments?

Summary answer: DANA system selects embryos with high implantation potential from an automatically parameter (UAD) based on the morphokinetic characteristics of those embryos that have implanted.

What is known already: Selecting the best embryo for transfer is crucial to achieve the final goal of in vitro fertilization treatments. Time-lapse technology has made possible to perform a deep and accurate analysis for optimal embryo selection. The usefulness of time-lapse monitoring greatly depends on the creation of predictive algorithms that could be effective in different clinical settings and after distinct IVF procedures.

Study design, size, duration: This is a multi-centred retrospective study designed in three phases for the validation of the DANA system. The patients were recruited from July 2015 to January 2018 in three IVI Clinic's centres. A total of 1312 IVF cycles and 5343 analysed embryos were included in this three-phase trial. A new parameter was developed to estimate embryo score: the average distance of each embryo to the centre of the cloud of KID embryos (UAD).

Participants/materials, setting, methods: Phase 1: Creation of the data cloud of KID (Known Implantation Data) embryos from 1676 transferred embryos. Timings of embryo cleavage and cell cycle lengths up to the 5-cell stage were included in the DANA software; Phase 2: embryo selection based on the DANA system and confronted with the embryologist manual annotations (389 transferred embryos); Phase 3: Validation of DANA automatic embryo selection, without embryologist's intervention (147 transferred embryos).

Main results and the role of chance: The implantation rate of the 1021 KID embryos from phase 1 were distributed as follows: grade A 34%, grade B 25%, grade C 24%, and grade D 19%. In phase 2, a classification ranking according to the unit average distance (UAD) values was established: High (UAD $<$ 0.447), Medium (UAD = 0.447 – 0.998) and Low (UAD $>$ 0.998) implantation potential. The pregnancy rates for these groups were 55%, 45%, and 33%, respectively ($p < 0.001$). In phase 3, the 147 transferred embryos were classified according to their UAD values as grade A (UAD $<$ 0.50), grade B (UAD = 0.50 – 0.66), grade C (UAD = 0.67 – 1.03), and grade D (UAD $>$ 1.03); most implanted embryos were found in Groups A, B, and C (UAD $\leq$ 1.03), whereas the implantation rate in Group D (UAD $>$ 1.03) was significantly lower: 46% vs. 25%, respectively ($P = 0.037$).

Limitations, reasons for caution: External and prospective validations are required to confirm the reproducibility and generalizability of this automated system.

Wider implications of the findings: The model can analyse data obtained in different settings, and therefore is supposedly applicable to any clinic, standardizing the interpretation of embryo development. The great innovation of DANA is that the system learns as the database grows with each new patient cycle, becoming more and more accurate (machine learning).

Trial registration number: not applicable

P-165 Trophectoderm cell cycle, inner cell mass area, blastocyst expanded diameter and pronuclei migration associated with implantation potential: description of novel embryo parameters through time-lapse technology.

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Study question: Can we improve the outcomes of the *in vitro* fertilization (IVF) cycle by studying new embryo development variables through time-lapse technology?

Summary answer: Novel analyzed variables showed a clear influence over the implantation rate. Optimal value ranges have been established for each variable, based on the implantation potential.

What is known already: Time-lapse technology allows consecutive observation of embryo development and provides morphological and morphokinetic information. Several studies have shown that timing of the most relevant events in embryo development are correlated with embryo preimplantation development and subsequent implantation rates. However, not all seemingly good quality embryos lead to implantation, raising the need to define new selection variables. Trophectoderm cell cycle length, inner cell mass area, blastocyst expanded diameter and pronuclei migration have already been assessed with the time-lapse incubator Embryoscope Plus® in a preliminary study performed by our research group. Currently, we have doubled the sample size reaching stronger results.

Study design, size, duration: A retrospective analysis, including 541 patients, was conducted. 765 embryos transferred were evaluated out of 2540 viable embryos. Single embryo transfer (SET) was performed for 476 of them. All of them were cultured in a time-lapse incubator (Embryoscope Plus®, Vitrolife, Denmark).

Participants/materials, setting, methods: 476 transferred embryos from younger than 38-year-old women were evaluated with the Embryoviewer®. Morphokinetic parameters were annotated during the embryo development by senior embryologists and the drawing tools were used to measure new variables, including: speed of the pronuclei migration, blastocyst expanded diameter, inner cell mass (ICM) area and trophectoderm cell cycle length. Data obtained was assessed in terms of clinical outcome and statistically analyzed with ANOVA test and Chi-squared test (SPSS 21 software).

Main results and the role of chance: This extensive analysis of morphokinetic parameters already known showed the optimal range for each variable, which give the embryos the highest implantation rate. Events associated with the late-stage embryo development are the most significant **t8**($<$62.83h), **tM**($<$93.15h), **tSB**($<$102.4 h) and **tB**($<$108.12h). New variables analyzed also showed values associated with different clinical outcomes. Implantation rate improved significantly ($p<.05$) as the blastocyst expanded diameter increased (**44,5%**for $<$158 µm vs. **52,3%**for 159-173 µm vs. **63,1%**for 174-187µm vs. **69.6%**for $>$188 µm). According to our data, embryos with ICM area larger than 2716µm^2 achieved better implantation rates than smaller ones (**60.20%**for $>$2716 µm^{2}vs **57.20%**for $<$2716 µm^2). In terms of speed of pronuclear migration, values from 0.21 µm/h to 3.32 µm/h were found, being the fastest ones, which displayed highest implantation rates. Trophectoderm cell cycle length was shorter than blastomere cell cycle. Moreover, the shorter the trophectoderm cell cycle the better (**64.4%**implantation rate for $<$8.66 h vs. **58.15%** for $>$8.66h).

Limitations, reasons for caution: Impact of each variable over the implantation rate has been individually assessed so far. In addition, although we used a high quality time-lapse system supplied with different plans, the embryonic three-dimensional morphology made the evaluation of some events difficult, mainly those which are related to pronuclei appearance and fading.

Wider implications of the findings: Analysis of the interaction among variables would be necessary to confirm the relevance of each parameter. A possible subsequent consolidation of the novel variables in embryo selection could lead to the development of new algorithms improving the selection of the best quality embryo.

Trial registration number: Not applicable

P-166 Impact of embryo replacement position (ERP) on euploid frozen embryo transfer (FET) outcomes

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Study question: Does the position of the euploid blastocyst, measured as distance from the fundus (DFF) of the uterine cavity (mm) affect the implantation potential?

Summary answer: There is a negative correlation between the DFF and the probability of pregnancy and implantation, while no effect was seen on the miscarriage rate.

What is known already: The optimal ERP of a euploid blastocyst is one of the last hurdles of an ART treatment. However, the ideal location within the uterine cavity is still being debated. While it has been claimed that the transfer site does not affect the implantation potential, most of the studies found that the highest

pregnancy rates are obtained when the embryo is placed in the upper or middle area of the uterine cavity. Unfortunately, due to heterogeneity of parameters between different studies in which embryos with unknown ploidy state were transferred, objective assessment on the real effect of the ERP is difficult.

Study design, size, duration: This single center retrospective cohort study included a total of 455 single/double euploid FET cycles between March 2017 and November 2018. 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 longitudinal distance between the fundal endometrial surface and the air bubble after transfer with a K-soft COOK catheter was measured and pregnancy, implantation and miscarriage rates were recorded.

Participants/materials, setting, methods: The following explanatory variables were analyzed: age, Anti Müllerian hormone (AMH), body mass index (BMI), endometrial thickness, quality of the trophectoderm, difficulty of the transfer (requirement of additional instrumentation), presence of mucus or blood, day 5 or day 6 biopsy, single (SET) or double (DET) embryo transfer, cycle preparation (natural cycle - NC or hormone replacement therapy - HRT). The primary aim was to detect if the implantation potential is affected by the DFF.

Main results and the role of chance: The patients were on average 33.5 years old. The FET was performed in a NC (n=137) or HRT cycle (n=318). Of the 455 transfers, 325 (71.4%) resulted in a pregnancy and 262 (57.6%) in a clinical pregnancy leading to an implantation rate of 61.6%. Initially, we performed bivariate basic comparison tests between all explanatory variables and the pregnancy, implantation and miscarriage outcomes. When comparing the cycle preparation, number of embryos transferred and the quality of the embryos, the pregnancy and implantation rates were significantly higher in NC (p=0.0117 and p=0.0077), after DET (p=0.0049; and p=0.0026) and for high quality embryos (p=0.0039 and p=0.0072).

After performing a multivariate logistic regression analysis to consider the effect of all explanatory variables on the DFF, a negative effect between DFF and pregnancy (p=0.0084) and implantation (p=0.0065) was found. When all variables remained constant, the increase of one mm of DFF increases the odds ratio of pregnancy by 0.902 and of implantation by 0.903, implying that the probability of pregnancy and implantation decreases as the fundus distance increases. No statistical significance of DFF was found for the miscarriage outcome (p=0.2166), however, the presence of only 38 miscarriage cases is insufficient to make an adequate evaluation.

Limitations, reasons for caution: Besides the retrospective design of the study, superiorly the full length of the cavity should have been measured to estimate the exact position within different uterine sizes. Also, a higher number of miscarriages is needed to find a possible effect of the distance from the fundus on this parameter.

Wider implications of the findings: The depth of embryo replacement inside the uterine cavity may influence the pregnancy and implantation rates and should be considered as an important factor to improve the success of IVF cycles.

Trial registration number: NA

P-167 Impact of equilibration duration during oocyte vitrification protocol: preliminary results of a prospective observational study.

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Study question: Does the duration of equilibration step during oocyte vitrification protocol influence survival rate and biological outcomes after oocyte warming and micro-injection?

Summary answer: The equilibration duration during oocyte vitrification protocol might influence survival rates after warming but does not impact further embryo development of surviving oocytes.

What is known already: Oocyte cryopreservation is a valuable technique in the field of female fertility preservation (FP) as well as oocyte donation programs. Numerous studies have already analyzed biological and clinical outcomes following oocyte vitrification. Regarding technical aspects, open versus closed carriers have mainly been investigated. However, oocyte vitrification protocols commercially available do describe various durations of the equilibration step (ranging from 6 to 10 minutes (min)). To date, a potential influence of this variability on the outcomes after oocyte warming has never been investigated.

Study design, size, duration: This prospective-observational-monocentric study has been in progress since 2014, including all oocyte cryopreservation cycles. Vitrification/warming (n=64) were performed using commercialized media (Kitazato, Japan). During equilibration done according to the manufacturer's procedure, nine oocytes maximum were deposited by three in three drops of equilibration solution (ES). After 6min, the vitrification step was initiated for the first three oocytes (duration 1min), and the following oocytes straw-by-straw were vitrified immediately thereafter, respectively after 7 and 8min of equilibration.

Participants/materials, setting, methods:

Oocyte vitrification/warming required patients' written informed consent. To date, 64 couples underwent the whole procedure of warming and ICSI. The allocation of oocytes per straw depended on the total number of mature oocytes available, explaining the variable number of oocytes in groups 6/7/8min. Survival, fertilization, embryo quality at Day-2 and suitability for transfer/cryopreservation were assessed for each oocyte and compared according to the duration of equilibration (6/7/8min).

Main results and the role of chance: Briefly, the included patients were on average 36 years of age and underwent their 1.8th ICSI cycle. Indications for oocyte cryopreservation were: oocyte accumulation program (46%); oocyte donation (33%); FP prior to cancer treatment (3%), for endometriosis (10%) or poor ovarian reserve (1%); and absence of spermatozoa on the day of ICSI (7%). Overall, 388 oocytes were warmed, and 329 of them survived (survival rate (SR)=84.8%). The analysis according to the equilibration step duration showed a slight difference in terms of SR: 82.5% (188/228) in group "6min" *vs.* 85.6% (101/118) in group "7min" *vs.*95.2% (40/42) in group "8min" (global p-value=0.06). Interestingly, SR after 8min of equilibration was significantly or close to significantly higher than after respectively 6min (p=0.02) and 7min (p=0.07). Regarding biological outcomes after ICSI of the surviving oocytes, fertilization rate (68.1 *vs.*74.5 *vs.* 60.6%, p=0.29), Day-2 top-quality (28.9 *vs.*37.1 *vs.*19.0%, p=0.23) and good-quality embryo rates (43.0 *vs.*47.1 *vs.*33.3%, p=0.52) and rates of embryos suitable for transfer/cryopreservation (70.3 *vs.*67.1 *vs.*52.4%, p=0.28) were statistically similar whatever the duration of the equilibration phase.

Limitations, reasons for caution: Further investigation on a higher number of cycles and particularly on a higher number of oocytes in group "8min" is needed to clarify a potential impact of the equilibration duration on biological outcomes after oocyte warming. Furthermore, clinical outcomes should be analyzed.

Wider implications of the findings: If the duration of equilibration had a confirmed impact on oocyte survival, larger investigations should be performed on the different vitrification media commercially available. Then, manufacturers' recommendations on vitrification protocol should be amended accordingly.

Trial registration number: Not applicable

P-168 Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants

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Study question: What is the efficiency of different types of germline nuclear transfer (NT) to prevent the transmission of mitochondrial DNA (mtDNA) variants in a mouse model?
Summary answer: First/second polar body transfer (PB1/2T) outperforms both maternal spindle transfer (ST) and pronuclear transfer (PNT) in reducing the transmission of mtDNA variants.
What is known already: Eliminating mtDNA disease's transmission may nowadays be possible by using NT in the germline. However, scientific evidence to compare different NT techniques to overcome mtDNA diseases within the same study is lacking. Recent studies have demonstrated the feasibility of ST, PNT and PB1T in humans, but conventional PB2T remains challenging due to difficulty of identifying the female pronucleus in human zygotes.
Study design, size, duration: We performed four types of NT (PB1/2T, ST and PNT) between B6D2F1 mice (n=91) (intra-strain NT) and between NZB/OlaHsd (n=52) and B6D2F1 (n=47) mice (inter-strain NT). We evaluated the efficiency based on embryonic development potential and the level of mtDNA carry-over. Moreover, using mouse MII (n=334) and human *in vitro* matured (IVM) (n=216) oocytes, we explored two novel protocols (PB2T-a and PB2T-b) to optimize PB2T procedures, and compared their efficiency with conventional PB2T.
Participants/materials, setting, methods: We conducted PB1T, ST and PNT by transferring nucleus from oocytes/zygotes into enucleated counterparts. PB2 was transplanted into half-enucleated zygotes (conventional PB2T), and into enucleated oocytes (PB2T-a), and into single-pronucleus zygotes (PB2T-b). The reconstructed embryos were cultured to assess embryonic development. Some reconstructed oocytes were examined for spindle formation using polarized microscopy, and for spindle morphology by confocal analysis. Polymerase chain reaction (PCR) followed by next generation sequencing (NGS) was performed to measure mtDNA heteroplasmy.
Main results and the role of chance: Polarized and confocal analysis revealed that the majority of PB1T and ST oocytes re-formed MII-like spindles, most of which exhibited normal spindle-chromosomal structures (90.1% and 89.7%, respectively). Following ICSI, both PB1T (89.0%) and ST (87.3%) yielded high blastocyst rates similar to an ICSI control (88.4%). Additionally, PB2T-b showed promising result with a blastocyst rate of 86.5%, which was higher than conventional PB2T (70.7%) ($P < 0.05$); in contrast, PB2T-a failed to form blastocysts probably owing to a high abnormal spindle rate (28.0%) ($P < 0.05$). PNT zygotes (92.0%) exhibited comparable blastocyst formation rates to those of PB1T, PB2T-b and ST embryos. Comparing intra-strain NT to inter-strain NT, we found no significant reduction in embryonic development. By analyzing artificial mixtures of mtDNA with different molar ratios, we confirmed that NGS had a high sensitivity in detecting mtDNA heteroplasmy with a detection threshold of ~0.5%. Analysis of NT-generated blastocysts using NGS revealed that both PB1T (0.6182%) and PB2T-b (0.9089%) had significant lower levels of mtDNA carry-over than those of ST (3.565%) and PNT (4.132%) ($P < 0.01$). Finally, extrapolation of novel PB2T-b to human IVM oocytes resulted in 12.9% of reconstituted embryos successfully developing to blastocysts similar to control ICSI embryos (20.5%).
Limitations, reasons for caution: Our findings in the mouse model should be verified in human patients with known mtDNA diseases. In addition, invasive micromanipulation of human oocytes or zygotes may cause chromosomal or epigenetic abnormalities. Further study to ensure the safety of different NT technologies is therefore required.
Wider implications of the findings: Our study reveals that PB1/2T results in minimal mtDNA carry-over without compromising embryonic development, indicating that PB1/2T has potential to reduce risk of transmitting diseased mtDNA. Since PBs are normally discarded, PB1/2T could be applied in combination with ST or PNT to improve the efficiency and number of available embryos.
Trial registration number: Not applicable.

P-169 Evaluation of the effects produced by Styrene (volatile organic compound- VOC) over the human pre- embryo development.

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Study question: Can specific concentrations of Styrene, a very common IVF VOC, affect the human embryo development and ploidy?
Summary answer: Embryos cultured along with IVF- ambient air- related concentrations of Styrene have worst survival rates and morphology score when evaluated at blastocyst stage (day 6).
What is known already: Polystyrene, a synthetic aromatic hydrocarbon polymer made from the monomer styrene is present in the plastic-ware used in IVF laboratories. Styrene may persist even after the process of devolatilization, and its presence is known to affect the embryo development in animal models but no specific effects on humans have been described yet. Previous analysis have demonstrated that there are negative effects over human embryo development when exposing embryos to Benzene and Limonene,. Specific threshold levels at which most of the common pollutants in the IVF's ambient air can cause harm to cultured human embryos, have not been determined either.
Study design, size, duration: Prospective study with 115 day-3 (D3) human embryos. These have been allocated in four experimental groups; a. 40 embryos were cultured in the control (C) culture media. At the same time, 75 embryos were cultured and exposed to three Styrene concentrations based on previous IVF's environmental characterizations: b. Exterior (SE: 165,8∗10^-5ppm), c. Laboratory (SL: 981∗10-^5ppm) and d. Double Laboratory (SDL: 1962∗10^-5ppm). All embryos were cultured till day-6 (D6) so they could reach the blastocyst stage.
Participants/materials, setting, methods: This developmental toxicity test includes D3 embryos, with 6 to 10 cells and less of 25% of fragmentation, which have been exposed by contaminating the blastocyst culture medium with three doses of Styrene (under 1% of the occupational limit value, OLV). After thawing, 25 embryos were exposed to each dose up to D6 of development, when embryo morphology was evaluated. Then biopsy of trophectoderm (TE) was performed for ploidy analysis of expanded blastocysts through NGS.
Main results and the role of chance: A total of 115 D3 human embryos were donated for this VOCs research. In general, 66,7% of embryos developed to blastocyst stage after being exposed to Styrene while 90% of the embryos developed in C group (p= 0.006). Individually, all three doses of Styrene affected similarly the development: SE and SL 68% (p= 0.0460) and SDL 64% (p= 0.0228).

53,3% of embryos exposed reached the expanded- hatched blastocyst stage compared to 75% of the C group (p= 0.028). SDL doses affected significantly the expansion of the embryos: 48% (p= 0.0350).

Inner cell mass (ICM) and trophectoderm (TE) good quality scores (A-A, A-B, B-A, B-B) were only registered on 26,7% of Styrene embryos group compared to 40% of the control group, not being significant (p= 0.2048). A-A score embryos were obtained in both groups.

A total of 55 embryos that reached expanded- hatched state were analyzed for aneuploidies. Aneuploidy rates were not significant between Styrene (n=35) and Control (n=20) groups (28,6% vs. 25% respectively); however when analyzing the effects of specific doses, the embryos that were exposed to the to the highest dose of styrene (SDL) reached up to 40% of aneuploidies compared to 25% of the C group. (p= 0.4311).
Limitations, reasons for caution: Developmental and ploidy rates were obtained by direct contamination of the culture medium with VOCs, specifically Styrene in this experiment. For this reason the results do not represent the exact conditions of an IVF laboratory.
Wider implications of the findings: The association of the specific morphologic and chromosomal effects produced by common IVF VOCs, over the human pre- embryos will allow to establish a database with regulatory institutions, with accurate occupational limit values for human embryo culture.
Trial registration number: Not applicable

P-170 ICSI outcome of infertile men with round-headed sperm: is ICSI effective?

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Study question: is ICSI an effective treatment for infertile male with round-headed sperm syndrome?

Summary answer: based on current knowledge, ICSI consider as main therapeutic procedure for these patients.

What is known already: patients with round-headed spermatozoa are infertile due the lack of acrosome in sperm structure, so acrosome reaction which is necessary for sperm entering to oocyte, do not take in part. More than 20 years studies showed that with ICSI, it could be possible to overcome infertility for about 25% of patients with this certain pathology worldwide. However, approximately all these studies, except one, applied on few numbers of patients. Reports showed a 24.3% fertilization rate, 22.1% pregnancy rate, and 12.2% delivery rate after ICSI cycles of these patients.

Study design, size, duration: in this study, we followed-up ICSI outcome of infertile round-headed sperm men who referred to Royan institute, Tehran, Iran, between years 2008 and 2016. This study, to date, is the largest one in assessing treatment of round-headed sperm patients.

Participants/materials, setting, methods: This is a retrospective study on ICSI outcome of 163 infertile couples which male partner has more than 50% round head spermatozoa, referring to Royan institute (Tehran, Iran) between years 2008 and 2016.

Main results and the role of chance: from all 163 couples, 53 underwent 66 ICSI cycles, achieving 38% fertilization rate. Afterward, 43 couples had a total number of 60 embryo transfers consists of transferring 41 fresh embryo and 19 frozen embryo. The chemical pregnancy rate was 27% (18 pregnancies out of 66 cycles; 11 from fresh and 6 from frozen embryos,), and clinical pregnancy rate showed a value of 24% (16 out of 66 cycles; 9 from fresh and 6 from frozen embryo transfers). Finally, 16 successful deliveries achieved from clinical pregnancies, which means a 24% delivery rate, with total 21 normal live births, 15 boys and 6 girls. notably, 3 patients with successful deliveries, have more than 90% round-headed sperm in their spermogram, may indicate that even patients with severe round-headed sperm syndrome could have benefit from ICSI procedure.

Limitations, reasons for caution:

Genetics evaluations of round-headed sperm syndrome, revealed that *DPY19L2* alterations, even the gene deletion, is the main genetics feature of this syndrome; however, we did not have access to patients to see whether they have this disorder or not, specially in those who have successful pregnancy and delivery.

Wider implications of the findings: These results, means that it could be hope for round-headed sperm patients to enjoy being parents. Also, some studies reported that ICSI along with assisted activated oocyte (AOA) could be more effective than ICSI alone, but the side effects of AOA should be considered.

Trial registration number: none

P-171 NOVEL APPROACH IN CRYOPRESERVATION OF HUMAN EMBRYOS AT THE MORULA STAGE WITH INCOMPLETE COMPACTION

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Study question: Is it possible to obtain a high survival rate after cryopreservation not fully compacted morulae?

Summary answer: Removing of excluded cell and fragmentation can increase the survival rate of not fully compacted morulae after cryopreservation.

What is known already: It has been shown that mosaic embryos may form partially compacted morulae in order to exclude aneuploid cells, which subsequently undergo apoptosis. Cryopreservation of embryos at the morula stage which had fragmentation more than 20% have led to significantly lower post-thaw survival rate compering with fully compacted morulae. These excluded fragments could be subject to major damage than compacted part by cryopreservation factor. The necrotic factor released from these injury cells may impact the survival rate of embryo and their further development.

Study design, size, duration: The investigation was performed in the ART-clinic of reproductive medicine since January 2015 to November 2018. The study involved 352 morulae with different compaction degree obtained in ICSI cycle from 70 patients with mean age 313 ± 4.2 years. 189 morulae were cryopreserved by conventional vitrification procedure (group I) and 163 morulae were underwent removing of excluded cells were prior to vitrification according with a written consent of patients (group II).

Participants/materials, setting, methods: All the morulae were graded into five groups by two embryologist independently: 1 – fully compacted morulae, 2–4 – incomplete compacted morulae (compaction were about 75, 50, 25 % of whole embryo respectively) and 5 –morulae with no compaction. The osmotic reaction in cryoprotectant media during equilibration time in vitrification procedure of all morulae groups, their survival and blastocyst formation rates after cryopreservation were evaluated taking into account embryo compaction degree.

Main results and the role of chance: The excluded fragments of morulae with not full compaction has been shown to have a low osmotic response to cryoprotactant solutions during equilibration time whereas the compacted part of embryo responds to the changes of osmotic pressure as a single whole. The survival rate of vitrified morulae of grades 1-5 were (98 ± 6.2), (85 ± 4.1), (38.2 ± 4.4), (50 ± 5.8) and (22.2 ± 4.4)% respectively. The blastocyst formation rate of morulae after warming positively correlated with their compaction degree ($r = 0.32$). Removal of excluded cells and fragments of morulae prior to cryopreservation led to an increase of their post-thawing survival rate up to (93.1 ± 4.1) and (75 ± 8.8)% and blastocyst formation rate up to (85.2 ± 10.4), (59.4 ± 5.2)%, $p<0.05$ in grades 2 and 3 respectively.

Limitations, reasons for caution: Limited number of cases of the study group. Further studies are recommended.

Wider implications of the findings: This study revealed that removing of excluded cells and fragmentation from morulae with incomplete compaction prior cryopreservation can increase the survival and blastocyst formation rates and as result improve the cumulative pregnancy rate.

Trial registration number: not applicable

P-172 Adverse results in oocyte donation cycles using cryo-banked eggs

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Study question: Is the oocyte survival rate an adequate prognostic factor of good outcome in oocyte donation cycles?

Summary answer: Low oocyte survival rates after warming lead to worse clinical outcomes in oocyte donation cycles.

What is known already: Oocyte vitrification is performed routinely and successfully in IVF and oocyte donation programs. The cryotop vitrification method for human oocytes described by Kuwayama et al. (2005) had reported a 91% survival rate. Other authors supported this finding (Cobo et al. 2010, Solé et al. 2014) but, in some cycles, it has been observed that this percentage is significantly diminished without any apparent explanation.

Study design, size, duration: This is a retrospective study including 535 oocyte donation cycles in which 6 or more eggs were warmed between December 2014 and December 2017. We compared vitrification/warming cycles with good survival rates (>60%), the high survival group, with cycles with low survival rates (≤60%), the low survival group.

Participants/materials, setting, methods: The participants were consenting donors and recipient couples from the oocyte donation program. Primary end-points were clinical pregnancy rate, miscarriage rate and live birth rate. Additionally, donor demographic baseline characteristics, donor ovarian stimulation parameters, type of ovarian stimulation treatment and embryo developmental characteristics were analysed.

Main results and the role of chance: 476 cycles (89%) were included in the high survival group and 59 cycles (11%) in the low survival group. There were no differences between the two groups in terms of donor demographic baseline characteristics and in donor ovarian stimulation parameters except for E2 on day of triggering (2188.58±1259.62 vs 2890.91±1504.71 p=0.004). No differences were observed regarding the type of ovarian stimulation treatment.

No differences were observed in laboratory outcomes: fertilization rate 76% (95% CI:74.5-77.6) vs 71.2% (95% CI:65.9-76.4), ongoing embryo rate 52.3% (95% CI:50.2-54.4) vs 53.3% (95% CI:46.1-60.6) and top-quality embryo rate 28.6% (95% CI:26.5-30.8) vs 22.1% (95% CI:15.9-28.2) in high survival group and low survival group respectively.

In general terms, 97.9% cycles had ongoing embryos, with a 44.7% clinical pregnancy rate, 20.9% miscarriage rate and 34.2% live birth rate.

When analyzing the clinical results per cycle, significant differences were observed regarding clinical pregnancy rate (46.4% vs 30.5% p=0.020) and live birth rate (35.9% vs 20.3% p=0.017), but not in miscarriage rate (21.2% vs 25% p=0.753) between groups. When comparing the data per embryo-transfer, not statistically significant differences were found regarding clinical pregnancy rate (48.7% vs 34.6% p=0.054); only the live birth rate showed significant differences (37.7% vs 23.1% p=0.038).

Limitations, reasons for caution: This is a retrospective study. A larger sample would probably be required to further strengthen the differences.

Wider implications of the findings: The results of this study suggest that the group of vitrification/warming oocyte donation cycles with low survival rate display similar laboratory outcomes but worse clinical results than cycles with survival rate >60%. New data are needed to ascertain the reasons for low survival rates after oocyte vitrification.

Trial registration number: Not Applicable

P-173 Success rates of hatching/hatched blastocyst transfer in frozen embryo transfer (FET) cycles.

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Study question: Is spontaneously hatching-blastocyst transfer more beneficial than hatched-blastocyst transfer in FET cycles?

Summary answer: Hatched-blastocyst transfer is more beneficial compared to hatching-blastocyst transfer in FET cycles. Hatched-blastocyst transfer in FET cycles can be considered as a superior method.

What is known already: Blastocyst stage embryo transfer has become a preferred practice in recent years. However, there is only limited amount of data available on cryopreservation of hatching and hatched-blastocysts.

Study design, size, duration: A total of 2,320 frozen-thawed blastocyst transfer cycles between 2016 and 2018 in CHA Fertility Center Seoul station, Seoul, Korea, was evaluated. All of the frozen and thawed blastocysts that were hatching or hatched were included in this retrospective cohort study.

Participants/materials, setting, methods: Day5 or day6 blastocysts were frozen and thawed using 2-step vitrification and 4-step warming protocols with EG and DMSO as cryoprotectants. Blastocyst survival post thaw was defined as more than 50% of ICM and TE cells remaining intact and re-expansion after culture for 3-4 hours before transfer. IR and PR were evaluated and compared between subgroups by ≤37 vs. >38 years old, the gradeA vs. B and the number of transferred embryos SET vs. DET.

Main results and the role of chance: SET cycles of Grade-A embryo, PR and IR were significantly higher in hatched-blastocysts compared to hatching-blastocysts in the group of ≤37 years old, PR 70.59% vs. 63.02%, p=0.019, and IR 66.34% vs. 56.61%, p=0.0035. In the group of >38 years old, PR was significantly higher in hatched-blastocyst transfer (58.70% vs. 41.75%, p=0.017), while there was no significance in IR for both groups. SET cycles of Grade-B embryo, only IR was significantly higher in hatched-blastocysts (54.10% vs. 38.87%, p=0.029). In the group of >38years old, PR and IR were significantly higher in hatched-blastocysts compared to hatching-blastocysts, 52.94% vs. 29.76%, p=0.066, and 47.06% vs. 17.86%, p=0.069, respectively. DET cycles of Grade-A embryo, there was a trend of higher PR and IR in ≤37 years group (83.78% vs. 77.35%, p=NS; 59.46% vs. 50.29%, p=NS). In >38years group, PR was significantly higher in hatched-blastocyst compared to hatching-blastocyst (84.21% vs. 36.96%, p=0.00009), and no significance in IR. DET cycles of Grade-B, PR and IR were significantly higher in hatched-blastocysts compared to hatching-blastocysts in both age groups; PR 78.13% vs. 56.94%, p=0.0039, IR 50.0% vs. 26.39%, p=0.00009 in ≤37 years group; PR 100% vs. 31.17%, p=0.04, IR 100% vs. 20.83%, p=0.00001 in >38 years group.

Limitations, reasons for caution: Due to small number of patients in the age group of >38 years old, a larger study is needed to fully support our results.

Wider implications of the findings: This study suggests that the transfer of hatched-blastocysts is more beneficial compared to hatching-blastocysts in FET cycles. Further study is needed with larger number of patients, especially in older age group.

Trial registration number: Not applicable.

P-174 The blastocyst score: a strong predictor of frozen euploid blastocyst transfer outcome

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Study question: What is the relationship between the blastocyst score, which integrates a blastocyst's morphologic grade with its rate of development, and frozen euploid blastocyst transfer outcome.

Summary answer: The blastocyst score is a predictor of frozen euploid blastocyst transfer outcome.

What is known already: With advanced genetic molecular technology and improved culture conditions, current trends in IVF practice are shifting to an increase in the use of blastocyst culture, preimplantation genetic testing for aneuploidy (PGT-A), and freeze-all cycles. However, the role of blastocyst morphology and day of blastocyst development in PGT-A embryo selection is controversial.

Study design, size, duration: This is a retrospective cohort study of 2,193 euploid frozen blastocyst transfer (FET) cycles performed between October 2013 and October 2018. We developed the blastocyst score, a numeric ranking system, by integrating a numerical score for the blastocyst morphologic grade (based on expansion, ICM, and TE) with the day of blastocyst development (D5 or D6).

Participants/materials, setting, methods: This study includes 2,193 FET cycles (1,901 SET, 291 TET, and 1 triple ET) from 1,462 patients (age 36.4±4.2). On days 5 or 6, blastocysts were biopsied and tested using aCGH/SNP/NGS. They were vitrified and transferred in programmed or natural cycles. The average blastocyst score was calculated for each transferred blastocyst. Clinical pregnancy and pregnancy loss rates were measured. Odds ratio and logistic regression analysis were used for statistical analysis.

Main results and the role of chance: The average transfer number of BL was 1.1±0.3, with an average blastocyst score of 7.4±2.6. Overall, the clinical pregnancy rate was 56.6% (1,241/2,193), with a pregnancy loss rate of 22.5% (340/1,512). Logistic regression analysis showed that the average blastocyst score was the strongest predictor of clinical pregnancy (p<0.00000), followed by the number of BL transferred (p=0.00011). The method of fertilization (ICSI/IVF), oocyte age and source (donor/autologous; fresh/frozen), sperm source (ejaculate/donor/surgical), transfer day, and type of FET cycle (programmed/natural) were not correlated with pregnancy. Interestingly, for pregnancy loss, the FET transfer protocol was the strongest predictor (programmed/natural OR=5.22; 95%CI 4.01-6.83), followed by the average blastocyst score (Unit OR=1.17; 95% CI 1.11-1.24).

Limitations, reasons for caution: The study is limited by its retrospective nature.

Wider implications of the findings: The blastocyst score is a predictor of euploid blastocyst pregnancy outcome. It can serve as a simple ranking tool to improve embryo selection by quantitatively assessing blastocyst quality. It also has potential applications in the personalization of ART treatment, particularly in the new era of artificial intelligence.

Trial registration number: None

P-175 Ideal strategy of trophectoderm biopsy for preimplantation genetic testing for aneuploidies (PGT-A) cycle.

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Study question: Is the timing of trophectoderm biopsy associated with successful pregnancy after the embryo transfer of blastocyst subjected to preimplantation genetic testing for aneuploidies (PGT-A)?
Summary answer: Trophectoderm biopsy(PGT-A) before or after the cryopreservation may be the optimal strategy for better clinical outcomes compared to the fresh trophectoderm biopsy without cryopreservation.
What is known already: Embryo biopsy and fresh embryo transfer are traditionally performed in the PGT-A cycle. However, before embryo transfer, the time allowed for genetic analysis of the specimens is restricted, particularly after blastocyst biopsy. Cryopreservation of blastocysts after biopsy instead of fresh transfer permits more sufficient time for performance of molecular diagnosis. The effect of cryopreservation and warming procedures on clinical outcomes in PGT-A cycle has not been effectively studied.
Study design, size, duration: This retrospective study included women that underwent IVF with PGT-A from January 2016 to October2018. 2098 blastocyst from cycles were subjected to trophectoderm biopsy for performing array comparative genomic hybridization (CGH) test. Embryos were cultured to expanded blastocyst stage and underwent trophectoderm biopsy on day 5 to day 6 of embryo development. Cycles with complete PGT-A diagnosis were 349, 178 of which had embryo transfer. The performance of different groups of PGT-A patients was evaluated.
Participants/materials, setting, methods: The groups were divided into three; first group (n=80 transfer/310 cases) contained fresh blastocysts that biopsied for PGT-A without cryopreservation followed by embryo transfer. In the second group (n= 175 transfer/ 176 cases),the blastocysts were initially biopsied then proceeded with vitrification and warming before the embryo transfer. The last group (n=94 transfer/126 cases), the cryopreserved blastocysts were warmed and biopsied prior to ET.
Main results and the role of chance: The total pregnancy and implantation rates of fresh blastocyst biopsied group was 43.8% (35/80), 37.9%(39/103); the cryopreserved-warmed-biopsied blastocyst group showed 53.7% (94/175), 47.3% (107/226); and finally biopsied and cryopreserved-warmed group showed 52.1% (49/94), 46.5% (59/127); respectively. Second group and third group are significant higher pregnancy rate than first group, (p=0.022). Also, The second(biopsied and cryopreserved-warmed) group and third (cryopreserved-warmed–biopsied)group showed higher numerical implantation rate than the first group.
Limitations, reasons for caution: In the case of biopsy after warming, it is difficult to determine the number of warming embryos and re-cryopreservation of surplus euploid embryo could increase embryo damage.
Wider implications of the findings: Using recently available data, when faced with the option of fresh embryos, before or after trophectoderm biopsy for PGT-A, our result supported performing the biopsy before or after embryo cryopreservation and warming.
Trial registration number: Not applicable.

P-176 Finding an optimal algorithm for predicting human embryo development using deep learning and high-resolution time-lapse cinematography (hR-TLC)

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Study question: What kind of algorithm is most suitable for predicting human embryo development using deep learning?
Summary answer: Analyzing hR-TLC of human embryos by deep learning with an algorithm using time-series images achieved 86% prediction accuracy, a 12% improvement versus using individual images.
What is known already: Predicting the development of good-quality embryos is essential for human assisted reproductive technologies such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), therefore analysis of the dynamic morphology of embryos using time-lapse imaging has become widespread. In particular, deep learning has been one of the major methods of analysis, but the optimal algorithm for such use of deep learning is still unclear.
Study design, size, duration: From April 2003 to November 2008, 100 normally fertilized embryos from conventional IVF and ICSI were cultured at 37°C at pH 7.35 ± 0.02. For both types of embryos, 40 hours of images of the embryos in culture were taken, starting from the time of second polar body extrusion, by hR-TLC using an inverted microscope with differential interference contrast. Approximately 2,000 photographs were taken every 2 minutes, and these were used for deep learning.
Participants/materials, setting, methods: Embryo quality was evaluated at the four-cell stage using modified Veeck's criteria. 100 embryos (50 good quality, 50 poor quality) were used to verify prediction accuracy (45 for training and 5 for validation per quality group). Two types of deep learning prediction algorithms were applied: a non-time-series model (convolutional neural network [CNN]), and a time-series model (long short-term memory [LSTM]). Cross-validation was repeated 10 times and the average rate of prediction accuracy was calculated.
Main results and the role of chance: First, when time-lapse images for 31 hours from second polar body extrusion were applied, the average rate of prediction accuracy was 72% for the CNN model (mean sensitivity, 0.72; mean specificity, 0.72) and 68% for the LSTM model (mean sensitivity, 0.78; mean specificity, 0.58). Second, when time-lapse images for 25 hours from pronuclear formation were applied, the average rate of prediction accuracy was 71% for the CNN model (mean sensitivity, 0.74; mean specificity, 0.68) and 72% for the LSTM model (mean sensitivity, 0.71; mean specificity, 0.74). Finally, when images for 5 hours from pronuclear disappearance (syngamy) were applied, the average rate of prediction accuracy was 77% for the CNN model (mean sensitivity, 0.76; mean specificity, 0.78) and 86% for the LSTM model (mean sensitivity, 0.84; mean specificity, 0.86). Therefore, the algorithm method using the LSTM model applied to the time-lapse images for 5 hours after syngamy was the most suitable for predicting embryo quality by deep learning, and increased prediction accuracy by approximately 12% when compared with the method using the CNN model.
Limitations, reasons for caution: The accuracy of our findings is limited because of the small number of embryos and images that we used. The optimal time and period used to predict human embryo development by deep learning analysis might be different if the numbers of embryos and the amount of training were increased.
Wider implications of the findings: The accuracy of prediction of good-quality embryos could be improved by updating deep learning and increasing the number of embryos applied to the LSTM model. Furthermore, in the future it may be possible to predict embryo quality at early embryonic stages before syngamy.
Trial registration number: Not applicable.

P-177 Comparison of clinical outcomes between day 5 early-middle expanding blastocysts (E-MB) versus day 6 fully expanded-hatched blastocysts (F-HB) transfer in vitrified-warmed cycles

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Study question: The aim of this study is to compare clinical outcomes between day 5 E-MB and day 6 F-HB transfer in vitrified-warmed cycles.
Summary answer: Day 5 E-MB transfer showed a significant increase in the rates of biochemical pregnancy, clinical pregnancy, and implantation compared with those of day 6 F-HB.
What is known already: Improved embryo culture system now makes more feasible the possibility of obtaining blastocysts after in-vitro fertilization. It is

known that fully expanded blastocysts represent a higher pregnancy rate than early blastocysts on day 5. Therefore, it is recommended that day 5 early blastocysts are additionally cultured as a strategy to increase pregnancy rates. However, little is known whether day 6 F-HB is also superior to day 5 E-MB in vitrified-warmed cycles.

Study design, size, duration: A retrospective cohort study of 308 cycles was performed from January 2014 to march 2017. The cycles with advanced maternal age (≥40 years) and poor quality blastocysts (grade CC) were excluded. We compared the rates of biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and implantation between day 5 E-MB and day 6 F-HB transfer in vitrified-warmed cycles

Participants/materials, setting, methods: The quality of vitrified blastocysts was evaluated by Gardner and Schoolcraft's classification. The cycles were divided into two groups based on the expansion grade of the blastocysts on day 5 and day 6 (Day 5 E-MB: expansion grades 2 and 3, n = 123 vs. Day 6 F-HB: expansion grades 4, 5 and 6, n = 185).

Main results and the role of chance: Characteristics of the cycles had no statistical differences in maternal age at ovum pick-up, maternal age at blastocysts transferred, infertility diagnosis, and number of blastocysts transferred.

Rates of biochemical pregnancy, clinical pregnancy, and implantation in day 5 E-MB were significantly increased than those of day 6 F-HB transfer (Table 1). In addition, ongoing pregnancy rate was showed that day 5 E-MB tends to be higher than day 6 F-HB transfer (Table 1).

Table 1 Comparison of clinical outcomes between day 5 E-MB and day 6 F-HB

	Day 5 E-MB	Day 6 F-HB	p-value
Cycles (n)	123	185	
Biochemical pregnancy (%)	43.1 (53/123)	31.9 (59/185)	0.045
Clinical pregnancy (%)	33.3 (41/123)	22.7 (42/185)	0.039
Ongoing pregnancy (%)	25.2 (31/123)	17.3 (32/185)	0.092
Implantation (%)	38.2 (47/123)	24.3 (45/185)	0.009

Limitations, reasons for caution: This study has a limitation. Cycles were divided into only two groups, because of its small sample size. Therefore, further study with larger sample size is needed to confirm pregnancy rates according to the degree of expansion of blastocysts (grades 1, 2, 3, 4, 5, and 6, respectively).

Wider implications of the findings: The day of vitrification rather than the degree of expansion is considered more important factor related to pregnancy rate in vitrified-warmed cycles. Therefore, we recommend vitrifying day 5 E-MB rather than additional culture to day 6 in vitrified-warmed cycles.

Trial registration number: Not applicable

P-178 To what extent does the survival rate of blastocysts following freeze and thaw affect the likelihood of pregnancy?

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Study question: To assess whether the blastocyst survival rate after freeze and thaw affects the likelihood of pregnancy

Summary answer: High blastocyst survival rate in a frozen embryo transfer (FET) cycle is positively correlated with an increased clinical pregnancy rate.

What is known already: The embryo freezing and thawing during IVF is well established process. Studies on embryos at the cleavage stage showed that the percentage of survival of the embryo after freeze and thaw process has an impact on a clinical pregnancy rate. However, to the best of our knowledge there is no published evidence in literature studying the impact of blastocyst survival rate on IVF outcome.

Study design, size, duration: Retrospective study conducted on over 800 embryo transfers performed in a Tertiary Assisted Conception Unit in 2018. Inclusion criteria were: top grade blastocyst (Gardner grading 3CC and above), single embryo transfer (SET), embryos surviving at least 50% and showing signs of re-expansion within 2 hours after thaw. All cycles from preimplantation genetic diagnosis, egg donation and egg freezing cycles were excluded.

Participants/materials, setting, methods: The total number of 356 frozen cycles was analysed. No patients had more than one cycle included. Female patient age ranged between 24 and 45 (mean age: 33.7+/-3.8) at the time of embryo freeze. Embryo survival rate was stratified into following groups: 50-59%, 60-69%, 70-79%, 80-89%, 90-99% and 100%. Clinical pregnancy rate (CPR) was then also adjusted to female age.

Main results and the role of chance: The result shows that there was a strong correlation between CPR and blastocyst survival rate. If the survival rate was under 80%, in spite of presence of re-expansion, the overall CPR was only 11%, whereas CPR was as high as 47% with embryos showing survival of 80% and above. These results were consistent when the data was adjusted by female age at freeze. The sample size is relatively large, however the proportion of embryos with survival below 80% was smaller.

Limitations, reasons for caution: The study was retrospective and some unadjusted variables could be a cause of bias. However, to the best of our knowledge, this is the biggest data set reported until today on this topic.

Wider implications of the findings: This data does suggest that survival of the blastocyst embryo under 80% results in significantly lower chance of pregnancy. This information should be discussed with the patient and option of double embryo transfer could be considered, when clinically appropriate

Trial registration number: not applicable

P-179 Application of PLCZ for patients with repeated unfertilization after ICSI.

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Study question: How to decrease the unfertilization rate of ICSI oocytes?

Summary answer: PLCZ injection seems to rescue the ICSI oocytes lacking activation potency and is beneficial for improving clinical outcome of fertilization failure.

What is known already: Most fertilization failures can be resolved by ICSI, however following ICSI fertilization failure still occurs in 20-30%. Fertilization failure in ICSI results mainly from oocyte activation deficiency. Assisted oocyte activation (AOA) can induce transient Ca2+ increase in the oocyte. AOA is an efficient procedure for infertile couples with a history of low or failed fertilization after ICSI treatment. Various AOA have been reported including mechanical, electrical and chemical activation. The most popular assisted activation agents in human eggs include electrical pulses and calcium ionophores, however there is still not an established AOA.

Study design, size, duration: We performed this retrospective study to investigate the usefulness of PLCZ injection for fertilization failure patients from 2014 December to 2016 August. We conducted the study to know the optimal PLCZ concentration incubation period from oocyte pickup to PLCZ injection (1) and cleavage rate among the PLCZ, IM, ES in parthenogenesis and ICSI (2).

Participants/materials, setting, methods: 1) To find a optimal PLCZ1 mRNA concentrations, parthenogenetic activation was examined with cleavage rate or monitoring of intra-cellular calcium oscillation.

2) 67 oocytes from 29 patients were used to compare the parthenogenetic cleavage between PLCZ1 and electrical stimulation (ES) or Ionomycin (IM). The three methods were applied to the ICSI oocytes from 38 patients (< 35 years old) who had repeated severe fertilization failure (less than 10%) more than two times.

Main results and the role of chance: 1) Parthenogenetic activation rate was very low (0-23%) in the concentrations of 0.01 to 10 ng/μl. At the optimal concentration of 100 ng/μl, the activation rate was raised to 66.7% (n=18) and the pattern of Ca2+ oscillations by PLCZ injection was similar to that of normal ICSI.

2) In parthenogenesis, no significant difference in PN formation was found among the three methods. However, cleavage rate was the highest in the PLCZ1 group (Table 1).

3) PLCZ was more effective on the development of male pronucleus in the ICSI oocytes than other methods (Table 2), suggesting that appropriate Ca2+ oscillation is required for normal fertilization. The pregnancy and miscarriage rates did not differ among the PLCZ1, IM and ES (25.0% and 0%, 6.7% and 100%, 18.2% and 50.0% respectively) because of the small sample size.

Table 1 Comparison of clinical outcomes between day 5 E-MB and day 6 F-HB

Parthenogenesis	No. of oocytes	Day 1 (1PN+2nd PB)	Day3 (>= 7 cell)
PLCZ1	35	65.7% (23)	60.1% (14/23)
IM	15	66.7% (10)	40.0% (4/10)
ES	17	52.9% (9)	44.4% (4/9)

Table 2 Comparison of clinical outcomes between day 5 E-MB and day 6 F-HB

ICSI	No. of oocytes	Day 1 (2PN+2nd PB)	Day3 (>= 7 cell)
PLCZ1	87	60.9% (53)	60.9% (53)
IM	78	29.5% (23)	24.4% (19)
ES	56	30.4% (17)	26.8% (15)

Limitations, reasons for caution: Birth of healthy offspring and reproduction of healthy second generation from mouse oocytes activated by PLCZ was reported in 2008. However, human births have not been reported after using this method. The safety of PLCZ injection to human health should be further examined for future clinical applications.

Wider implications of the findings: Current poor clinical outcome of ROSI is likely to de derived from insufficient oocyte activation. Application of PLCZ to oocyte is a more physiological activation than that electrical oocyte stimulation and it will be beneficial to improve the clinical outcome of ROSI.

Trial registration number: UMIN Clinical Trials Registry: UMIN000020860

P-180 The effect of high humidity culture conditions over embryo development: a continuous embryo monitoring assessment

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Study question: Since in vivo culture, culture conditions are humid. Our aim was to know how high humidity might affect embryo development comparing to dry conditions by using single step culture media in a continiuous embryo monitoring incubator (CEM)

Summary answer: Our results suggested that culture conditions with high humidity atmosphere did not affect embryo development but increase the pregnancy and implantation rate

What is known already: Oil overlay has supported successful use of a dry incubator for culture human embryos, preventing changes in the pH, temperature. However, dry conditions may affect the osmolality due to the evaporation of culture media. Therefore, the use of humid conditions avoid osmolality changes. In a previous study we found statistical differences in terms of blastocyst rate. Pregnancy and implantation rate was affected but remained not significantly.

Study design, size, duration: A total of 7544 embryos from 1043 patients, from ovum donation programme and own oocytes and that were culture on a time lapse incubator system (Geri genea biomedix, Australia) were included in a retrospective and multicentric study (three IVF units and 5 CEM incubators) from 2016 to 2018

Participants/materials, setting, methods: This CEM incubator has 6 separated small incubators. Three of them works in a dry atmosphere (DC) and the other 3 in humid conditions (HC). In the dry chambers, the embryos from 478 patients were cultured and under HC a total of 558. Retrospectively, blastocyst, good morphology blastocyst rate, pregnancy, implantation and miscarriage rate were evaluated.

Main results and the role of chance: In a previous study we found that culture conditions with high humidity atmosphere promoted embryo development and reproductive outcome. This time, with increased sample size, we didn´t found any differences in embryo development. By performing an stratified analysis, humid conditions were equaly distributed by clinic and treatment (mulivariable analisis). We had very similar blastocyst rate when the embryos were culture under high HC; 71.3% vs 71.0% DC). Also the proportion of blastocyst with good morphology was very similar 38.1 % in HC vs 37.7 % and DC. The ongoing pregnancy rate (OPR) was higher in HC vs DC (52.5 % vs 47.7% respectively), additionally the implantation rate slightly better in HC 54.85 vs 52.7 but but remained not significant.

Limitations, reasons for caution: The retrospective nature of the study may limit the conclusion although sample size is remarcable. A prospective reandomize study may solve the remaining questions surrounded this topic.

Wider implications of the findings: Our results would suggest that HC may increase the reproductive outcome of our patients when using single step media in CEM incubators although effects are limited and still need to be confirmed with a larger sample and improved designs.

Trial registration number: NA

P-181 Day of vitrification, blastocyst developmental stage and trophectoderm quality are strongly associated with survival and implantation in vitrified blastocyst cycles: analysis of 12064 warmed blastocysts

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Study question: Which fresh-embryo blastocysts parameters are related to survival and implantation rates following vitrification and warming?

Summary answer: Day of vitrification, developmental stage and trophectoderm (TE) quality are parameters directly related to survival and implantation of warmed blastocysts.

What is known already: Due to the relatively recent but exponential increase in blastocysts cryo-transfers, a clearer guide of the factors affecting the outcome is needed. Blastocyst morphology is associated with clinical outcomes in fresh and vitrified cycles. Additionally, fresh blastocysts developed on day 5 of culture give rise to higher pregnancy rates than embryos reaching the blastocyst stage on day 6. However, it is not clear if delayed blastulation and embryo morphological quality can impact the final outcome in vitrified cycles. This study aimed to evaluate the effect of the fresh embryo parameters on survival and implantation in cryo-transfers of vitrified/warmed blastocysts.

Study design, size, duration: This was a multicentric retrospective study including 12064 vitrified-warmed blastocysts transferred from January 2017 to December 2018. No PGT-A cycles were included.

Participants/materials, setting, methods: Blastocysts were allocated to different categories according to: i) day of vitrification (5 and 6); ii) blastocyst expansion degree: cavitated (BC), fully expanded (BE) and hatching out of zona (BHi); iii) TE quality (A, B, and C); and iv) oocyte origin (donor and autologous). Survival and clinical outcomes were compared between groups using chi-square test and 95% confident intervals were calculated.

Main results and the role of chance: General survival rate was significantly lower in blastocysts vitrified on **day 6 *vs.* day 5**: 90.3% (95%CI: 94.9 – 95.8) *vs.* 95.4% (95%CI: 89.1 – 91.5). After analyzing in the different morphological categories, these differences were maintained in the following groups: BE B, 92.5% (95%CI: 90.6 – 94.4) *vs.* 95.3% (95%CI: 94.6 – 95.9); BE C, 87.5% (95%CI: 84.7 – 90.3) *vs.* 94.0% (95%CI: 92.4 – 95.6) and BHi B, 90.2% (95%CI: 87.5 – 92.9) *vs.* 95.2% (95%CI: 94.1 – 96.3). In the same way, implantation rate was significantly lower in blastocysts vitrified on day 6 *vs.* day 5 both in donor oocyte cycles: 31.9% (95%CI: 29.1 – 34.7) *vs.* 47.2% (95%CI: 45.8 – 48.6) and in autologous oocyte cycles: 26.2% (95%CI: 23.1 – 29.2) *vs.* 49.7% (95%CI: 47.9 – 51.5). A greater degree of expansion decreases the survival rate: BC survival was significantly higher than that of BE and BHi (when TE was B or C). Although, implantation was lower for BC compared to BE and BHi (when TE was B or C). Regarding **TE quality,** both survival and implantation were significantly decreased in blastocysts with TE catalogued as C. Finally, the **oocyte origin** had no impact on survival.

Limitations, reasons for caution: The retrospective nature of this study and the subjectivity of the morphological evaluation may be a limitation, although the magnitude of the sample size may counteract this limitation.

Wider implications of the findings: Survival and implantation were impaired in delayed embryos. Warming day-5 should be prioritized. Furthermore, the degree of expansion when vitrifying is closely related to success: BC embryos show higher survival but lower implantation rates and should be cultured after warming to allow them to expand prior to the embryo transfer.

Trial registration number: Not applicable

P-182 The higher the number of times follicular flushing is repeated, the lower the probability of oocyte maturation, without an effect on fertilization and embryo cleavage.

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Study question: Is the probability of oocyte maturation, fertilization and cleavage associated with the number of times follicular flushing is repeated during oocyte retrieval?

Summary answer: The higher the number of times follicular flushing is repeated, the lower the probability of oocyte maturation, without an effect on fertilization and embryo cleavage.

What is known already: Although follicular flushing has not been shown to increase oocyte retrieval rate compared to no flushing, it is still a procedure performed in many IVF centers. Currently, it is not clear whether there is an association between the number of times follicular flushing is repeated per follicle and the probability of oocyte maturation, fertilization and cleavage.

Study design, size, duration: A retrospective observational study was conducted from November 2017 until December 2018. Ninety women undergoing ovarian stimulation with GnRH antagonists and gonadotrophins for intracytoplasmic sperm injection (ICSI) were recruited and contributed 563 oocytes. Oocyte donation or PGD cases were excluded.

Participants/materials, setting, methods: Oocyte retrieval was performed 36hrs after hCG by using double-lumen needle. Follicular flushing was performed up to 5 times when no oocyte was retrieved in the initial aspirate. Oocytes were categorized into two groups depending on the number of times follicular flushing was repeated (Group A: 1-2 times, Group B: 3-5 times). Values are expressed as mean (SD) or median (95% CI) depending on their distribution. Due to multiple comparisons, p-level was set at 0.01.

Main results and the role of chance: The number of COCs retrieved per patient without flushing, in Group A and in Group B was 2 (95%CI: 2-3), 3 (95%CI: 2-4) and 1 (95%CI: 1-2), respectively. Overall 4 COCs (95%CI: 3-6) were retrieved.

The number of MII-oocytes retrieved per patient without flushing, in Group A and Group B was 2 (95%CI:2-2.6),2 (95%CI:2-3.3) and 1 (95%CI:0.3-1), respectively. Overall 3 (95%CI:2-5) MII-oocytes were retrieved.

Maturation rate of oocytes retrieved per patient without flushing, in Group A and Group B was 100% (95%CI:100-100), 100% (95%CI:82.9-100) and 58.3 (95%CI:7.3-100), respectively. Overall maturation rate was 96.5% (95%CI:85.7-100.0).

Generalized linear model (GLM) showed no difference in the probability of an oocyte being mature comparing no flushing vs group A (p=0.068). However, a significant decrease in the probability of an oocyte being mature was present comparing no flushing with group B(p=0.001),and groups A and B (p=0.007).

Moreover, GLM showed no difference in the probability of fertilization comparing no flushing vs group A(p=0.88) or group B(p=0.55), as well as group A vs group B(p=0.60),and no difference in the probability of cleavage comparing no flushing vs group A (p=0.20) or group B (p=0.32), as well as group A vs group B (p=0.03).

Limitations, reasons for caution: This is a retrospective observational study with oocytes retrieved after repeated follicular flushing and thus the further outcome of embryos blastulation or implantation and pregnancy could not be reliably examined.

Wider implications of the findings: Although oocyte maturation rate is significantly lower when follicular flushing is repeated 3-5 times as compared to 1-2, mature oocytes can still be retrieved with similar probabilities of fertilization and cleavage. Nevertheless, the clinical significance of repeated flushing for the achievement of pregnancy needs to be further evaluated.

Trial registration number: not applicable

P-183 Rescue in vitro maturation of oocytes shows potential to improve IVF outcome for patients under 39 years of age

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Study question: Can IVM-Rescue improve IVF outcome for patients with more than 50% of immature oocytes obtained at retrieval?

Summary answer: The IVM-R can help increase the number of blastocysts available for transfer only for patients under 39 years old. For elder there were no advantages.

What is known already: Rescue in vitro maturation (IVM-R) is currently not a routine procedure in IVF treatment. For patients with low ovarian reserve or advanced maternal age the oocyte retrieval a large often results in a large portion of immature ones (MI and GV stage) which are routinely discarded. Most oocytes released from the follicular environment are able to undergo meiotic maturation to metaphase II spontaneously when placed in a suitable culture medium. They can provide an additional pool of cells to increase the chances of obtaining transferable embryos.

Study design, size, duration: It was a retrospective study that included data for 88 patients (median age 36 years, range 28-46) with diagnosis of infertility who were undergoing IVF treatment between January 2018 and December 2018 at INVICTA Fertility Centre, Poland with at least 50% of immature oocytes obtained at retrieval.

Participants/materials, setting, methods: Oocytes were obtained from patients with standard pick-up procedure. Immature oocytes were divided into two groups: MI (168) and GV (215) and cultured 24-48 h in standard IVF medium. When they reached MII stadium they were fertilized by ICSI. Presence of 2 pronuclei was estimated 18 hour after ICSI procedure. Blastocysts were vitrified on day 5 or 6 day after they become MII.

Main results and the role of chance: The study group included 60 patients under 39 years old and 28 who were 39 or older. There were 145 MI and 155 GV oocytes in the younger group and 23 MI and 56 GV in the older group. In the younger group 104 MI (71.1%) and 67 GV (43.2%) successfully matured into MII and 17 MI (73.9%) and 22 GV (39.3%) in the older group. Blastocysts were obtained only in the younger group.

Considering patients under 39 in the MI subgroup there were 14 blastocysts (22.9% of fertilized and 13.5% of matured oocytes) and in the GV subgroup there were 4 blastocysts (21.1% of fertilized and 6.0% of matured oocytes).

It is also worth noting that 7 patients who obtained blastocyst from IVM-R had no blastocysts from matured oocytes from the same cycle (including 3 who had only immature oocytes retrieved). In 2 cases patients only obtained blastocysts from GV oocytes.

Additionally 6 blastocysts from matured oocytes were transferred and in 2 cases resulted in an ongoing pregnancy.

Limitations, reasons for caution: The study is limited by sample size. A higher sample size could be used in future studies to corroborate the current findings.

Wider implications of the findings: IVM-R seems promising for patients under 39 and shows potential of significantly affecting results of their treatment by increasing the number of blastocysts. Further studies with larger study groups are required to confirm the results and determine if IVM-R could be included as part of the standard treatment process.

Trial registration number: not applicable

P-184 The correlation between re-compaction post de-compaction (RCPDC) of embryos and implantation (IR) and live birth rate (LBR) in fresh embryo transfers.

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Study question: Does the occurrence of RCPDC influence clinical outcomes?

Summary answer: Implantation and live birth rate, but not miscarriage rate (MR), significantly correlate with the exhibition of RCPDC of embryos observed at the morula stage.

What is known already: No previous studies have been conducted examining the possible association between RCPDC of human embryos and clinical outcome. Extracellular calcium is known to be necessary during embryonic development for its role in cell-cell adhesion, whereas intracellular free calcium ($[Ca^{2+}]i$) has been shown to be essential for embryos undergoing compaction. However, causative factors for RCPDC at the morula stage are currently unknown. A study conducted in mouse embryos reported that during experimental decompaction, $[Ca^{2+}]i$ concentration increases and simultaneously cytoskeleton-related proteins E-cadherin, fodrin, and calmodulin relocate. The study suggested $[Ca^{2+}]i$ acts on the cytoskeleton achieving maintenance of the compacted status.

Study design, size, duration: This is a retrospective study conducted between June 2011 and September 2017. Transferred Day 4/5 embryos of known implantation outcome were included, all fresh treatments, cultured in EmbryoScope® (Vitrolife). There were 2474 embryo transfers (ETs): 103 RCPDC ETs (97 single-, 6 double-ETs), and 2371 non-RCPDC ETs (1244 single-, 1088 double-, 39 triple-ETs). Embryos were divided according to compaction degree: fully compacted (M1), and partially compacted (M2). IR, MR, LBR was compared between groups (RCPDC/non-RCPDC, M1/M2).

Participants/materials, setting, methods: Patient treatment included conventional insemination or intracytoplasmic sperm injection. No statistical significance was found in demographics between the RCPDC and non-RCPDC groups (mean female age of oocyte provider 34.5±5.8 vs. 35.0±3.9 years, p=0.1532, and mean number of oocytes 10.6±4.1 vs. 10.3±4.6, p=0.8005).Environmental culture conditions were consistent throughout for RCPDC and non-RCPDC groups, whereas culture media heterogeneity was reflected in both groups. Statistical analyses were conducted using Fisher's exact test (two-tailed) and t-test.

Main results and the role of chance: IR was significantly increased in the RCPDC group compared with the non-RCPDC group, regardless of the compaction degree at morula (67/109 (61.4%) vs. 1365/3537 (38.6%); p=0.0154). Odds ratio was 2.54, 95% CI: 1.7158-3.7553, p<0.0001. The LBR was also significantly higher in the RCPDC group than the non-RCPDC group (48/109 (44.0%) vs. 958/3537 (27.1%); p=0.0261. Odds ratio was calculated to 2.12, 95% CI: 1.4409-3.1143, p=0.0001. No statistically significant difference (p=0.7807) for MR was found between RCPDC (31.7%), non-RCPDC (30.4%) cases. Moreover, no statistical difference was found between M1 embryos of RCPDC and non-RCPDC groups for IR (63.4% vs. 46.7%, p=0.2320), LBR (45.1% vs. 35.8%, p=0.5511), and MR (27.1% vs. 23.3%, p=0.5959). Furthermore, no statistical difference was found between M2 embryos for IR (55.6% vs. 32.4%, p=0.2385), LBR (40.8% vs. 20.5%, p=0.1220) and MR (36.6% vs. 38.2%, p=0.4298) for RCPDC and non-RCPDC groups. The lack of statistical significance is likely due to the small number of cases for each category.

Limitations, reasons for caution: The main limitation of the study is the small sample size of the RCPDC transferred embryos. Additionally, the study is limited to its retrospective design and the heterogeneity of culture conditions.

Wider implications of the findings: Our data shows an association of embryos exhibiting RCPDC to implantation potential and live birth, without a higher miscarriage rate. The identification of a positively correlated time-lapse marker to clinical outcome could be possibly routinely introduced in practice and utilised upon considering embryo selection for fresh transfers.

Trial registration number: Not applicable.

P-185 Dynamics of embryo multinucleation as an early predictor of euploid status following PGT-A -morphokinetic analysis

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Study question: Do embryos with multinucleated cells (MNC) have a self-correction mechanism that possibly results in euploid embryos?

Summary answer: Multinucleated embryos are prone to chromosomal aberrations. This feature self-corrects before 4-cell stage resulting in higher euploidy rates, when compared to embryos showing persistent MNC.

What is known already: Blastomere multinucleation is commonly observed in early embryo development. This characteristic seen in embryos have been associated with diminished embryo potential, reduced blastocyst development, significantly lower implantation and higher miscarriage rate. The presence of multinucleation in early human embryo development during the first and second mitotic division are generally considered as abnormal. Some studies using FISH and arrayCGH have shown that 50-100% of MN embryos are aneuploid, complex aneuploid or mosaic. However, resent studies using advanced chromosomal screening have shown that re-analysing on D5 the D3 embryos considered as abnormal due to MNC observed reduction in aneuploidy in these embryos.

Study design, size, duration: A retrospective morphokinetical analysis of 166 known PGT-A (NGS) blastocysts was performed. Presence of MNC was annotated from the 2-cell to morula stage. Analysis consisted of comparing the stages of appearance of multinucleation, number of cells with MN and the stage at which MN disappears in conjunction with chromosomal outcome of the MN blastocysts. The mean female age was 38.51 ± 3.8(SD 3.8) years

Participants/materials, setting, methods: Patients undergoing PGT-A cycles due to advanced maternal age, previous miscarriage, recurrent failed attempts or previous aneuploid pregnancy in a single IVF centre. IVF Inseminated or Intracytoplasmic sperm injected oocytes were cultured in the Time Lapse monitoring incubator. Embryos were annotated from D1 until D5/6 of development. Only embryos reaching the blastocysts stage were biopsied for PGT-A screening. Data were analysed by chi-square statistic using SPSS v23 statistics software

Main results and the role of chance: Analysis was performed based on 166 blastocysts; of which 106 (63.4%) displayed MNC during their early development and 60 (36.6%) embryos did not present this feature. Of the embryos presenting MNC: 25/106 (23.58%) were euploid, 78/106 (73.5%) aneuploid and 3% did not produce a conclusive result. Blastocysts with no MNC: 24/60 (40%) euploid, 34/60 (56.6%) aneuploid and 3.4% no result. Comparison of both groups highlighted the correlation between MNC and ploidy status (p 0.0249). Furthermore, euploidy was analysed considering the disappearance stage of MNC. Euploidy rates were the highest when MNC self-corrected at the 2-4 cell stage, progressively decreasing the longer MNC are observed falling to nearly 0% when MNC persisted past the 10-cell stage. Hence, the later the embryo becomes mononucleated, the higher the odds for it to be chromosomally abnormal (OR 8.54 (CI 5.6-12.7), p<0.0001).

Limitations, reasons for caution: The sample size was small involing a few number of biopsied blastocysts. Larger studies are required for the morphokinetic analysis.
Wider implications of the findings: Multinucleation,embryos have been shown to be chromosomally normal using NGS. Perhaps, the disappearance of these MNB at an early stage could be an indication of cellular repair mechanism or sperm and eggs spindle packaged separately which should converge during the next mitotic division. These embryo/s should be considered for Transfer.
Trial registration number: N/A

P-186 A novel predictive model to estimate the number of oocytes required for obtaining at least one euploid blastocyst for transfer: The ART Calculator

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Study question: Is it possible to develop a predictive model to estimate the number of oocytes required to obtain at least one euploid blastocyst for transfer in infertile couples undergoing IVF/ICSI?
Summary answer: Our novel predictive model estimates the minimum number of mature oocytes needed to achieve at least one euploid blastocyst for transfer.
What is known already: The POSEIDON group (**P**atient-**O**riented **S**trategies **E**ncompassing **I**ndividualize**D** **O**ocyte **N**umber) has introduced 'the ability to retrieve the number of oocytes needed to achieve at least one euploid embryo for transfer' as an intermediate marker of successful outcome in IVF/ICSI cycles. A clinical predictive model to estimate the POSEIDON's marker -and that provides a revised estimate of the probability of achieving this outcome when fewer than the predicted number of oocytes are obtained after one or more oocyte retrieval cycles- would be invaluable for both patient counseling and establishment of a working plan with a clear goal for management.
Study design, size, duration: We analyzed clinical and embryonic data of 347 infertile couples who underwent IVF/ICSI with the intention to have trophectoderm biopsy for preimplantation genetic testing for aneuploidy (PGT-A) from February 2016 to June 2017. A total of 2,520 mature oocytes were injected, resulting in 882 blastocysts that were subjected to PGT-A. PGT-A was mainly used for reasons of advanced maternal age, repeated implantation failure, and for patients concerned about the euploidy status of their embryos.
Participants/materials, setting, methods: Participants were consecutive couples undergoing their first treatment cycle. We only included patients with a complete IVF/ICSI record. Included patients had at least one metaphase II oocyte (MII) retrieved. The oocytes were inseminated for own use and all resulting viable blastocysts were biopsied and analyzed by next-generation sequencing (NGS). We used the negative binomial distribution to model the number of euploid blastocysts and adaptive LASSO (Least Absolute Shrinkage and Selection Operator) method for variable selection.
Main results and the role of chance: The fitted model selected female age, sperm source, and number of mature (metaphase II) oocytes as predictors ($p<0.0001$). Female age was the most important factor for predicting the probability of a blastocyst being euploid given each mature oocyte (loglikelihood of age [adjusted for sperm source]: 30.9; df=2; $p<0.0001$). The final model was developed using logistic regression analysis and internally validated by the holdout method. Its predictive ability assessed by the area under the ROC curve was 0.715. Using the final model and mathematical equations, we calculated the individualized probability of blastocyst euploidy per mature retrieved oocyte and the minimum number of mature oocytes required to obtain at least one euploid blastocyst —with their 95% confidence interval [CI]— for different probabilities of success. The predicted probabilities of a mature oocyte turn into a euploid blastocyst decreased progressively with female age and was negatively modulated overall by use of testicular sperm from men with non-obstructive azoospermia across age ($p<0.001$). A calculator was developed to make two types of predictions automatically, namely (1) the minimum number of mature oocytes for at least one euploid blastocyst, and (2) the chances of having a euploid blastocyst based on the actual number of mature oocytes collected/accumulated.
Limitations, reasons for caution: There is a need to validate its prediction ability externally to confirm generalizability. Our estimations cannot be generalized to patients undergoing cleavage-stage embryo transfer as the study was based on blastocyst biopsies and NGS analysis. The model should be used with caution to decide whether a patient should undergo treatment.
Wider implications of the findings: This tool can help healthcare providers to counsel infertility patients about the individualized oocyte number needed to optimize the chances of having a euploid blastocyst for transfer, thus shaping patients' expectations. Also, it may guide clinicians on a risk-shared decision about ART treatment options aimed at achieving the oocyte number.
Trial registration number: Not applicable

P-187 A comparison of Primovision and EmbryoScope+ time-lapse imaging systems on laboratory performance indicators.

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Study question: Is there a difference in laboratory performance indicators between Primovision and EmbryoScope+ time-lapse systems?
Summary answer: Cleavage rate and embryos developmentally on target on d3 and d5 was significantly higher with EmbryoScope+, and rates of embryo transfer cancellation were significantly lower.
What is known already: Many IVF centres now employ time-lapse imaging systems to monitor embryo development and to help predict the most viable embryo for transfer. There are a number of time-lapse imaging systems which have been developed including closed systems such as EmbryoScope+, or Primovision which can be fitted into the laboratories existing incubators. Within our laboratory, Primovision is fitted within a Heraeus Cytoperm 2 incubator. It is thought that EmbryoScope+ is at least comparable to other standard incubation methods with regards to laboratory performance indicators.
Study design, size, duration: A prospective cohort study, collecting data on fresh treatment cycles utilising Primovision or EmbryoScope+ time-lapse systems from d1 to d6 of culture between July 2017 and Aug 2018. Each time-lapse system was used on alternating days. Cycles utilising donor oocytes were excluded. The Primovision group included 157 cycles and 1007 embryos, whereas the EmbryoScope+ group included 246 cycles and 1718 embryos. Pregnancies were followed up until 7 weeks gestation when an ultrasound scan was performed.
Participants/materials, setting, methods: Cycle data collected included: female age, AMH, endometrial thickness, cycle number, insemination method, number fertilised, cleavage rate, embryo development rate on d3 (defined as >6c) and d5 (defined as cavitating and blastocyst formation), good blastocyst development rate, embryo transfer cancellation rate (due to no suitable embryos available), freeze all rate, number of embryos transferred, utilisation rate, implantation rate (IR), clinical pregnancy rate (CPR), multiple pregnancy rate (MPR) and pre-clinical miscarriage rate.
Main results and the role of chance: Patient age (36.53 vs. 36.18, $P>0.05$), cycle number (2.1 vs. 2.06, $P>0.05$), AMH (17.95 vs. 17.90, $P>0.05$) and endometrial thickness (10.65mm vs. 10.68mm, $P>0.05$) were comparable for the EmbryoScope+ and Primovision groups respectively. The number of embryos (6.98 vs. 6.41, $P>0.05$), embryos transferred (1.59 vs. 1.64, $P>0.05$), and the proportion of IVF/ICSI/split cases (113/246, 131/246, 2/246 53.25/45.93/0.81% vs. 75/157, 81/157, 1/157 51.59/47.77/0.64%, $P>0.05$) also did not vary significantly between the EmbryoScope+ and Primovision groups, respectively.

The embryo transfer cancellation rate (17/157, 10.83% vs. 5/246, 2.03%, $P<0.01$) was significantly lower with EmbryoScope+, compared to Primovision respectively. The cleavage rate (987/1007, 98.01% vs. 1706/1718, 99.30%, $P<0.01$) and embryo development rate on day 3 (739/1007, 74.87% vs. 1347/1718, 78.96%, $P<0.05$) and day 5 (507/1007, 51.37% vs. 979/1718, 57.39%, $P<0.01$) was significantly higher with EmbryoScope+, compared to Primovision respectively.

No difference in good blastocyst development rate (326/1718, 18.98% vs. 166/1007, 16.48%), utilisation rate (687/1706, 40.27% vs. 369/987, 37.39%,

P>0.05), IR (118/357, 33.05% vs. 89/225, 39.56% P>0.05), MPR (17/89, 19.10% vs. 16/68, 23.53%, P>0.05), pre-clinical miscarriage rate (28/117, 23.93% vs. 19/87, 21.84%, P>0.05) or CPR/treatment cycle (excluding freeze-alls) (89/229, 38.86% vs. 68/154, 44.16%, P>0.05) was observed between the EmbryoScope+ and Primovision groups, respectively.

Limitations, reasons for caution: Live birth rates should be followed up when the data is available to see if there is a difference in the final outcome between the Primovision and EmbryoScope+ groups. Furthermore, large randomised controlled trials are needed to confirm the results of this study.

Wider implications of the findings: Our findings support other studies that suggest EmbryoScope+ is at least comparable to other standard incubation methods, and may promote improved laboratory performance indicators. This may be due to a more stable culture environment with the use of EmbryoScope+.

Trial registration number: Not applicable

P-188 An improvement in blastocyst quality using temperature variation treatments during in vitro embryo culture

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Study question: Does temperature variation treatments during *in vitro* culture improve blastocyst quality?

Summary answer: Temperature variation treatments improve blastocyst quality and enhance blastocyst rate.

What is known already: It is known that temperature, among other factors, can affect a variety of aspects in the oocyte and embryo function, particularly meiotic spindle stability and embryo metabolism. Basal body temperature (BBT) is the lowest temperature reached by the human body during rest. Even though BBT has been associated with natural family planning and timing of ovulation, the effects of BBT variation during day and night on embryo development is unknown. Moreover, studies have demonstrated a gradient of temperature in the female reproductive tract of several mammalian species.

Study design, size, duration: Fresh mouse zygotes (N=854, 6 repetitions) were cultured for 96 hours in continuous culture media incubated at 37°C in control group (C group), in Treatment 1 group (T1 group) at 37°C during the day and 35.5°C during the night and Treatment 2 group (T2 group) at 38.5°C during the day and 37°C during night, 90% humidity and 6% CO_2. Number of blastocysts and the assessment of embryo quality were analyzed on day 5 of development.

Participants/materials, setting, methods: Female mouse C57BL/6J were superovulated with PMSG and ECG. Males and females were placed to mate for natural fertilization. Zygotes were collected from the tubes and then cultivated (15 embryos/droplet) for 96 hours in continuous culture media and incubated under the 3 temperature variation treatments (Control, T1 and T2 group). Blastocyst rate (N=459) and the assessment of embryo quality, through Morphological Assessment (N=444) and TUNEL Test (N=181) were analyzed on day 5 of embryo development.

Main results and the role of chance: T2 group (38.5°C/37°C) presented a significant (p=0.0001) higher blastocyst rate (63.30%), when compared to T1 group (48.12%) and C group (49.14%). Additionally, considering the TUNEL test, T2 group presented a significant (p=0.0001) lower apoptotic rate (0.04+0.03) when compared to T1 group (0.10+0.08) and C group (0.06+0.04). Regarding the total number of cells of the blastocysts, which is the reference for the TUNEL analysis T2 group also presented a significant (p=0.0001) higher number of total cells (133.24+36.17) when compared to T1 group (83.42+37.37) and C group (116.61+41.93). Considering the Morphological Assessment (SART criteria), T2 group presented blastocysts with a significant (p=0.0001) higher grade (2.21+0.68) when compared to T1 group and a similar grade when compared to C group (2.28+0.70). In summary, blastocysts from T2 group showed better results in all assessments: blastocyst rate, TUNEL test and Morphological Criteria, indicating that with a temperature variation treatment of 38.5°C for 12 hours and 37°C for the next 12 hours not only more embryos reached the blastocyst stage but also, the blastocysts showed enhanced quality.

Limitations, reasons for caution: This study was only carried out in the mouse model, so further investigations are needed on embryos of larger mammalian species.

Wider implications of the findings: More studies are necessary to elucidate the effects of temperature variation treatments on embryo development *in vivo* and *in vitro,* which could lead to novel, safer methods of mammalian embryo culture *in vitro*.

Trial registration number: N/A

P-189 Trophoblast plugs: How do they stay together and why do they break up?

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Study question: How do the mechanical influences of blood flow impact trophoblast plugging and unplugging of the spiral arteries in early pregnancy?

Summary answer: High cell-cell interaction forces maintain trophoblasts in cohesive plugs, but any asymmetries/weak points produce high flow channels that lead to a cycle of plug disintegration.

What is known already: During early pregnancy trophoblasts colonise and remodel maternal spiral arteries, ensuring adequate delivery of oxygen-rich blood to the placenta later in pregnancy. During this process, trophoblasts form plugs that occlude spiral arteries, preventing maternal blood flow to the placenta until the late first trimester. Inadequacies in these processes are associated with pregnancy disorders. We have shown that trophoblast plugs play crucial roles in spiral artery remodelling by creating a low shear stress environment that promotes trophoblast migration (driven by chemotactic signals). However, our understanding of the cellular interactions involved in maintaining trophoblast plugs, and how/why they disintegrate/dislodge is almost non-existent.

Study design, size, duration: A 3D agent-based computational model of trophoblast migration within plugged spiral arteries was developed, and used to simulate trophoblast behaviour within plugs in response to fluid shear stress and chemotactic signals. Shear stress conditions were informed by our previously published model of haemodynamics in these vessels. In the model, blood flow acts in the opposite direction to chemotactic force, meaning that the flow acts to reduce the speed of trophoblast migration along the artery.

Participants/materials, setting, methods: The net force experienced by each cell/agent in the model was defined as the sum of F_{random} (cell movement in the absence of stimuli), F_{social} (interactions with other cells) and $F_{environment}$ (the force of environmental influences such as arterial walls, chemotaxis, and shear stress). Each force was parameterised using both stochastic data from *in vitro* imaging of trophoblast migration under fluid shear stress, and previous studies investigating mechanical interactions between cells in close proximity.

Main results and the role of chance: How do plugs stay together? When cell-cell interaction forces are high, trophoblasts in plugs remained tightly clustered and little cell movement out of plugs occurs. Decreasing cell-cell interaction forces increases cell migration out of plugs along the vessel wall. Thus, for trophoblast plugs to remain intact (as occurs *in vivo*), cell-cell interaction forces must be relatively high.

How/why do plugs break up? When cell-cell interaction forces are low, plugs progressively open from the centre as cells migrate towards and along the vessel wall. Conversely, when cell-cell interaction forces are high, cells remain in cohesive plugs. If a plug region becomes cell-free (here due to asymmetry in migration, *in vivo* also potentially due to cell death), flow in that region increases, forming channels similar to those reported histologically. Once channels are evident, local increases in flow stimulate further trophoblast migration away from the channels, further increasing flow and reinforcing the cycle of plug breakdown. In this scenario, flow drives plug dispersion, and if total volumetric blood flow increases, as is expected as the larger arcuate/radial arteries remodel late in the first trimester, plug break-up occurs more rapidly. Thus, increases in blood flow may act as a stimulus to induce plug dispersal.

Limitations, reasons for caution: The model was parameterised using in vitro data that is not able to simultaneously capture the influence of all the

factors involved in regulating trophoblast behaviour in vivo (e.g. shear stress, chemotaxis, 3D cell-cell interaction forces).
Wider implications of the findings: This model enabled us to simulate trophoblast behaviour and visualise potential scenarios leading to plug dispersal. These data provide a physiological mechanism by which the channels observed in trophoblast plugs *in vivo* may contribute to their dispersal in the late first trimester.
Trial registration number: Not applicable

P-190 Morphokinetic characteristics of embryos originating from extremely small follicles: a prospective study.

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Study question: Is the developmental potential of oocytes and embryos derived from extremely small follicles (<10mm) different from those originated in larger follicles?
Summary answer: Embryos originated from small follicles are not different than embryos from larger follicles, as assessed by morphokinetic parameters in time lapse system.
What is known already: There is no data concerning morphkinetic parameters of embryos developed from oocytes derived from very small follicles.
Study design, size, duration: Prospective study, A university affiliated tertiary hospital, IVF unit, PGD referral center.
Participants/materials, setting, methods: We documented follicle aspirations of 98 patients. Aspiration of follicles larger and smaller than 10 mm was undertaken separately and the development of embryos was followed up using different wells for each embryo. We recorded: oocytes retrieved, maturation, fertilization rate, cleavage rate, morphokinetic parameters, transfers, embryo freezing, oocyte freezing and biopsy rate for preimplantation genetic diagnosis (PGD).

Quality was evaluated using time-lapse imaging technology. Day 3 KIDScore was calculated and comparison was made between groups.
Main results and the role of chance: Small follicles compared to large follicles displayed lower recovery rate (45% vs. 74%, P<0.0001), fewer matured oocytes (37.5% vs. 61.7%, P<0.0001), higher rates of GV oocytes (20.7% vs., 3.7%, P< 0.0001), and lower fertilization rate (43.7% vs. 63.3%, P<0.0001. However, morphokinetic variables were similar between embryos that originated from either small or large follicles. Median KIDscores were identical for embryos from small or large follicle origin.
Limitations, reasons for caution: assessment was limited to day 3 embryos.
Wider implications of the findings: In view of our findings, physicians should bear in mind that small follicle aspiration might yield good quality embryos.
Trial registration number: not applicable

P-191 The EGFL7/NOTCH pathway: a novel regulator of the endometrium-blastocyst dialog

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Study question: Is the EGFL7/NOTCH pathway involved in the regulation of endometrial receptivity and embryo implantation, influencing fertility?
Summary answer: EGFL7, NOTCH1, NOTCH-target genes are reduced in the endometrium of patients experiencing unexplained recurrent miscarriages-(uRM) and recurrent implantation failure-(RIF). EGFL7-overexpressing trophoblast spheroids demonstrate increased adhesion
What is known already: Several NOTCH family members have been identified in the endometrium, where they regulate cellular events involved in feto-maternal communication. Reduced expression of Notch1 in endometrial stromal cells inhibits decidualization, and its aberrant activation in the mouse uterus downregulates the expression of progesterone receptor, leading to infertility. The secreted factor Epidermal Growth Factor Like Domain 7 (EGFL7) has been recently demonstrated as a novel regulator of the NOTCH pathway. We previously demonstrated that EGFL7 is expressed by mouse blastocyst and by mouse and human trophoblast cells, and that it regulates their migration and invasion ability by activating NOTCH1
Study design, size, duration: The presented work is a translational study which so far enrolled 60 women stratified into 3 experimental groups: controls, uRM, RIF. Endometrial biopsies have been collected to compare the activation of the EGFL7/NOTCH pathway. From part of these samples, endometrial cell cultures have been obtained and used to determine the ability of recombinant EGFL7 to activate NOTCH signaling. Spheroids of EGFL7-expressing trophoblast cells have been used to study the role of EGFL7/NOTCH crosstalk in implantation
Participants/materials, setting, methods: Women 42 years of age or younger were enrolled. Endometrial biopsies were obtained, and partly processed to characterize the EGFL7/NOTCH pathway expression by both qRT-PCR and immunofluorescence (IF); part of the samples were processed for the preparation of cell cultures used for functional studies. The human trophoblast cell line HTR8 was induced to over-express EGFL7 (HTR8-E7), and spheroids (to resemble the human blastocyst) were obtained to elucidate the contribution of the EGFL7/NOTCH pathway in implantation
Main results and the role of chance: Our qRT-PCR results demonstrate that the expression of EGFL7 is significantly reduced in both RIF and uRM endometrial samples compared to controls. A parallel reduction is also observed for NOTCH1 and the NOTCH target genes HEY1 and HES1, suggesting a correlation between the downregulation of the EGFL7/NOTCH1 pathway and implantation defects. Immunofluorescence analysis of control samples demonstrates that EGFL7 is expressed by endothelial and glandular epithelial cells, and consistent with the qRT-PCR results, in RIF and uRM such expression is strongly reduced. Supplementation of endometrial cultures with hrEGFL7 up-regulates the expression of the NOTCH target genes HES1 and HEY2 in a time dependent manner, indicating an EGFL7-mediated, time-dependent activation of the NOTCH signaling in endometrial cells. Trophoblast spheroids over-expressing EGFL7 show a higher ability to adhere and spread on gelatin and on endometrial cells than control spheroids, suggesting that trophoblast derived EGFL7 may play a role in regulating implantation. In order to avoid serendipitous findings, we have performed our analyses on a sufficiently large number of samples and repeated each experiment at least three times; in all experiments we have also always included the appropriate controls
Limitations, reasons for caution: The reported results may have some limitations: - *in vitro* cultures might per-se influence gene expression, not faithfully representing the *in vivo*; -for ethical reasons no human blastocysts could be used to prove trophoectoderm-expression of EGFL7; -trophoblast spheroids may be a too simplified model of the blastocyst, not including ICM
Wider implications of the findings: Our results provide new insights into the etiopathology of implantation defects and pregnancy complication by identifying alteration in EGFL7 expression and secretion and dysregulation of NOTCH signaling as leading causes of RIF and uRM. Our results might have therapeutic relevance, with EGFL7/NOTCH pathway being target for medical intervention
Trial registration number: Not Applicable

P-192 Evaluation of the intracytoplasmic sperm injection (ICSI) results in recurrent total fertilization failure (TFF)

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Study question: Are there any differences regarding parameters of in-vitro-fertilization (IVF) treatment process, semen analyses and sperm functions between cases with recurrent TFF versus fertile pairs?

Summary answer: TFF is associated with the male and female age, basal FSH value, the amount of gonadotropin used during ovarian stimulation and properties of gametes.
What is known already: In ICSI applications, the causes of TFF include the mitosis errors, sperm aster formation defect, sperm decondensation defects, defects in PN formation, morphological abnormal oocytes and oocyte activation defects.
Study design, size, duration: In this retrospective study, semen parameters and sperm functions, oocyte morphology, basal hormone levels and treatment processes were compared in 32 cases with second time TFF after ICSI application and 91 fertile control cases treated between 2014 and 2018.
Participants/materials, setting, methods: A total of 123 patients, who underwent ovarian stimulation with antagonist protocol at Memorial IVF Center, Istanbul, Turkey, were evaluated. Exclusion criteria included polycystic ovaries, endometriosis, azoospermia and globozoospermia. The primary endpoint was basal hormone measurements and used amount of gonadotropins. Secondary endpoints were oocyte maturity, morphology and oocyte counts.
Main results and the role of chance: The male age (36.9±5.0 y vs 34.8±4.5 y, p=0.041), female age (33.5±5.1 y vs 30.8±3.9 y, p=0.004), basal FSH value (7.2±3.7 IU/L vs 5.7±2.0 IU/L, p=0.008) and the amount of gonadotropin used during ovarian stimulation (2725±1127 IU vs 2171±817 IU, p=0.004) were significantly higher in the TFF group than the fertile controls. Basal AMH level (1.8±1.7 ng/mL vs 3.8±2.8 ng/mL, p=0.002), total oocyte count (4.4±4.3 vs 9.6±4.4, p<0.001), M2 oocyte count (0.3±0.8 vs 2.5±2.7, p<0.001), quality oocyte count (2.7±3.1 vs 4.4±3.0, p=0.008) and normal ZP (2.6±2.8 vs 7.4±3.6, p<0.001) were lower in TFF cases compared to the fertile controls. There was no statistically significant difference between the two groups in terms of semen parameters and sperm function.
Limitations, reasons for caution: The main limiting factor of this study ins the small number of cases.
Wider implications of the findings: The oocyte-cytoplasm and zona-pellucida abnormalities, high FSH and low AMH values, and high-dose gonadotropin administration may be associated with TFF. Since the mean-female age was less than 35 and the oocyte count was lower in the TFF-group, the probability of TFF should be considered also in young patients with ovarian-insufficiency.
Trial registration number: 2018/BUEK-1.2.2018/34

P-193 Perishing IVF embryos: Evaluating different approaches in light of the bioethical status of the preimplantation embryo.

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Study question: How do In Vitro Fertilization (IVF) laboratories worldwide perish supernumerary embryos?
Summary answer: Discrepancies in management of supernumerary embryos involve practice, timing, and allowing couples' involvement in the procedure. Practitioners recognize the need for a universal protocol implementation.
What is known already: Perishing surplus embryos in an IVF clinic constitutes a routine procedure for embryologists. These surplus embryos may be originating from fresh cycles, as poor-quality embryos fail to be qualified for embryo transfer or cryopreservation. Alternatively, they could be embryos diagnosed with genetic or chromosomal abnormalities following Preimplantation Genetic Testing, or cryopreserved embryos in storage that patients choose to perish. Current literature lacks data regarding the different practices employed in discarding embryos in IVF laboratories, as there is no universal protocol or guideline.
Study design, size, duration: A web-based questionnaire titled 'Anonymous questionnaire on embryo disposal practices' was designed employing the "google-forms" format in order to ensure anonymous participation of IVF professionals around the world. Demographic information regarding the name of the Country and, where applicable, the State the IVF clinic was based. During a 3 months data collection period 440 answers from 56 different countries were acquired.
Participants/materials, setting, methods: In an effort to reach a worldwide audience, the authors employed the contact platforms available in websites of respective associations and organizations, or used the provided contact email of clinics to reach IVF professionals. The questionnaires' link was posted online employing social media platforms while individual associations of embryologists were contacted worldwide. The answers were collected and analysed in order to unfold trends and patterns on embryo disposal practices around the globe.
Main results and the role of chance: The questionnaire was divided into three sections. The first section focused on how IVF practitioners perform embryo disposal of surplus embryos. In both fresh and frozen cycles, the majority of practitioners dispose of embryos by placing them directly in a biohazard bin for both fresh and frozen cycles (38.6% and 37.0% respectively). Moreover, 66.2% of practitioners perish the embryos separately, case by case, at different time points during the day. Over half of embryologists (53.7%) wait until Day 6 to perish the surplus embryos, while 65.9% do not implement a special allocated incubator space as a designated waiting area prior to the disposal procedure. Interestingly, 65.1% reported that this is a witnessed procedure. The second section of the survey assessed the various approaches employed in order to satisfy patient requests or requirements in regards to embryo disposal. The majority of embryologists (93.6%) do not employ different protocols for different groups of patients, but 18.6% reported that they have been asked to perform a ceremony for these embryos. The third section assessed the embryologists' perspective with 57.7% of participants stating that embryology practice would benefit from a universal protocol.
Limitations, reasons for caution: Employing google forms platform, reflecting an individual opinion was enabled and current practice was linked to the stated Country. The language barrier may have served as an obstacle reaching Countries where English is not spoken. Despite the anonymity, social reasons may have led some respondents not to mention certain practices.
Wider implications of the findings: The variety on the practices may be attributed to both moral and bioethical perceptions surrounding the preimplantation embryo's bioethical stance. This study provides insights into embryo disposal practices and raises questions on the standardization and regulation of clinical practices while highlighting the need for concurring on a universal protocol.
Trial registration number: Not applicable

P-194 Rab10 regulates actin-dependent cortical granule migration for fertilization in mouse oocytes

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Study question: As cortical granule (CG) migration and exocytosis are important for meiotic maturation and fertilization, we investigated possible factors and mechanism regulating these processes.
Summary answer: We found a vesicle traffic protein Rab10 could affect myosin Va powered CG migration through RhoA-ROCK pathway-mediated actin assembly.
What is known already: During fertilization, cortical-area-distributed CG exocytosis takes place and CG contents are released into the perivitelline space, which could modify the zona pellucida (ZP) and make ZP stiff for blocking polyspermy. CGs are recognized deriving from Golgi complex and then are migrated to the cortical area waiting for fertilization. During this process, microfilaments play important roles both in vertebrates and invertebrates. Besides, CGs also could bind to Rab11a vesicles powered by myosin Vb and then are transported to the destination. RhoA-ROCK pathway are verified to regulate actin dynamics and Rab10 are reported to regulate endosome transport.
Study design, size, duration: RNAi was used to perform the knockdown of Rab10. Rab10 T23N and Rab10 Q68L site-mutant plasmids were used to overexpress inactive or active form of Rab10. At least 50 oocytes were used to

detect actin assembly and CG migration after microinjection of Rab10 siRNA and in-vitro transcribed cRNA. All experiments were performed at least three times.

Participants/materials, setting, methods: We used immunofluorescent staining and western blotting to examine the effects of Rab10 on oocyte meiotic maturation and fertilization.

Main results and the role of chance: siRNA-mediated attenuation of Rab10 expression led to a significant ($P<0.05$) decline of mouse oocyte maturation (the first polar body extrusion rate). Rab10 disruption did not affect spindle organization ($P>0.01$), but significantly decreased cytoplasmic microfilament actin ($P<0.01$). Compared to overexpression of active Rab10 (Rab10-GTP), Rab10 knockdown and overexpression of inactive state Rab10 (Rab10-GDP) could delay the CG migration to cortical area ($P<0.05$). Further, overexpression of inactive Rab10 could downregulate the expression of factors in RhoA-ROCK pathway, which are proved to be important for actin assembly. At last, we detected more Rab10-knock down oocytes presented polyspermy than control group and Rab10-GTP overexpression group ($P<0.05$). Taken together, these observations hint at a key role of Rab10 in the regulating of actin-dependent CG migration and fertilization.

Limitations, reasons for caution: Only in vitro investigation was conducted in our research and Rab10 knock out was lethal at embryo development stage in mouse.

Wider implications of the findings: Our findings indicated a vesicle traffic protein Rab10 participating in regulating oocyte maturation and blocking polyspermy. This information could contribute to a better understanding of fertilization mechanism.

Trial registration number: not applicable

P-195 Challenges in oocyte vitrification and fertility preservation: female age and oocyte quality

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Study question: Is there an age limit for patients to undergo oocyte freezing for fertility preservation and how many oocytes should be retrieved?

Summary answer: A birth rate of 28.6% in patients ≥39 years with Ø 5.1 oocytes warmed encourages the future application of oocyte vitrification in fertility preservation.

What is known already: Nowadays oocyte vitrification is widely used for fertility preservation, however clinical outcome published remains scarce, especially for IVF-patients accumulating oocytes (low responders) and cancer patients. Several publications report a dramatically increased amount of oocytes needed to achieve a pregnancy with increasing female age. However, the oocyte yield we can clinically expect is often far below these numbers. Further, IVF-patients often show multifactorial subfertility. Facing the poor knowledge on clinical outcome, this study aimed to analyze the impact of age, oocyte quality and infertility on IVF-success after aseptic oocyte vitrification to estimate clinical relevance of oocyte freezing at advanced female age.

Study design, size, duration: This retrospective study encompassed data of 6 years (2011-2016). A total of 43 aseptic oocyte vitrification / warming cycles were included. Patients were grouped according to female age: ≤38 and ≥39 years. Primary outcome parameters were survival rate (SR) after warming, fertilization rate (FR) and blastocyst development. Second endpoint included pregnancy rate (PR) and birth rate (BR).

Participants/materials, setting, methods: Oocyte vitrification was indicated by medical reasons (e.g. fertility preservation in ultra-low responder patients, before cancer treatment, or oocyte accumulation due to severe male factor infertility). Aseptic vitrification of oocytes was performed around 2 hours after oocyte pick-up with a standardized protocol (VitriSafe and FertiPro solutions). Warming was done in sucrose solutions according to the manufacturer's protocol.

Main results and the role of chance: Patients underwent a mean of 1.3 stimulation cycles for oocyte retrieval. Thereby, a total of 376 mature MII oocytes were aseptically vitrified and subsequently warmed for embryo transfer. When comparing the age groups, a slightly diminished SR was found in patients ≥39 years compared to the younger group (77.9% vs. 83.8%). No differences in FR (70.1% vs. 73.7%) or blastocyst rate were observed between both groups (42.2% vs. 39.6%). Differences were found in PR (35.7% vs. 56.5%) and BR (28.6% vs. 43.5%) for patients ≥39 or ≤38 years, respectively. Reduced incubation time before vitrification and subequently increased time after warming before ICSI increased the yield of top-quality blastocysts (58.1% vs. 37.7%).

Limitations, reasons for caution: Results might not be fully applicable to other vitrification protocols or sulutions. Larger studies should confirm the results obtained in this retrospective study.

Wider implications of the findings: Aseptic vitrification avoids cross-contamination with pathogens and chemicals during vitrification, cryo-storage, and warming, rendering this technique especially valuable in fertility preservation involving prolonged storage-times. Our results encourage to perform oocyte vitrification in patients with reduced ovarian reserve or advanced maternal age. Refined lab-techniques might improve future outcome of oocyte vitrification.

Trial registration number: not applicable

P-196 Are blastocyst development rates and quality related to chromosomal aneuploidy?

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Study question: Are blastocyst development rates and quality related to chromosomal aneuploidy?

Summary answer: Our data shows that blastocyst quality and developmental rate correlate well with the ploidy status of the examined embryo.

What is known already: The goal of IVF is to maximize clinical pregnancy rates without increasing multiple pregnancy rates. Preimplatation Genetic Screening (PGS) allows the selection of chromosomally normal blastocysts with possibly better implantation potential. However, PGS is an invasive procedure with some limitations. For this reason, it would be interesting to assess whether non-invasive embryo selection techniques, such as those based on morphology and developmental rate, could reliably reflect the ploidy of the embryo for transfer. This knowledge could help us to avoid unnecessary biopsies in future. Controversial data have been reported so far and no clear conclusion has been made yet.

Study design, size, duration: PGS data, from IVF and ICSI cycles performed at Hammersmith Hospital, between January 2013 and December 2018 were collected. Retrospective analysis of 256 blastocysts [170 on Day 5 (D5) and 86 on Day 6 (D6)] was performed. The ploidy status of blastocysts of different quality on D5 and D6 was compared.

Participants/materials, setting, methods: Laser assisted trophectoderm biopsy was performed on D5 or D6 depending on the timing of blastocyst formation. Blastocysts were analysed either with comparative genomic hybridization or next generation sequencing. Blastocyst grading was performed using the Gardner's system. Biopsied blastocysts were divided into three categories based on their morphology [good quality-GG (AA,AB,BA,BB), average quality-AQ (AC,CA,BC,CB), poor quality-PQ (CC)]. Mosaic embryos were excluded. Chi-squared test was used for statistical analysis and significance was considered if $p<0.05$.

Main results and the role of chance: Our data shows significant difference in ploidy rates when D5 blastocysts were compared to D6 blastocysts, with the D5 blastocysts showing significantly higher euploidy rate [40% D5 euploid rate (n=68/170) vs 18.6% D6 euploid rate (n=16/86), $p=0.00026<0.05$]. Additionally, the euploidy rate was significantly higher for good quality blastocysts compared to lower quality ones [GQ euploidy rate 50% (n=60/120) vs AQ euploidy rate 18.47% (n=17/92) and PQ euploidy rate 15.9% (n=7/44), $p=0.00001<0.05$]. Then, embryos of similar quality were compared on D5 and D6. There were no significant differences in ploidy rates when good quality blastocysts where compared on D5 and D6 [euploidy rates: GQ on D5 and D6, 50.49% (n=51/101) vs 47.36% (n=9/19), $p=1>0.05$] or when average quality blastocysts were compared on D5 and D6 [euploidy rates: AQ embryos on D5 and D6, 21% (n=12/57) vs 14.28% (n=5/35), p=0.5815]. However, when poor quality blastocysts where compared on D5 and D6, poor quality blastocysts showed significantly lower euploid rate on D6 [euploidy rates: PQ blastocysts on D5 and D6, 41.66% (n=5/12) vs 6% (n=2/32), $p=0.011<0.05$].

Limitations, reasons for caution: A larger samples size would be required to confirm our findings. Also, confounders such as patient age, which could

possibly affect outcomes, were not considered within our analysis. The biopsy technique itself could be another limitation as the trophectoderm sample analysed may not be representative of the entire embryo.
Wider implications of the findings: Morphology and developmental rate are associated with ploidy status**.** Nevertheless, our data shows that poor prognosis blastocysts could also be euploid. This information could be beneficial when no good quality blastocysts are available. Also, PGS could improve the efficiency of traditional selection by avoiding using good quality but aneuploid embryos.
Trial registration number: not applicable

P-197 Embryo transfer under sedation: Five years of experience at Imperial College London

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Study question: What were the indications and outcomes for women who underwent an Embryo transfer under sedation (ETUS) in our Centre over the last 5 years?
Summary answer: Our data shows that ETUS is a viable option for women with a range of medical and non-medical indications with reassuring pregnancy outcomes.
What is known already: ETUS is a recognised option for women in specific circumstances. However, most of the literature is focused on sedation or anesthesia in Transvaginal oocyte retrieval (TVOR), but there are few reports about ETUS. A Brazilian group reported 2 cases of ETUS in patients with vaginismus and they described some reasons why sedation was requested by patients such as pain and extreme anxiety as well as other medical causes as cervical stenosis or trouble inserting the catheter (Do Carmo B, 2018;22(1):35-41). Our study represents the first report in the literature about a group of patient who underwent ETUS.
Study design, size, duration: A retrospective descriptive study of consecutive patients who underwent ETUS at Wolfson Fertility Centre (WFC), Hammersmith Hospital London, between January 2014 and December 2018 (60 months). The variables analysed included: age, IVF/ICSI or Frozen embryo replacement cycle (FERC) protocol, reason of sedation, clinical pregnancy rate, live birth rate, type of delivery.
Participants/materials, setting, methods: We analysed 3,766 embryo transfer cycles which were carried out at WFC between January 2014 and December 2018. 100 patients were identified in that period. The causes were divided in three groups: Medical, non-medical and non-identified
Main results and the role of chance: The average age of the patients was 34.5 years old (range 24 – 43 yo). 64 patients underwent fresh embryo transfer (64%) and 36 patients had FERC (36%). Causes were divided in three groups: (A) medical causes (50 patients) and (B) non-medical causes (44 patients) and in 6 patients a cause was not identified (C). In group A, 37 patients had history of previous difficult ET or a difficult mock ET, 5 patients had trachelectomy, 5 patients presented cervical stenosis, one patient had history of female genital mutilation, one patient who had a spasm in the TVOR and one patient who had a severe vulvar Crohn's disease. In group B, we included 31 patients with vaginismus, 12 patients who requested sedation due to anxiety and one patient who was virgo intacta.

Overall, Clinical pregnancy rate in ETUS was 41%, which is comparable to the patients who had ET without sedation (37,9%). 28 patients had live birth (12 in Group A, 11 in Group B, 4 in Group C) and two are still ongoing. 19 births were by caesarean section (67.8%), 6 patients had forceps (21.4%) and 3 vaginal deliveries (10.7%)
Limitations, reasons for caution: Due to the difference in group sizes between ETUS and standard ET, it was not possible to perform a statistical analysis in this study, however results seems to be comparable to ET without sedation. Live birth rates are not available as some pregnancies are still ongoing.
Wider implications of the findings: To the best of our knowledge, this is the first analysis about Embryo transfer under sedation in the literature and confirms that it is a viable option for women with a wide range of indications, not only for medical, but also for non-medical reasons, such as vaginismus and anxiety.
Trial registration number: no trial registration

P-198 Embryo cleavage rate is enhanced by extending oocyte- cumulus cell contact: a randomized sibling oocyte study

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Study question: Does increasing oocyte- cumulus oophrous contact before denudation improve reproductive outcome?
Summary answer: Cleavage rate significantly increases after preincubation of oocyte for 2 hours before denudation and injection,but no effect was found on pregnancy and implantation rates
What is known already: Optimal embryonic developmental competence depends on proper oocyte maturation. Nuclear maturation is usually achieved in vivo and can be assessed by the time of ovum pick up, however, cytoplasmic maturation cannot be readily assessed and might be incomplete. Cumulus cells, surrounding the oocyte might play a role in the oocyte cytoplasmic maturation. Several studies that compared denudation timing were retrospective, did not evaluate the interval within first 2 hours of OPU, neither evaluated all laboratory and clinical outcomes.
Study design, size, duration: Randomized sibling oocyte study including 160 ICSI cycles carried out between January 2017 and June 2018 in a private fertility center; the integrated fertility center of Alexandria.
Participants/materials, setting, methods: A total of 2621 oocytes were randomized into two groups according to denudation timing, after 1 hour or 2 hours of OPU. MII oocytes injection performed immediately after denudation in each group. Each patient had 1-2 fresh embryo transfer (ET) from the best available embryos at day 5, from either group 1 or group 2,those required mixed ET were excluded. For all patients, OPU performed 35-36h post hCG administration after treatment with long agonist protocol.
Main results and the role of chance: The mean woman's age was 29.5 years (SD 5.10). No significant difference in maturation rate between group I (81.7%) and group II (78.7%, P= 0.055). The total fertilization rate between the two groups was comparable 72.6% & 73.4% for group I and group II respectively (P= 0.675), while the cleaved embryos/ diploid zygotes in group I (94.7%) were significantly lower than those in group II (99.1%, P<0.001) also cleaved embryos/MII was 68.7% in group I and 72.7% in group II, (P=0.045). Both groups had no significant difference in quality of embryos at day 2, day 3 and day 5. The available blastocysts at day 5 were comparable (59.2% vs. 57.6% for group I and II respectively, P=0.536). The proportion transferred grade I blastocysts from group I (61.9%) were similar to group II (66.4%) (P>0.05). The clinically pregnant females and implantation rates were not significantly different between both groups. Clinical pregnancies were 50% and 51.5% for group I and group II respectively, p= 0.854) and implantation rates 35.7% & 35.9% in group I and group II respectively (P= 0.977).
Limitations, reasons for caution: Only good prognosis patients were enrolled, so these results may not be generalized to all infertile couples. In addition, we cannot provide any guidance for patients undergoing either day 3 embryo transfers or treated with antagonist protocol.
Wider implications of the findings: Our findings underline the importance of oocyte- cumulus cells contact for cytoplasmic maturity. Although the impact of higher cleavage rate after 2 hours of preincubation does not affect clinical pregnancy rate in the fresh cycles, it increases the probability to achieve a live birth after utilization of all cryopreserved embryos.
Trial registration number: Alexandria University, Faculty Of Medicine, Research committee

P-199 An additional post-thaw culturing of cryopreserved full blastocysts (Gardner's 3) can improve clinical pregnancy rates

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Study question: Can an additional post-thaw culturing of blastocysts affect the ART outcome of a single, cryopreserved, full-blastocyst (Gardner's classification 3) transfer (BT)?
Summary answer: An additional 24-hour culturing that follows the thawing of cryopreserved blastocysts, either early or full, improves the clinical pregnancy rate.
What is known already: According to Gardner's classification, embryo blastocyst development stages advance in the following order: early, full, expanded, and hatching. Blastocysts are usually cryopreserved at the full, expanded or hatching stage. Pregnancy rates using full blastocysts (Gardner's 3), however, are lower than the rates of expanded (Gardner's 4) or hatching (Gardner's 5) blastocysts.

Indeed, the reported pregnancy rate for the transferral of early or full blastocysts ranges from 25 to 35%, while the range for expanded or hatching blastocysts averages between 45 and 55%.
Study design, size, duration: This study was approved by the Local Ethics Committee. From January 2016 to December 2018, this study recruited 560 single BT cycles. In this study, the cryopreservation stage of the blastocysts included only full (Gardner's 3) and expanded versions (Gardner's 4). Hatching blastocysts (Gardner's 5) were not included. Endometrial preparation involved a hormone replacement cycle. Blastocysts were transferred 118 to 122 hours following progesterone initiation.
Participants/materials, setting, methods: The patients, who received single BTs with cryopreserved full blastocysts, were divided into two groups: The first group involved 409 BT cycles that used thawed full blastocysts on the same day (same-day group); the second group involved 151 BT cycles that used expanded or hatching blastocysts after full blastocysts had been thawed and cultured for 24 hours (additional-culturing group).
Main results and the role of chance: The average ages of the women in the same-day and additional-culturing groups were 37.5 ± 3.9 and 37.4 ± 4.2 years (NS) with previous BT attempts that averaged 1.6 ± 1.2 and 1.8 ± 1.5 (NS), respectively.

The clinical pregnancy rate for the additional-culturing group was 45.0% (68/151), which was significantly higher than that for the same-day group (35.4% [145/409], p=0.03). In the additional-culturing group, 21 women miscarried for a miscarriage rate of 30.9%. By contrast, 31 women miscarried in the same-day group for a miscarriage rate of 21.3%. There were no other significant differences between the two groups.

The pregnancy rate in our clinic using previously frozen, expanded (Gardner's 4), or hatching (Gardner's 5) blastocysts averaged 51.7% (849/1641) during the same period. Compared with this figure, the rate for the same-day group was significantly lower (p<0.01), but the rate for the additional-culturing group was comparable (p=0.36). With regard to full blastocysts, however, the clinical pregnancy rate for BT cycles with an additional regimen of post-thaw culturing was significantly higher than for that without additional culturing.
Limitations, reasons for caution: This study was an observational retrospective study. All transferred blastocysts were evaluated by morphological analysis rather than by chromosomal testing, because the Japanese Society of Obstetrics and Gynecology has imposed restrictions on the use of preimplantation genetic testing.
Wider implications of the findings: An optimal window of opportunity for the implantation of expanded or hatching blastocysts may be formed by decidualization of the endometrium approximately 120 hours following progesterone initiation in hormone replacement cycles. BTs using previously frozen full blastocysts will likely miss this window for implantation.
Trial registration number: None

P-200 Hyaluronan-enriched embryo transfer medium for frozen-thawed embryo transfer: a double-blind randomised controlled trial

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Study question: Does the use of hyaluronan-enriched embryo transfer medium (HETM) improve the live birth rate of frozen-thawed embryo transfer (FET) compared to standard medium?
Summary answer: The use of HETM compared to control does not improve the live birth rate of FET.
What is known already: Some studies suggest that the use of HETM when compared to transfer medium containing low or no hyaluronan improved clinical pregnancy and live birth rates of in-vitro fertilization (IVF). However, data on FET are limited.
Study design, size, duration: We performed a double-blind randomised controlled trial involving 550 women undergoing FET in two centres from April 2016 to April 2018. The sample size was chosen to detect a difference of 12% in the live birth rate with a power of 80% and type I error of 0.05. Women with donor oocyte/embryo treatment or pre-implantation genetic testing were excluded.
Participants/materials, setting, methods: Women under the age of 43 at the time of IVF undergoing FET were randomised into two groups in a 1:1 ratio: HETM group and control group. The HETM group used HETM (hyaluronan concentration 0.5 mg/ml) while the control group used a conventional medium (hyaluronan concentration 0.125 mg/ml). Up to two embryos/ blastocysts were replaced. Each woman took part once only. The primary outcome was the live birth rate per randomised woman.
Main results and the role of chance: Basic characteristics including age of women, BMI, and number of embryos replaced were comparable in the two groups. Thirteen women (8 in HETM group, 5 in control group) did not have FET because the embryos did not survive upon thawing. One woman in the HETM group had FET cancelled due to fever. One in the HETM group withdrew after randomisation and received conventional medium. All 550 women were included in the intention-to-treat analysis, with a mean of 1.4 embryos/blastocysts transferred in both groups. The live birth rate per randomised woman was 25.5% (70/275) in the HETM group versus 25.5% (70/275) in the control group (p = 1.00). The clinical pregnancy rate was 34.2% (94/275) in both groups (p = 1.00). The multiple pregnancy rate was 13.8% (13/94) in the HETM group versus 19.1% (18/94) in the control group (p = 0.33). One ectopic pregnancy occurred in each group. Other secondary outcomes including on-going pregnancy rate, miscarriage rate and incidence of obstetric complications were similar between the two groups. Subgroup analyses for different embryo stage (cleavage stage embryos versus blastocysts) and different types of endometrial preparation showed no statistically significant difference in the live birth rate between the two groups.
Limitations, reasons for caution: The study assessed the role of HETM in an unselected population of infertile women. While this made the results generalizable to the general infertile population, the results did not address specific population e.g. women with recurrent implantation failure.
Wider implications of the findings: In this study, the use of HETM did not improve the live birth rate following FET.
Trial registration number: ClincialTrials.gov: NCT02725827

P-201 External validation of a commercially available morphokinetic algorithm

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Study question: The aim of this retrospective study was to validate the KIDscore5 algorithm using an external dataset and assess its comparative value to standard morphological grading.
Summary answer: The KIDscore5 algorithm is predictive of implantation and live birth when retrospectively applied to an external dataset, which justifies its use prospectively.
What is known already: The widespread use of time-lapse incubators has proven safe and effective and has led to the development of morphokinetic algorithms. External validation of published algorithms has produced variable results, with several authors concluding that morphokinetic algorithms have

limited applications without replication of the original culture conditions. The KIDscore5 algorithm is a commercially available adjunct to the Embryoscope. It was developed using a large cohort of embryos, from multiple centres and is designed to be generally applicable. To the best of our knowledge, this is the first external validation of this morphokinetic algorithm to guide embryo selection.

Study design, size, duration: This is a retrospective analysis of all treatment cycles performed in a single centre, June 2015-June 2016. Patients who underwent a fresh blastocyst transfer, whose embryos were of known implantation outcome (KID embryos), were included. When the fate of the embryo could not be confirmed patients were excluded. Data were obtained from 529 cycles for 524 patients. Clinical pregnancy was confirmed by presence of a fetal heartbeat. Live births/miscarriages were confirmed by patient reporting.

Participants/materials, setting, methods: Following ovarian stimulation, oocyte retrieval and in-vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment, all fertilised oocytes were cultured, observed and annotated in the Embryoscope until embryo transfer. All patients in the study had at least two good quality embryos on Day 3 and therefore had a blastocyst transfer. Embryo selection was based on morphological score. Retrospective additional annotation and application of the KIDscore5 algorithm was carried out for all 651 KID blastocysts.

Main results and the role of chance: 651 KID blastocysts were transferred on Day 5, for 524 patients who completed 529 fresh treatment cycles. IVF or ICSI was used to create 296 (45.5%) and 355 (54.5%) embryos, respectively. 407 (76.9%) SETs and 122 (23.1%) DETs were performed. Overall implantation rates (IR) and live birth rates (LBR) were 36.4% and 31.0%, respectively. Of the 651 KID embryos, 601 (92.3%) had all necessary annotations available for the KIDscore5 algorithm to be applied.

Logistic regression modelling was performed, with either the presence of a fetal heartbeat (FH) or a live birth (LB) as the outcome and KIDscore as the predictor. Logistic regression models were fitted between presence of FH or LB and KIDscore or morphology score. Statistically significant relationships were observed between both FH and LB and KIDscore ($p<0.001$), and both FH and LB and morphology score ($p<0.001$). There was no evidence of a difference in selecting successful embryos between KIDscore and morphology score in terms of IR ($p=0.3394$) or LBR ($p=0.5729$).

Overall, when predicting implantation or LB, there was no evidence that morphology score added anything over KIDscore, or vice-versa. However, it does appear that for fair/poor morphology embryos KIDscore may provide useful information when choosing between similar embryos.

Limitations, reasons for caution: This is a single centre, retrospective analysis and therefore results may not be universally applicable. Application of the algorithm is only available to Embryoscope users, for a fee, as the algorithm is not published and can only be applied using the Embryoscope Viewer platform.

Wider implications of the findings: This is the first study that externally validates the KIDscore5 algorithm. These results support using the algorithm prospectively. The algorithm's clinical usefulness may be limited when choosing between higher quality embryos but may be more effective for choosing between lower quality embryos; which requires further investigation.

Trial registration number: Not applicable

P-202 To transfer or not to transfer: this is the question

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Study question: How do patients manage the situation of having only a mosaic embryo for transfer?

Summary answer: Genetic counselling is fundamental to make an informed decision. However, the way to proceed when only a mosaic embryo is available varies widely among patients.

What is known already: The existence of mosaic embryos has been known for many decades. However, only recently new technologies have allowed such feature to be detected in Preimplantation Genetic Testing for Aneuploidies cycles. It has been reported that mosaic embryos can result in healthy live births with no post-natal affectations so far, but the potential risks of such embryos cannot be dismissed. Therefore, the clinical management of a situation when the only option for the patient is a mosaic embryo is challenging. Most studies have been focused on clinical outcomes but very few have presented actual data on how patients confront this situation.

Study design, size, duration: Retrospective observational study performed on 383 Preimplantation Genetic Testing for Aneuploidies cycles (PGT-A) from October 2017 to December 2018. The incidence of cycles in which the only option for transfer was a mosaic embryo was assessed. Such cycles were carefully analysed in terms of patients' decision making on the possibility of transferring mosaic embryos.

Participants/materials, setting, methods: Cytogenetic constitution results of 1602 embryos were obtained by NGS Veri-Seq PGS method (Illumina). Mosaicism was reported when the percentage of abnormal cells for an affected chromosome ranged from 30-70%. Consultation with an internal genetic counsellor was offered to all patients with mosaic embryos to discuss the potential expected outcomes and associated risks related to the transfer of mosaic embryos. All available reproductive options were considered.

Main results and the role of chance: Twenty-four percent of the cycles (95/383) had at least one embryo diagnosed as mosaic. However, in just 4.4% of the cycles (17/383) the only transferable option was a mosaic embryo. Moreover, from the cycles with available euploid embryos, 2.1% (6/288) have transferred all such embryos without success and had to face the decision of transferring or not a mosaic embryo.

In total, 23 patients have had to decide whether to transfer or not their mosaic embryos during the time the study was conducted.

Almost all patients followed the recommendation of receiving genetic counselling (91.3%). Only 2 patients refused the genetic counsellor advice as they were determined to repeat a PGT-A cycle before considering the transfer of mosaic embryos.

The patients' decisions were the following: 26.1% (6/23) of the patients decided to transfer their mosaic embryo, 39.1% (9/23) decided to keep them cryostored while performing a new PGT-A cycle and 34.8% (8/23) opted to discard the embryos.

Up to date, 5 out of 6 mosaic embryos have been transferred. Two of them resulted in an ongoing clinical pregnancy (2/5, 40%).

Limitations, reasons for caution: A limited series of cases is presented. More data are needed for conclusive results. No correlations with factors potentially affecting the decision could be established due to the sample size.

Wider implications of the findings: The safety of replacing mosaic embryos is still a matter of debate. A remarkable number of patients opt for conservative decision making. Uncertainty derived from mosaicism is difficult to manage, especially in the era when extensive and accurate genetic testing is available.

Trial registration number: not applicable

P-203 The impact of media/oil volumes and dish type on medium osmolality throughout embryo culture

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Study question: Does the dish type and media/oil volume used during embryo culture impact upon evaporation?

Summary answer: Use of a suitable media/oil volume alongside an optimal dish design results in greatly reduced evaporation and subsequent osmolality changes, reducing likelihood of embryo damage.

What is known already: The importance of avoiding potential stressors on gametes and embryos while *in vitro* has been well documented. Osmolality is a crucial media parameter and thus, it is imperative to ensure it is adequately controlled to avoid compromising embryo development. The risk of significant osmolality increase is exacerbated in monophasic culture systems due to the time embryos spend in culture without medium replenishment. Mineral oil has been used as an overlay for culture medium for decades, the core function of which is to minimise medium evaporation and resultant osmolality changes.

Study design, size, duration: Experiments were conducted to monitor the level of evaporation of medium and consequently the change on osmolality in the Geri® dish using various configurations of medium and mineral oil for the intended period of culture in the Geri® incubator, used without humidification for the purposes of this study. Each dish configuration was tested a minimum

of three times. Geri Medium and Sage Oil for Tissue Culture were used throughout.

Participants/materials, setting, methods: Gravimetric measurements were performed using a Sartorius MSU324S analytical balance. To ensure results were in accordance with those obtained when using an osmometer, method validation was conducted using an Advanced 3250 Osmometer. Weights were measured before and after the intended culture period, including standard equilibration time, of 5.7 days (136 hours). Various medium and oil configurations were used in 60mm Falcon and Geri dishes, both commonly used in ART laboratories.

Main results and the role of chance: Larger volumes of medium and oil resulted in decreased evaporation, consequently minimising medium osmolality changes over the 136-hour simulated culture period. Specifically, the use of 40 µL drops of medium with a 2 mL oil overlay in a Geri® dish led to considerable evaporation, equivalent to an average osmolality change of 58.6 mOsm/kg. An increase in the medium drop size presented some mitigation, with 60 µL drops and 2 mL oil resulting in average osmolality change of 40.1 mOsm/kg. The minimal average osmolality change (7.9 mOsm/kg) was observed when using 80 µL medium drops with 4 mL oil overlay.

Furthermore, selection of a suitably designed dish for embryo culture proved to be imperative. The average osmolality change observed using a 60mm Falcon dish (comprising nine, 20 µL medium drops and 4 mL oil) was 70 mOsm/kg. With 11 mL oil overlay, the maximum possible without spillage, average osmolality change was 25.8 mOsm/kg, still presenting a potential risk to developing embryos. To that end, results highlighted that not only are medium and oil volume critical for minimising evaporation, but that selection of an optimised dish for embryo culture is equally important in the process of preventing osmotic shock.

Limitations, reasons for caution: Our results may be confounded by a small sample size; a larger scale study would be beneficial to improve confidence in the average osmolality change values obtained.

Wider implications of the findings: We concluded that dish type and media/oil volumes are crucial to avoid osmolality changes during the embryo culture period. Therefore, the decision on those parameters should be considered in-depth to ensure optimal conditions for developing embryos and, potentially improve clinical outcomes.

Trial registration number: Not applicable

P-204 Can follicular Emmprin and Bmp 4 levels predict ICSI outcome?

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Study question: To evaluate the relationship of clinical pregnancy rates with Bone Morphogenic Proteins 2-4-7, Growth Differentiation Factor 9 and Emmprin levels in follicular fluid of intra cytoplasmic sperm injection patients.

Summary answer: Clinical pregnancy rates after ICSI may be associated with follicular fluid levels of Emmprin and BMP4.

What is known already: Bone Morphogenic Proteins (BMPs) and Growth Differentiation factor-9 (GDF9) which are members of transforming growth factor beta (TGF-beta) super family, have critical roles in oocyte and follicular maturation. Mutations affecting the function of these proteins lead to dysregulated signaling, thus interfering with the success of human conception [6. Emmprin (extracellular matrix metalloproteinase inducer), also called Basigin (CD147), is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. Lack of Emmprin has been shown to be related with infertility in both male and female mice. Emmprin plays roles in oocyte maturation, fertilization, implantation and early embryonic development.

Study design, size, duration: Follicular fluid of seventy-seven patients who underwent ICSI procedure was collected during the oocyte retrieval procedure. And follicular fluid levels of BMP 2, BMP 4, BMP 7, GDF 9 and Emmprin (Basigin) were measured and compared for clinical pregnancy rates.

Participants/materials, setting, methods: The study analyzed the follicular fluid samples of 79 patients who underwent ICSI procedure for tubal factor or unexplained infertility. The first retrieved leading follicle's fluid was collected and used for analysis. Human BMP 2, BMP 4, BMP 7, EMMPRIN/CD147 and GDF 9 enyzme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm Co. Ltd, China) was used for the quantitative measurement. BMP 2-4-7 levels were expressed as ng/ml, Emmprin and GDF 9 levels were expressed as ng/L.

Main results and the role of chance: A total of 79 follicular fluid samples were examined. Of these, 23 (29.1%) were the ones obtained from women who achieved clinical pregnancy and the remaining 56 (79.9%) were the ones obtained from women who did not.

The median *BMP 4* level was significantly higher in women who achieved clinical pregnancy than in those who did not ($p=0.03$), whereas the median *Emmpirin* level was significantly lower in women who achieved clinical pregnancy compared with those who did not ($p<0.001$). The levels of other proteins studied were similar between the groups.

When *transformed levels of BMP 4 and Emmprin* were included together in logistic regression, AUC was found to be 0.70 (95% confidence interval: 0.55, 0.84, $p= 0.03$). A threshold of 0.14 had a sensitivity of 67% and specifity of 67%.**Limitations, reasons for caution:** Evaluating BMP2/BMP7, GDF9/BMP15 heterodimers can be more reasonable instead of evaluating homodimers because of the heterodimers' are more active and more potent in follicular development.

Wider implications of the findings: Follicular fluid assessment can be a research area for prediction of the ICSI outcomes. Follicular fluid proteins which have critical roles in oocyte and follicular maturation like as BMP 4 and Emmprin may be further assessed for choosing the most convenient retrieved oocyte for fertilization.

Trial registration number: N/A

P-205 Comparison between paraffin heavy oil and light oil covering on early human embryo culture: a time-lapse imaging

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Study question: Are there differences on early embryo morphokinetic parameters and abnormal cleavage events after culture under paraffin heavy oil and light oil?

Summary answer: We find significant difference in morphokinetic parameters after 4-cell stage, but no significant differences in abnormal cleavage events between the two groups.

What is known already: Mineral and paraffin oils are commonly used to overlay IVF media in order to maintain their stability throughout the embryonic culture. Indeed, without any oil overlay, the medium osmolality would quickly become too high and deleterious for embryo development. Despite the importance of an oil overlay, very few publications address this aspect and to our best knowledge, the Sifer study is the only one describing.Interested in human embryo development under several types of commercial oil. This study is the first to apply Time-lapse imaging (TLI) to embryo utilization rates after culture under paraffin heavy oil and light oil.

Study design, size, duration: Retrospective analysis of fertilized embryos (n=2885, 421 cycles) were cultured in time-lapse incubator from August 2017 to November 2018.

Participants/materials, setting, methods: This study was performed in 421 cycles (n = 2885) that had undergone ICSI treatment in an unselected population. They were culture in cleavage and blastocyst medium (MRC#D13 and 46; Maria Medical Foundation, South Korea) overlaid either by heavy oil (KITAZATO) or light (Ovoil™, Vitrolife). All embryos were cultured after ICSI insemination and assessed in a time-lapse incubator (EmbryoScope, Vitrolife) and annotated for the pattern time of cleavage.

Main results and the role of chance: We analyzed 10 parameters and abnormal cleavage events after culture under paraffin heavy oil and light oil. No significant differences were found from tPB2 to t3 between the two groups. However, Heavy oil-derived embryos developed significantly faster than those produced at t4 (38.9 vs 40.7, $P < 0.001$), t5 (47.8 vs 49.2, $P < 0.001$), t6 (50.6 vs 53.2, $P < 0.001$), t7(53.3 vs 57.2, $P < 0.001$), and t8 (55.3 vs 60.1, $P < 0.001$). Moreover, the cell cycle of cc2 (8.3 vs 9.0) and synchrony of the cell cycle of s2 (2.5 vs 3.5) were also significantly different (respectively; $P < 0.001$). We compared abnormal cleavage events in embryonic development. There were no differences between the two groups in the rate of multinucleated 2-cells (37.4% vs. 39.6%, $P = 0.292$), uneven 2-cells (18.6% vs. 19.7%, $P = 0.375$), or direct cleavage from 1 to 3-cells (26.1% vs. 25.1%, $P = 0.575$).
Limitations, reasons for caution: The study was monocentric and retrospective study. Data set size is small numbers. It should be extended to larger populations and additional morphokinetic parameters.
Wider implications of the findings: This study is the first observation thoroughly describing the development of embryos derived from culture under paraffin heavy oil and light oil using morphokinetic parameters and abnormal cleavage events collected until day 3.
Trial registration number: Not applicable.

P-206 Bisphenol S content in human follicular fluid and its effect on IVF outcomes

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Study question: Does presence of Bisphenol S in follicular fluid negatively affect early embryonic development and implantation rate of human embryos?
Summary answer: Our results document that high concentrations of Bisphenol S in follicular fluid are associated with higher rates of embryos that stopped their development.
What is known already: Human fertility is influenced by a range of chemicals permanently present in our environment. One of these chemical is Bisphenol S (BPS), which is commonly used. BPS is an endocrine disruptor that can influence variety of physiological processes. It has been documented that BPS can disrupt spindle formation in oocytes and thus negatively influence IVF and embryonic development in porcine oocytes. BPS is currently commonly used in manufacture of plastics and it is often chosen to replace Bisphenol A, which has been prohibited in some countries. A direct effect of BPS on meiotic maturation of human oocytes is still unclear.
Study design, size, duration: In this study we evaluated relation between concentration of BPS in follicular fluid, IVF outcome and early embryonic development in human. A total of 38 patients aged 26 to 42 years were included in this prospective study from spring to autumn 2018. Their follicular fluid was collected during transvaginal follicle puncture. BPS content was determined by the LC-MS method (liquid chromatography-mass spectrometry).
Participants/materials, setting, methods: Based on detection of BPS in follicular fluid, the set of patients was divided into two groups – with high content of BPS (>5ng/ml) and with low content of BPS (<5ng/ml). Number of oocytes collected, efficiency of IVF, number of embryos suitable for transfer or cryoconservation, number of abnormal embryos, quality of embryonic development, biochemical and clinical gravidity were evaluated in each of the groups. Statistical evaluation was performed using one-way ANOVA and chi-square test.
Main results and the role of chance: The examination detected presence of BPS in 35 samples of follicular fluid (92.1 % of all samples analysed). In 19 samples, the concentration was higher than 5 ng/ml (50.0 % of samples analysed). Subsequently, individual parameters of IVF were assessed. There were no significant differences found between the groups of patients with high and low concentration of BPS in number of oocytes collected (low BPS 8.3 vs. high BPS 7.4), efficiency of fertilization (low BPS 80 % vs. 72 % high BPS) and number of embryos usable after in vitro cultivation (low BPS 4.4 vs. 4.2 high BPS). In the patients with high concentration of BPS, a significantly higher share of abnormal embryos after in vitro cultivation was found (low BPS 1.3 vs. high BPS 2.3). Similarly in the in rates of biochemical (low BPS 36.8 % vs. high BPS 21 %) and clinical (low BPS 31.5 % vs. high BPS 15.8 %) gravidities were not statistically significant differences.
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: In this study, BPS was detected in more than 90 % of analysed follicular fluid samples. Our results indicate a potential negative effect of BPS presence for development of human embryos.
Trial registration number: Supported by MH CZ – DRO (FNBr, 65269705) and project AZV NV18-01-00544.

P-207 Cumulative Live Birth Rates (CLBR) in patients following Freeze-all policy are higher compared to patients who undergo fresh embryotransfer (ET) followed by frozen-thawed embryotransfers

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Study question: Is there any difference in the CLBR in patients following Freeze-all policy compared to patients who undergo first fresh and then subsequent frozen-thawed ETs?
Summary answer: The CLBR is higher in patients who follow Freeze-all strategy compared to those who undergo fresh and then frozen-thawed ETs of the available embryos.
What is known already: Elective freezing of all embryos, followed by frozen-thawed ET cycles emerged to prevent risk of Ovarian Hyperstimulation Syndrome and to allow endometrium recovery after Controlled Ovarian Stimulation (COS). The efficacy of embryo vitrification allowed Freeze-all policy to gain popularity. Previous studies have reported better In vitro Fertilization (IVF) outcomes after adopting the Freeze-all policy, instead of fresh ET, mostly in normal and high responders. Nevertheless, efficacy in terms of CLBR has not been studied yet.
Study design, size, duration: This prospective observational study includes two groups of patients. Group A concerned couples which followed Freeze-all Policy, with no fresh ET and up to 3 frozen-thawed ETs. Group B includes couples which completed one fresh ET and two subsequent frozen-thawed ETs. All patients followed blastocyst ET, with 1 or 2 embryos each time. The women's age was less than 40 and they all had at least 4 embryos available for ET.
Participants/materials, setting, methods: The study was performed from January 2016 until December of 2017 in Assisting Nature, Centre of Human Reproduction and Genetics, Thessaloniki, Greece. Group A included 87 couples with a mean female age of 32.8 years, while group B included 76 women with an average age of 33. All women followed COS with an antagonist protocol. No Preimplantation Genetic Testing (PGT), Testicular Sperm Extraction (TESE) cycles or poor responders (oocytes <4) were included in the study.
Main results and the role of chance: The CLBR was estimated for each group of patients for every ET (fresh ET, first frozen, cumulative of the first frozen ET/ second/ third frozen ET). The total CLBR was estimated for the two groups, regarding the total number of ETs in each case. X^2 test was used to compare live birth rates between the two groups. In group A the Live Birth Rate after the first frozen ET was estimated 57.5%, while the same rate after the fresh ET in group B was 39.5%. The total CLBR for all the completed ETs was 81.6% in group A and 76.3% in group B. The CLBR was significantly higher in group A compared to group B after the first ET (frozen versus fresh, $p<0.05$). Cumulatively, the live birth rates were again higher for the Freeze-all group ($p>0.05$). These results are consistent with previous published data which are in favor of ETs in a frozen cycle, subsequent to the stimulation cycle, as the endometrium receptivity and the hormone profile of the woman offer a better environment for a successful implantation. This indicates that women

considered normal or high responders have better chances of achieving live birth, if they follow Freeze-all policy.
Limitations, reasons for caution: No sperm quality was taken into consideration and no natural cycles were used for endometrium preparation for the frozen-thawed cycles. A higher number of cases is required in order to confirm the obtained results.
Wider implications of the findings: Adopting Freeze-all strategy after blastocyst culture can lead to a delivery in shorter period, comparing to a fresh ET. Moreover, the policy of Freeze-all can contribute to improve delivery outcome after IVF in terms of Cumulative Live Birth Rate, in a clinically significant way.
Trial registration number: N/A

P-208 Obstetric and perinatal outcomes: How far can in vitro culture influence it?

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Study question: Are obstetric and perinatal outcomes affected by embryo culture systems? Are singletons and twins similarly affected?
Summary answer: For singletons, *in vitro* cultured embryos presented similar obstetric and perinatal outcomes. For twins, group cultured embryos had better obstetric and perinatal outcomes.
What is known already: Newborns after assisted reproductive technologies (ART) usually have poorer perinatal outcomes if compared with newborns after spontaneous conception. Low birthweight (LBW), preterm birth (PTB), and proportion of large for gestational age (LGA) have been correlated to *in vitro* culture. Birthweight and inter-twin disparity have also been shown to be affected by culture medium in singletons and twins, suggesting a negative effect of *in vitro* culture. Previous analyses of perinatal outcomes in our center have revealed that single embryo culture in a time-lapse system (TLS) resulted in less PTB than single embryo culture in a standard incubation system (SI).
Study design, size, duration: A retrospective cohort of patients attending a fertility clinic in Germany between August 2014 and October 2018 were included in the study. Singletons and twins born after fresh ICSI cycles and cultured individually in a TLS (n=160 and n=94) were compared to singletons and twins after grouped embryo culture in a SI (n=128 and n=40). Patients with a vanishing twin were excluded from the study.
Participants/materials, setting, methods: Fresh ICSI cycles with embryo transfer were cultured either in the EmbryoScope© (n=864) or in a standard incubator (n=715). Fertilization, implantation, clinical pregnancy and live birth/ongoing pregnancy rates were compared among the groups. Obstetric and perinatal outcomes were compared among singleton and twin newborns in the groups. Z-scores were calculated based on customized birthweight data in a cohort of Dutch newborns. Statistical analyses were performed by Fisher´s exact test or Mann Whitney test.
Main results and the role of chance: There was no difference among female age and stimulation in the groups, although patients in the TLS had more oocytes (9.396±5.012 versus 9.055±5.993, p=0.0159) and consequently more zygotes in culture (3.469±1.747 versus 3.257±1.801, p=0.0152), embryos transferred (1.561±0.5012 versus 1.478±0.5055, p=0.001) and surplus blastocysts frozen (0.4549±0.8839 versus 0.3538±0.7701, p=0.0191) than the SI group. Nevertheless, the fertilization rate was significantly lower in the TLS group (62.72% versus 66.14%, p=0.0099). On the other hand, implantation (29.42% versus 25.45%, p=0.031) and clinical pregnancy rate (39.24% versus 33.85%, p=0.0278) were higher in the TLS group but no difference could be found for live birth/ongoing pregnancy rate (92.22% versus 92.69%, p=0.8712). We could not observe any significant differences in obstetric and perinatal outcomes for singletons among the groups. To avoid the birthweight paradox, we also compared the groups for the Z-scores and could not find any significant differences among the groups. Surprisingly, for twins, SI resulted in higher GA (37.08 weeks versus 35.99 weeks, p=0.0035), less PTB (20% versus 47.87%, p=0.0035), higher birthweight (2511±620.4g versus 2284±634.9g, p=0.0023), higher height (47.5±3.976cm versus 46.2±3.7cm, p=0.013) and less LBW (37.50% versus 59.57%, p=0.0236) than the TLS group. Data are presented as mean ± standard deviation or numbers (percentage).
Limitations, reasons for caution: This is a non-randomized retrospective analysis of a small cohort of patients. Furthermore, not all residual confounding factors could be considered, such as social background, smoking habits, length and cause of infertility.
Wider implications of the findings: Improving current incubation systems may be a valuable step towards more physiological and embryo-friendly *in vitro* culture. Fewer disturbances of embryos may favour not only embryo development *in vitro*, but also foetus growth and neonatal outcomes. However, positive effects of group culture should be considered to improve our current systems.
Trial registration number: Not applied.

P-209 Positive correlation trend between implantation potential of fresh Day 5 transfers, and a morphokinetic based blastocyst scoring system– a retrospective analysis of 318 treatments.

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Study question: Is there a correlation between implantation potential of fresh blastocyst transfer and a universal blastocyst morphokinetic ranking model (KIDScore D5 v2).
Summary answer: Retrospective analysis of 318 known implantation treatment cycles shows that there is a correlation trend between morphokinetic score and implantation potential.
What is known already: The use of time-lapse technology to improve embryo selection is increasing. A challenge of using selection and deselection models is that they may not perform equally as well when applied in a different laboratory setting. Furthermore, some of the reported models do not incorporate development events past cleavage stage and therefore may not be as applicable to Day 5 embryo evaluation. A new universal model for predicting potential of implantation has been developed using multicenter known implantation data of fresh Day 5 transfers (KIDScore D5 v2, Vitrolife A/S).
Study design, size, duration: Retrospective Time-lapse analysis of 318 treatments with 351 blastocyst transfers from September 2015 until December 2018 having Known Implantation outcome. All embryos were cultured in the EmbryoScope or EmbryoScope+ time-lapse systems. Embryo development was annotated in the EmbryoViewer software. Model scores were calculated and compared with actual implantation ratio.
Participants/materials, setting, methods: Females of age range from (24-46) with a median age of 37. KIDScore values (continuous scores range from 1-9.9) were grouped into roughly five equal data size sets for analysis, and implantation ratios for each category were calculated. Statistical analysis was performed with R v3.4.4. Differences between groups were tested using Wilcoxon rank sum test.
Main results and the role of chance: KID ratios for the grouped KIDScore value categories were as follows: (score range, implantation rate,n) (1.0-6.5,21.9%,64) (6.6-7.2,34.2%,73), (7.3-7.7, 40%, 65), (7.8-8.2,42%,73), (8.2-9.9, 64.5%, 76). There was a clear trend between KIDScore D5 values and implantation rate. The implantation rate of scores less than of 8.2 and those higher than 8.2 were statistically significant different ($p<0.001$).
Limitations, reasons for caution: Our clinic performs all cycles in a time-lapse settings and therefore makes use of time-lapse information to select embryo. This may present some bias in the embryos selected for transfer. Larger datasets are required to confirm these findings and to determine score differences which represent statistically significant differing implantation potential.
Wider implications of the findings: The use of a morphokinetic Day 5 scoring model may assist in prioritization embryo transfer when multiple equally transferable embryos are available for transfer in a fresh cycle.
Trial registration number: Not applicable

P-210 A time-lapse comparison between genetically balanced and unbalanced human embryos

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Study question: Are there differences between time-lapse variables of unbalanced human embryos and chromosomally normal embryos?
Summary answer: The time of pronuclei appearance (tPNa) and cleavage time to eight-cell stage (t8) are significantly delayed in unbalanced embryos compared with the normal embryos.
What is known already: Many morphokinetic parameters obtained from human embryos cultured in time-lapse imaging systems (TL) have been evaluated as potential biomarkers of embryo quality and successful implantation. They include static and interval time-lapse variables from the early cleavage stage to morula and blastocyst stages. However, it is still not fully clear whether balanced and unbalanced blastocysts differ in certain morphokinetic parameters.
Study design, size, duration: 214 blastocysts generated from ICSI between February 2016 and March 2017, derived from 98 patients were included in this cohort retrospective study. All embryos were cultured in single culture medium (Life Global) and a time-lapse system (EmbryoScope, Vitrolife, Sweden). After day 5 biopsy and preimplantation genetic screening (PGS) testing embryos were classified as balanced (chromosomally normal) and unbalanced.
Participants/materials, setting, methods: Fifteen morphokinetic parameters were analysed and compared between balanced (normal) and unbalanced embryos: time of pronuclei appearance (tPNa), time of pronuclei fading (tPNf), cleavage times (t2, t3, t4, t5, t6, t7, t8, t9), morulae formation time (tM), starting blastulation (tSB), full blastocyst stage (tB), expansion and hatching timing). Statistical analysis was performed by IBM SPSS Software v.21.
Main results and the role of chance: A total of 123 embryos (67.58%) were proven to be unbalanced and 91 blastocysts (32.41%) were chromosomally normal. There were no significant differences in women age, 3 Day FSH, BMI and embryo quality between the compared groups ($p>0.05$, Student t-test). However, there was a significant difference between normal and unbalanced blastocyst groups in two time-lapse variables – tPNa (6.45 vs. 6.85 hrs, respectively, P=0.037) and t8 (55.56 and 59.25 hrs, respectively, P=0.002).
Limitations, reasons for caution: The retrospective nature of this study is a limitation. Larger study with an increased number of embryos is needed to confirm our findings.
Wider implications of the findings: Specific time-lapse variables such as tPNa and t8 could be considered as potential predictors of chromosomal abnormalities. Morphokinetic embryo characteristics should be analysed and considered before a decision for taken a biopsy and PGS.
Trial registration number: Not applicable.

P-211 the effect of a transient premature luteinizing hormone surge without elevated serum progesterone on in vitro fertilization outcomes in a gonadotropin-releasing hormone antagonist flexible protocol

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Study question: What are the IVF outcomes of patients experiencing transient premature LH surge (PLHS) but without an elevated progesterone in a GnRH antagonist flexible protocol.
Summary answer: Even a transient PLHS without elevated serum progesterone will lead to a detrimental effect on the IVF/ICSI pregnancy outcomes in fresh embryo transfer cycles.
What is known already: Many researchers have concluded that IVF outcomes may be inversely related to serum progesterone levels >1.5ng/mL by affecting endometrial receptivity and damaging embryo quality.Previous studies have paid attention to the association between a poor pregnancy rate and PLHS, but they either did not exclude the accompanying elevated progesterone or only focused on the PLHS on the hCG day after GnRH-ant administration.

In addition, the live birth rate has not been reported. Whether a transient PLHS without progesterone>1.5ng/mL during ovarian stimulation has a negative effect on the IVF/ICSI outcome has not been fully explored.
Study design, size, duration: This was a retrospective cohort study including all women who underwent IVF/ICSI fresh cycles with a GnRH antagonist flexible protocol at Peking Medical College Hospital (PUMCH) between January 2016 to December 2017. Patients whose progesterone ≥ 1.5 ng/mL during ovarian stimulation were excluded. A total of 405 women who had undergone IVF/ICSI-fresh ET cycles were reported during this timeframe.
Participants/materials, setting, methods: A PLHS was defined as LH higher than threefold of it's basic level on day 2 of the same menstrual cycle with the absolute value > 5 IU/mL.119 patients (29.4%) reached the criteria for the diagnosis of PLHS with progesterone <1.5ng/mL, while the other 286 women (70.6%) were assigned to the control group.Logistic regression models were fitted to the clinical pregnancy rates and the live birth rates to reduce the relevant confounders.
Main results and the role of chance: The baseline characters and ovarian response were comparable between both groups, and they had harvested similar number of oocytes, an accordant oocyte maturation rate, and an equal rate of high quality embryo formation after 3 days of culture. However, the remaining embryos from the PLHS group, except for those freshly transferred, were less likely to be cultured into blastocysts compared to the control group (31.9% (176/551) vs 38.2% (454/1187), P = 0.011). Compared between PLHS and control group, the implantation rates were12.9% (30/233) vs 25.0% (141/536), P = 0.000; clinical pregnancy rates were 21.0% (25/119) vs 41.6% (119/286), P = 0.000 ; live birth rates were17.6% (21/119) vs 29.7% (85/286), P =0.012. After adjusting for age, BMI, bFSH, and infertility factors, the adverse effects were still as pronounced for the clinical pregnancy rate (OR = 0.39, 95% CI = 0.24-0.66) and live birth rates (OR = 0.54, 95% CI = 0.32-0.93.According to the POSEIDON Criteria, we found more women belonging to the Group II (age≥35; ovum retrieved<9) and less from the Group III (age<35; ovum retrieved≥9) experienced the PLHS, compared to the controls (Group II 20.2%(24/119) vs 11.9%(34/286), P=0.030; Group III 11.8%(14/119) vs 20.3%(58/286), P=0.041).
Limitations, reasons for caution: we only looked into fresh cycles in this study. PLHS possibly deteriorates the potential for embryo growth and implantation, and so are the cumulative live birth rates. Our hypothesis should be further explored in frozen–thawed cycles before it is extrapolated to multiple subsequent live births within the same IVF/ICSI cycle.
Wider implications of the findings: There is a strong inverse relationship between the transient PLHS and the pregnancy outcomes, although the surge was suppressed immediately with the antagonist and no elevated progesterone was generated. An earlier antagonist administration may be a possible preventive measure, especially for those women with advanced age but highly responsive ovaries.
Trial registration number: not applicable.

P-212 Maternal Body Mass Index impacts on morphokinetic parameters of embryo development: a time-lapse study.

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Study question: Does maternal body mass index (BMI) affect embryo morphokinetic parameters assessed by time-lapse technology?
Summary answer: Maternal overweight and obesity affect morphokinetic parameters during embryo culture and are associated with slower embryo development.
What is known already: Maternal obesity impairs natural and assisted reproduction regardless the occurrence of ovulatory disorders, being also associated with spontaneous abortions, preeclampsia, preterm delivery and gestational diabetes. The relative importance of the impact of high BMI on oocyte/embryo quality in the mechanisms undermining fertility is unknown. Static morphological analysis has not been capable to detect any impact of obesity on embryo quality. Since embryo developmental competence is not solely reflected by morphology, but also associated with develop-

mental speed, a dynamic evaluation of morphokinetic parameters appears necessary for a more reliable assessment of the impact of BMI on embryo competence.

Study design, size, duration: This is a retrospective study including 1528 intracytoplasmatic sperm injection (ICSI) cycles performed from January 2012 to December 2017, in which time lapse technology and static embryo morphology were utilized to assess embryo development across different BMI groups. Main morphokinetic endpoints were time intervals from the beginning of embryo culture to reach two (t2), three (t3), four (t4), five (t5) and eight (t8) blastomeres-stages. Multivariate linear regression was used to analyze continuous variables.

Participants/materials, setting, methods: Women included in this study were in their first ICSI cycle utilizing autologous oocytes. The cohort was divided into four groups: 593 embryos from 128 underweight women (BMI<18.50 kg/m^2) in group A; 5248 embryos from 1107 normoweight women (BMI 18.50–24.99 kg/m^2) in group B; 1053 embryos from 226 overweight women (BMI 25.00–29.99 kg/m^2) in group C and 286 embryos from 67 obese women (BMI≥30 kg/m^2) in group D.

Main results and the role of chance: A total of 1528 ICSI cycles and 7180 embryos were assessed with time lapse technology. No statistical differences across BMI groups were found for female age, male age, number of oocytes retrieved, percentage of mature oocytes and embryo quality score based on static morphology. Data were normalized for maternal age, paternal age and cause of infertility. Embryos from obese women showed a delay in t5 in comparison with normoweight women [50.84 h (46.31 - 55.29) *vs.* 49.24 h (45.69 - 53.22), adjusted p <0.05)], while embryos from both overweight and obese patients showed a delay in t8 in comparison with normoweight women [56.72 h (51.83 - 63.92) and 57.89 h (51.60 - 65.94) *vs.* 55.66 h (50.89 - 62.89), respectively, adjusted p <0.01]. These results suggest that maternal obesity and overweight slows embryo development during embryo culture, which can be observed by assessing embryo morphokinetics, but not static morphology.

Limitations, reasons for caution: We acknowledge that our study is limited by its retrospective nature and by the utilization of data generated in a single IVF center.

Wider implications of the findings: Our results suggest that standard embryo morphology is not sufficiently accurate to detect effects of obesity and overweight on embryo development. The use of time lapse technology may be useful to improve embryo selection and ART prognosis for obese and overweight patients.

Trial registration number: None

P-213 Dual buffering handling medium is better on Blastocyct development

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Study question: Does dual buffering handling medium has superiority over single buffering handling medium on in vitro oocyte handling?

Summary answer: Blastocyst development and cryopreservation of embryo rates are significantly higher in dual buffering medium. No differences in fertilization and on going pregnancy rates.

What is known already: Appropriate culture conditions is needed for gametes and embryos to minimize stress of in vitro conditions. Therefore a stable pH is crucial for oocytes which are lack of robust mechanisms especially without protective cumulus cells. Some laboratory procedures like oocyte pick-up, denuding oocytes or microinjection are susceptible to pH fluctations which can affect outcomes. Most IVF handling media utilize HEPES or MOPS to stabilize enviromental pH as single-buffering systems. Use of dual buffering system containing HEPES and MOPS together may have beneficial effects over single systems.

Study design, size, duration: Prospective randomized comperative study from 2018 to 2019. Fresh Tese+ICSI and Thaw Tese+ICSI patients excluded from the study. In total 385 fresh ICSI cycles randomized as 212 dual buffering medium(MOPS+HEPES) usage and 175 single buffering medium (HEPES) usage. In all the procedure like oocyte pick-up, denudation of oocytes and microinjection, one of these handling mediums used. When we see at least three good quality embryos on day three we extended the culture to day 5.

Participants/materials, setting, methods: We used as a dual buffering handling medium MHM (Irvine Scientific) and a single buffering medium QA with HEPES (CooperSurgical). Primary outcome measure was fertilization and ongoing pregnancy rates between the groups and also day 5 blastocycst development and cryopreservation rates was evaluated. Groups were comparable according to women age. Presence of fetal heart beats at 12 weeks of gestation assumed as ongoing pregnancy positive. Chi-square is the statistical test. P<0.05 is significant.

Main results and the role of chance:

Table A	MHM (MOPS+HEPES) n=212	QA (HEPES) n=175	P Value
Fertilization rates in general	% 69 (1090/1578)	% 66.5 (833/1252)	0.15
On going Pregnancy rates in general	% 42.4 (90/212)	% 39.4 (69/175)	0.54
Day 5 blastocycst transfer rate	% 52.8 (112 / 212)	% 40.5 (71/175)	0.016
Fertilization rates in day 5 blastocycst group	% 71.6 (802/1119)	% 69.1 (536 / 775)	0.2
On going Pregnancy rates in day 5 blastocycst group	% 55.3 (62/112)	% 53.5	0.8
Cryopreservation rates of good quality surplus embryos in general	% 50 (106/212)	% 37.1 (65/175)	0.011

According to our results there is no significant difference between fertilization rates and on going pregnancy rates in general groups. And also there isn't a significant difference in day 5 blastocycst formation groups according to fertilization and ongoing pregnancy rates. However day 5 blastocyst transfer rate is significantly increased with dual buffering handling media and cryopreservation of good quality surplus embryos increased significantly in dual buffering handling medium group (Table A).

Limitations, reasons for caution: This study is compared HEPES+MOPS medium and HEPES only medium, therefore may not fully reflect the situation of MOPS only buffered medium.

Wider implications of the findings: This study demonstrates that dual buffering handling medium(MOPS+HEPES) has more stable pH as a result with better and more blastocycst development than single HEPES buffering medium. And though more embryos for cryopreservation which may be good for PGD on the way for getting a healthy baby.

Trial registration number: not applicable

P-214 Granulocyte macrophage colony-stimulating factor (GM-CSF) influences pre-implantation embryos by modulating cell fate and cell identity in the trophectoderm

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Study question: Does GM-CSF influence expression patterns of cell lineage markers in murine blastocysts during pre-implantation development?

Summary answer: Mouse embryos cultured in medium supplemented with GM-CSF show a dose-dependent increase of ectopic expression of the pluripotency marker NANOG in trophectoderm cells.

What is known already: GM-CSF is a cytokine influencing the maternal-fetal interface and supporting placental development in mouse and human. It is expressed in epithelial cells of the endometrium under regulation of estrogens. A large clinical trial showed that GM-CSF supplemented embryo culture medium leads to an increased survival of embryos up to week 12. Especially in women with previous miscarriages GM-CSF supplementation of culture medium improved implantation rates. Animal and cell culture studies on isolated trophectoderm cells supported an effect on cellular expansion and pluripotency pathways after exposure to GM-CSF.

Study design, size, duration: Mouse zygotes were produced *in vivo* and cultured *in vitro* up to the blastocyst stage (day 4.5) in culture media supplemented with mouse and human recombinant GM-CSF (1, 2, 5, 10 and 20 ng/ml). A total of 715 blastocysts were fixed and subjected to immunohistochemical staining. Markers of the three cell lineages trophectoderm (CDX2), epiblast (NANOG) and primitive endoderm (SOX17) enabled selective scoring of absolute cell numbers. 604 immunostained blastocysts were eligible for further analysis.

Participants/materials, setting, methods: 68 female mice (B6C3F1, 5-10 weeks old) were superovulated by injection of 5 IU PMSG and 10 IU hCG and mated to males (C57BL/6J). Zygotes were isolated on day 0.5 post coitum, pooled and randomly allocated to KSOM(aa) with or without GM-CSF. Medium refreshment was applied on day 2.5.

Main results and the role of chance: 604 blastocysts were scored. Total blastocyst cell numbers changed significantly between KSOM(aa) with 0, 2 and 5ng/mL mouse GM-CSF and between 2 and 5ng/mL and 20 ng/mL ($p<0.005$; mean values: 0 ng/mL mGM-CSF: 80.2±18.8; 1ng/mL: 84.5±15.7; 2ng/mL: 90.9±16.7; 5ng/mL: 91.4±13.5; 10ng/mL: 86.1±14.9; 20ng/mL: 76.2±17.1). Total cell numbers did not change in human GM-CSF groups (mean values: 0 ng/mL hGM-CSF: 78.4±18.7; 1ng/mL: 73.9±14.8; 2ng/mL: 74.7±14.5; 5ng/mL: 75.7±13.4; 10ng/mL: 78.7±15.6; 20ng/mL: 68.0±17.7). 519 of 604 embryos (86%) showed normal expression of specific cell lineage markers, whereas 85 of 604 embryos (14%) showed an ectopic expression of NANOG among CDX2-positive trophectoderm cells at increasing concentrations of either mouse or human GM-CSF supplemented media. While embryos cultured in medium supplemented with mouse GM-CSF showed abnormalities only in the highest concentrations (KSOM(aa) +10ng/mL: 40%; +20ng/mL: 39%), embryos cultured in human GM-CSF showed abnormalities already from the lowest concentration onwards (0 ng/mL: 0%; 1ng/mL: 11%; 2ng/mL: 15%; 5ng/mL: 29%; 10ng/mL: 24%; 20ng/mL: 35%).

Limitations, reasons for caution: This study was performed in a mouse model and would need further investigation in other animal models or experiments with human embryos to elucidate and validate the influence of GM-CSF on ectopic NANOG expression and its consequences on trophectoderm cells during pre-and post-implantation development.

Wider implications of the findings: GM-CSF has led to an ectopic expression of the pluripotency marker NANOG in trophectoderm cells. This may be due to a change of identity because of a false reprogramming or due to change of location because of false positioning. Either way, these errors could impact the pre- and post-implantation processes.

Trial registration number: DFG NO 413/3-3 and BO2540/4-3

P-215 Pilot study of novel noninvasive imaging approach for determination of meiotic status in intact human cumulus-oocyte-complexes.

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Study question: Can the maturation status of intact human cumulus-oocyte-complexes (COCs) be determined noninvasively without removal of cumulus cells?

Summary answer: Combinatorial imaging using of intact COCs revealed properties of cell-cell and cell-zona adherence correlated with oocyte meiotic stage and quality in 36/38 samples tested.

What is known already: Cumulus cell metabolic support is vital to acquisition of developmental competence in human oocytes. It may be compromised when removing somatic cells to visualize maturity status and to enable ICSI. Resistance to gonadotropin stimulation, as occurs with advancing maternal age, often leads to the retrieval of immature oocytes that, once denuded, exhibit impaired developmental potential.

Study design, size, duration: Prospective cohort study, to evaluate oocyte maturity in intact COCs.

Participants/materials, setting, methods: Intact COCs retrieved from 11 patients undergoing routine IVF. Five (5) were oocyte donors (24-29 years), and 6 were infertility patients (36-43 years). Individual COCs were imaged under polarization, relief contrast, and bright field optics in 35mm glass-bottom dishes for 5-7 min at 36 degrees C, and incubated for 2-4 hours prior to stripping with hyaluronidase. Each sample was re-imaged, and mature oocytes underwent ICSI with resulting embryos cultured to day-3.

Main results and the role of chance: Combinatorial optics and image analysis revealed distinct changes in zona pellucida (ZP) and adhesion sites of cumulus cells to the outer surface of the ZP. In 36 of the 38 samples analyzed. Oocyte maturity status was predictable based on retardance values in the ZP (positive if mature in 18/24 M2s; negative in 10/12 GV, M1, or atretic oocytes). An inverse correlation was noted between stage of maturity and cumulus cell cortical birefringence for cells remaining attached to the ZP.

Limitations, reasons for caution: Descriptive study awaits confirmation that imaging conditions do not impair oocyte quality or adversely affect pregnancy chances after IVF. As a pilot study, this work is limited by low number of samples and need to optimize laboratory workflow.

Wider implications of the findings: If confirmed, noninvasive imaging approach may offer poor prognosis patients opportunities to conceive using autologous oocytes by allowing such oocyte to achieve maturation under conditions retaining cumulus cell somatic support. Moreover, this technique should aid in the optimization of *in vitro* maturation protocols for patients undergoing fertility preservation.

Trial registration number: n/a

P-216 Laser Assisted Hatching in cryopreserved blastocysts: a randomized study.

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Study question: Does LAH improve the clinical outcomes in embryo cryopreservation program?

Summary answer: LAH could enhance clinical pregnancy rates in vitrified/warmed embryo cycles.

What is known already: Assisted Hatching (AH) has been proposed as an useful tool to improve the outcomes of different groups of patients, like advanced Maternal Age, implantation failure, Zona Pellucida defects or frozen embryos transfers.

AH can be performed with two techniques: chemically or mechanically.

Not clear evidences have been published about real benefits of AH in previous mentioned groups(Some papers were published for and against, without find clear evidences of the benefits of AH).

With the appearance and improvement of the Laser devices for PGD cycles, Laser devices were proposed to do AH (LAH) taking into account the safety, effectivity and reliability of this technique.

Study design, size, duration: This RCT was taking out between September 2018 and December 2018. Patients underwent blastocyst (day 5/6) cryotransfer were considering. Patients underwent PGD cycles and day 3 frozen embryo transfers were excluded. A total number of 88 patients were included in the study: 44 LAH cycles and 44 without LAH.

Participants/materials, setting, methods: Oocyte origin (fresh/frozen, and autologous/donor), age and blastocyst quality were recorded, and randomizely, LAH or not were realized in every warmed blastocyst.

We correlated LAH with implantation: gestational sacs/embryos transferred. Clinical pregnancy(CP)was confirmed with the presence of fetal heart beat by ultrasound.

The size of the Zona Pellucida hole was standardized with the width of one-single 2 ms shoot in Octax Laser system (Vitrolife).

Chi-square test was performed as statistical analysis, and $p<0.05$ were considered statistically significant.

Main results and the role of chance: A total of 37 CP were achieved, 50% in LAH group and 34.9% in non LAH group (p=0.1306).

Regarding oocyte origin, in autologous cycles 9 pregnancies out of 21 cycles with LAH were achieved (42.8%) and 6 out of 17 cycles without LAH (35.3%; p=0.6353). In oocyte donated cycles, 13 out of 23 CP were achieved in LAH group and 9 out of 26 in non LAH cycles (56.5% vs 34.6 %; p=0.1239 respectively).

Donated oocyte cycles (49) were also divided according with oocyte status (fresh or frozen) and no statistical differences were found. With fresh oocytes, 5 CP were obtained (55.6%) using LAH, and 4 without it (30.8%; p=0.2450) and in the frozen-donated group, the results were similar (57.1 % vs 38.5 %; p=0.3317, respectively).

Limitations, reasons for caution: This preliminary data must be confirmed increasing the size of the population, in order to improve the robustness of the conclusions.

Wider implications of the findings: No differences were observed between groups, considering autologous and donated oocytes, and fresh and frozen oocytes. Nevertheless, we found a point in favour of performing LAH, and no detrimental effect.

Trial registration number: Not applicable

P-217 Live birth following embryo transfer using new media composed of human oviductal amino acid fluid for IVF-ET: A randomized trial.

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Study question: Is human oviductal amino acid medium more clinically effective in human IVF than the commonly used single step medium?

Summary answer: Media composed of human oviductal amino acids better enhance embryonic ability than the current single step media.

What is known already: Sequential media and single step media are the most widely used embryo cultures in human IVF. The amino acid concentrations of most of these media were set in 1959 by Dr. Eagle on the basis of somatic cell requirements such as HeLa cells and L cells. However, these animal based media do not reflect the requirements of human embryos. This may negatively impact on human embryo developments in vitro. Efficacy of media composed of amino acid concentrations of human oviductal fluid has yet undergo rigorous analysis.

Study design, size, duration: Human oviductal fluid samples were collected in our clinic laparoscopically from 28 women aged 26-39 years, and were analyzed to formulate the components of new embryo culture media. In 2017, medium composed of amino acid concentrations of human oviductal fluid became available in Japan. We conducted an RCT to evaluate the new medium using 2,986 embryos from 581 cycles of patients who underwent IVF or ICSI between August 2017 and December 2018.

Participants/materials, setting, methods: Before fertilization, the oocytes were randomly divided into two groups: cultured using the new medium composed of human oviductal amino acid (OVIT, FUSO Pharmaceutical Industries, Japan); and cultured using the current medium (Medium A). The embryo development status during culture period (day 0 to day 5) and clinical outcome after embryo transfer (ET) to the uterus were compared using OVIT and Medium A group. After the ET, subsequent outcomes of neonatal findings were evaluated.

Main results and the role of chance: Between the two groups, patient characteristics including women's age, husband's age, the number of previous IVF-ET failure cycles were not significantly different. The number of blastomeres on day 3 was significantly larger in the OVIT group than in the Medium A group (7.3±2.1 versus 7.1±2.2, $P<0.05$). The OVIT group showed significantly higher rates of blastocyst development than the Medium A group (60.5% (904/1,493) versus 56.2% (839/1,493), $P<0.01$). The number of embryos which were elected for ET or cryopreserved was larger in the OVIT group compared with the Medium A group (45.1% (673/1,493) versus 36.6% (547/1,493), $P<0.01$). Furthermore, in the OVIT group had higher implantation rates after vitrified - warmed ET than that in the Medium A group (37.4% (82/219) versus 31.6% (54/171)). Neonatal birth weight showed a similar level after the vitrified-warmed ET using the OVIT versus the Medium A: Birth weight of the OVIT group was 3,150.3±461.6 grams (n=21) versus the Medium A group was 3,127.8±387.0 grams (n=9). The ratio of male to female was 11/10 in the OVIT group (n=21), and 3/5 in the Medium A group (n=8). One trismus nascentium child was confirmed in the OVIT group.

Limitations, reasons for caution: Due to this research being conducted by only one clinic, the sample size is limited.

Wider implications of the findings: Our findings indicate that the new media composed of human oviductal amino acid may improve the embryo development and the implantation rate. More embryos were able to be transferred or frozen using the OVIT, thus, the chance of a successful pregnancy rate may be better than with the current media.

Trial registration number: UMIN-CTR (UMIN000030904)

P-218 Outcomes of in vitro fertilization-embryo transfer in poor ovarian responders with growth hormone-pretreated: a prospective cohort study.

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Study question: Are the IVF-ET outcomes different in poor responders after pretreatment with growth hormone compared to non-pretreatment women?

Summary answer: Pretreatment with growth hormone improves ovarian response to stimulation and embryological parameters in women with poor ovarian response in IVF-ICSI cycles.

What is known already: Growth hormone is used as an adjuvant therapy in ART for poor ovarian responders, which is associated with ovarian steroid and follicular development. It remains unclear, whether poor ovarian responders with growth hormone-pretreated who undergo IVF-ICSI have different response to ovarian stimulation and whether the reproductive outcomes in these women are different from those in women without pretreatment.

Study design, size, duration: A prospective cohort study was conducted at the Reproductive Medical Center of Peking University Third Hospital from Jun to July, 2018. The participants included poor-responder women who fulfilled the criteria defined by ESHRE consensus and had at least one controlled ovarian hyperstimulation cycle without oocyte or embryo achieved or non-pregnancy after embryo transfer. Overall 184 women were included in the study.

Participants/materials, setting, methods: Women who willing to receive treatment with growth hormone over 30 days averaging 2 IU per day were included in study group (n=92). Propensity score matching (PSM) method was used to choose control group in our database by matching age and body mass index (BMI) (n=92). The evaluation parameters included ovarian response and embryological parameters, and pregnancy outcomes after fresh embryo transfer.

Main results and the role of chance: Demographic characteristics were comparable between the two groups. The duration and amount of gonadotrophin therapy tended to be shorter in the participants treated with GH, but the difference did not reach statistical significance (p=0.104 and p=0.570, respectively). Peak E2 level were significantly higher in the GH group (p=0.003), but there was no difference in the mean endometrial thickness on the day of hCG trigger between the two groups (p=0.844). The mean number of retrieved oocytes was significantly higher after GH pretreatment (8.29±4.82), than in controls (6.02±3.27), p=0.000. The fertilization rate was significantly higher in women treated with GH than in controls, $p < 0.05$. The number of available embryos in the GH group was 3.89±2.97 and in control

group was 2.52±1.81, was significant difference in favor of GH pretreatment, p=0.000. In women treated with GH the implantation rate was 12.4% and in the control group, the implantation rate was 9.8%. Clinical pregnancy rate and ongoing pregnancy rate per fresh embryo transfer cycle were 17.9% and 16.1% in women treated with GH, and 12.3% and 10.5% in controls, respectively. Miscarriage rate was 10.0% in women from the GH group and 14.3% in controls.

Limitations, reasons for caution: The important limitation of our study was its small sample size. Due to the study duration constraints, follow-up did not achieve live births.

Wider implications of the findings: One month pretreatment with GH increases ovarian response to stimulation and improves oocyte and embryo quality in low prognosis patients with diminished ovarian response. There is a possible beneficial effect on clinical pregnancy, but this needs to be confirmed in larger studies.

Trial registration number: Not applicable

P-219 Does increased number of oocytes retrieved improve the cumulative live birth rate in women aged 40-44 years?

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Study question: Does increased number of oocytes retrieved improve the cumulative live birth rate in women aged 40-44 years?

Summary answer: The cumulative live birth rate per ovum pick-up was maximal in women aged 40-44 years whose number of oocytes retrieved was beyond 20.

What is known already: Advanced maternal age is a strong predictor of live birth in IVF treatment, especially for those who are over 40 years old. It is clear that the age-related reduction in fecundity is primarily due to oocyte aging, which includes the decrease in oocyte number and the increase in aneuploidy rates.

Study design, size, duration: A retrospective cohort study of 1660 IVF/ICSI cycles was performed at the Reproductive Medicine Center of the sixth affiliated hospital of Sun Yan-Sen University from 2013 to 2017. Outcome data were compared with t-test, Chi-squared test and Cochran-Armitage test for trend.

Participants/materials, setting, methods: Infertile patients aged from 40-44 years were categorized into five groups based on the number of oocytes retrieved (0-5, 6-10, 11-15, 16-20 and >20 oocytes). For the purpose of this study, cycles involving oocyte donation, oocyte thawing or patients who had not achieved a live birth but still had frozen embryos left were excluded from our analysis.

Main results and the role of chance: Basic characteristics of live birth group and no live birth group:

There was no significant difference in duration of infertility, insemination method, BMI or fertilization rate between live birth group and no live birth group. The number of oocyte retrieved(8.0±6.07 vs. 4.75±3.7, P<0.05) and transferable embryo (4.53±3.43 vs. 2.53±1.95, P<0.05) was significantly higher in live birth group

Women in live birth group was significantly younger (41.06±1.15 vs. 41.67±1.30, P<0.01).

Cumulative live birth in five groups:

The overall cLBR was 14.7 % per ovum pick up. There was a positive correlation between the number of oocytes retrieved and cLBR. The cumulative live birth in five different groups was 9.8%(0-5), 18.9%(6-10), 24.6%(11-15), 45.7%(16-20) and 80.0%(>20), respectively. Statistical testing by means of Cochran-Armitage test of trend revealed a trend that cLBR was elevated with the increasing number of oocytes (P<0.05).

Limitations, reasons for caution: As a retrospective study, our analysis depends on previously recorded data which means certain variables could not be collected.

Wider implications of the findings: Women over 40 years of age are faced with diminishing opportunities to achieve a live birth. Our results suggest that increased number of oocytes retrieved improves the cumulative live birth rate.

Trial registration number: not applicable

P-220 Optimising a vitrification programme – lessons learned

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Study question: Have changes made to a vitrification programme improved blastocyst survival rates and clinical outcomes?

Summary answer: Use of Cryotop, Embryo Glue as post-warming medium and artificial blastocoel collapse using laser significantly improved the blastocyst survival rates and clinical outcomes.

What is known already: In the UK the drive to reduce multiple pregnancy rates has lead to an increase in single embryo transfers. Therefore, a good vitrification programme is essential to preserve surplus good quality blastocysts. It is known that survival rates can be influenced by storage devices (closed vs open systems) and use of artificial blastocoel collapse (mechanical or laser). There is a lack of information on the use of different post-warming media and their impact on clinical outcomes. Also there is an ongoing debate as to whether vitrified blastocysts can be safely stored in a vapour phase of nitrogen.

Study design, size, duration: A retrospective data analysis of frozen embryo transfer (FET) cycles performed between May 2010 and December 2018. A total of 947 day 5 blastocysts were thawed in 722 FET cycles. Since February 2013 the majority of embryos have been stored in a vapour phase. Treatment cycles (n=64) using High Security Vitrification (HSV) straws were only included for the comparison of the storage devices; due to significantly reduced clinical outcomes they were excluded from further analysis.

Participants/materials, setting, methods: Patients (mean age 34.7±4.7 years) underwent a hormone regulated FET cycle. Top quality (grade ≥3Bb) blastocysts were vitrified using Medicult Vitrification solutions and HSV straws (closed system) or Irvine Scientific solutions and Kitazato Cryotop (open system). From March 2016, blastocoels were artificially collapsed using laser prior to vitrification. Embryos were transferred after 2 - 3 hour incubation post-warming, in either culture medium (CM), CM with 20% human serum albumin (CM20%) or Vitrolife Embryo Glue (EG).

Main results and the role of chance: Survival rates (SR) and clinical outcomes: pregnancy rates (PR), clinical pregnancy rates (CPR) and implantation rates (IR), have progressively improved since our vitrification programme was introduced in 2009. Changing from HSV straw to Cryotop did not improve SR (87% vs 89% respectively) but significantly (P<0.01) improved clinical outcomes (PR: 21% vs 57%; CPR: 11% vs 43%; IR: 12% vs 39%, respectively). Embryos stored in a liquid phase of nitrogen (n=101) had significantly (P<0.05) greater SR (96%) compared to vapour stored (n=496) embryos (92%) however, clinical outcomes did not vary. Embryo Glue significantly (P<0.01) improved SR and clinical outcomes compared to CM and CM20% (SR: 92% vs 86% and 80%; PR: 65% vs 55% and 49%; CPR: 51% vs 42% and 40%; IR: 49% vs 41% and 39%, respectively). Laser artificial blastocoel collapsing further increased SR to 97% (P<0.01) and clinical outcomes (PR to 70%, CPR to 56% and IR to 54%), although not statistically significant. Overall, EG had the most positive impact on PR (OR 1.57, 95% CI 0.95-2.6, P=0.18) and laser artificial blastocoel collapsing on CPR and IR (OR 1.6, 95% CI 0.95-2.6, P=0.18; OR 1.4, 95% CI 0.91-2.15, P=0.124, respectively) irrespective of liquid nitrogen phase.

Limitations, reasons for caution: Not a randomised controlled trial. The group sizes relatively small for some variants and uneven distribution of cycles between groups. Patient's age was not taken into consideration.

Wider implications of the findings: In accord with current literature, open system and laser artificial blastocoel collapsing significantly improved survival and clinical outcomes. Other factors such as post-warming medium also play a role in the vitrification programme and should be considered. Blastocysts can be stored in a vapour phase of nitrogen without compromising success rates.

Trial registration number: Not applicable.

P-221 Fresh cycle outcome as a possible predictive factor of post-warming survival and pregnancy in the same cohort of egg donation cycles. A retrospective study.

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Study question: Within the same donor cohort, can a previous fresh oocyte cycle predict post-warming survival and clinical outcome of a sibling vitrified oocytes cycle?

Summary answer: Clinical outcome of a donor fresh cycle can not estimate neither the survival rate nor the pregnancy rate of a later vitrified sibling oocytes cycle

What is known already: Clinical outcomes with vitrified and fresh oocytes are comparable in egg donation cycles.

Donor's oocyte survival can not be estimated by baseline characteristics, oocyte morphology, storage time or stimulation parameters. Only vitrification within 2 hours post follicular puncture has shown to be significant.

Actually, the pregnancy rate increases progressively with the number of vitrified eggs consumed.

Study design, size, duration: Retrospective study performed since August 2012 to September 2018.

A total of 3425 mature oocytes (MII) from 154 egg donations were distributed between synchronous recipient (1808 MII inseminated in fresh cycles) and asynchronous recipient (1617 vitrified MII).

Participants/materials, setting, methods: On the retrieval day, after denudation, the oocytes assigned to the synchronous recipient were inseminated and the rest, assigned to the asynchronous recipient, were vitrified. Vitrified eggs were inseminated by ICSI 2-3 h post warming and the embryo transfer was performed on day 3. Survival rate, fertilization rate and clinical outcome were reported.

$\chi 2$ test and Mann-Whitney test were used for the statistical analysis, both two-tailed with a significance level set to $\alpha = 0.05$.

Main results and the role of chance: A previous positive pregnancy with fresh oocytes does not affect either the oocyte survival rate (77.7% vs. 78.8%), the fertilization rate (72.2% vs. 71.9%) nor the pregnancy rate (44.7% vs. 36.8) of a later vitrified sibling oocytes cycle. No significant differences were found.

Limitations, reasons for caution: Oocytes from healthy young donors were used in this study, so the results should not be extrapolated to others populations.

Wider implications of the findings: No factors can predict post-warming survival or the clinical outcome in donors.

Oocytes from a donor which are able to get a fresh pregnancy are not more resistant to vitrification and do not have any implications in the clinical outcomes of the later thawing cycles.

Trial registration number: none

P-222 Spent culture media from embryos transferred at day 5 show significant altered protein profile subject to ART outcome possibly allowing non-invasive embryo assessment

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Study question: Do media from embryos show a significant altered protein profile subject to IVF outcome?

Summary answer: Media from implanted embryos show a significant altered protein profile compared to media from non-implanted embryos with MCP-1, SELE and PECAM-1 as possible key proteins

What is known already: Successful implantation requires a receptive endometrium a well as a competent blastocyst. In order to improve ART outcome, non-invasive embryo assessment is gaining more and more attention. Progress in methodology allows collecting more information about blastocyst metabolism. However, so far no satisfying analysis tool has been established to determine the secretome, mainly due to the small volume of blastocyst media used in IVF culture. The aim of this study was to investigate if protein secretion differs between blastocysts with consecutive implantation compared to those without implantation and to analyze possible interactions between these proteins.

Study design, size, duration: In this prospective study, 69 spent culture media from embryos transferred at day 5 were collected from patients undergoing IVF/ICSI-treatment at the University Hospital Heidelberg between 04/15-10/18. The study was approved by the Ethical Committee of Heidelberg University (S572/2014). Embryos were cultured using Sidney IVF® blastocyst medium (Cook K-SIBM-20) until 09/16 or CSC-Complete-Medium (Fujifilm Irvine Scientific 90165) since 10/16. Exclusion criteria were the absence of informed consent, PCOS, endometriosis and maternal age > 41 years

Participants/materials, setting, methods: Dependent on the treatment outcome, medias were subsequently divided in two groups: (1) from embryos who implanted successfully ($n = 37$) and (2) from embryos without implantation ($n = 32$). 92 proteins were measured simultaneously using the Proximity Extension Assay (PEA) technology with the Olink® CVDIII panel employing oligonucleotide-labeled antibodies. Results are presented in Normalized Protein eXpression (NPX) values, which is an arbitrary unit on a log2-scale. Statistical analysis was performed using students-t-test and Fishers-exact-test.

Main results and the role of chance: There were no significant differences in maternal age (33.95 ± 4.01 years vs 35.85 ± 3.65 years), maternal BMI (26.20 ± 4.84 kg/m^2 vs. 24.29 ± 2.73 kg/m^2), embryo quality, infertility treatment (ICSI in 59.45% vs 50.00%), percentage of frozen IVF-cycles (16.2% vs 15.6%) or percentage of single-step-medium used (48.64% vs. 46.86%).

Media from implanted blastocysts showed significantly higher expression of ephrin type-B receptor 4 (EPHB4), activated leukocyte cell adhesion molecule (ALCAM), cystatin b (CSTB), monocyte chemotactic protein 1 (MCP-1), bleomycin hydrolase (BMH), tissue Inhibitor of Metalloproteinase-4 (TIMP4), CC-chemokine ligand 24 (CCL24), selectin E (SELE), first apoptosis signal (FAS), junctional adhesion molecule-A (JAM-A), paraoxonase 3 (PON3), plateled-derived growth factor (PDGF) subunit A and platelet endothelial cell adhesion molecule-1 (PECAM-1) compared to media from blastocyst without subsequent implantation. The highest relative expression change in media from implanted embryos compared to media from embryos without implantation can be demonstrated for PECAM-1 (1.48). We analyzed the potential protein network using STRING database (version 11.0) and found the following proteins coexpressed with each other: MCP-1 (with SELE and FAS), SELE (with MCP-1 and PECAM-1) and PECAM-1 (with SELE and potentially with EPHB4).

Limitations, reasons for caution: Main limitation of this study is the fact that two different culture media were used, even if the distribution in between the groups was similar (single-step medium in 48.64% vs. 46.86%). Due to the innovative methodology, defining a threshold for the use as biomarker remains to be assessed.

Wider implications of the findings: Our results may be used for individual non-invasive embryo assessment. In literature, especially EPHB 4, ALCAM, MCP-1, BMH, CCL24, FAS and PDGF-A have already been described in early embryonic development and metabolism. Therefore, these proteins together with SELE and PECAM-1 indicate possible biomarkers for non-invasive embryo assessment in the future.

Trial registration number: not applicable

P-223 Impact of day 5 vs. day 6 blastocyst transfer on the pregnancy outcome of frozen thawed donor recipient cycles

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Study question: To compare the pregnancy outcome and miscarriage rates in day 5 versus day 6 frozen thawed donor recipient embryo transfer cycles.

Summary answer: Similar initial pregnancy rate, however statistically higher miscarriage rate in day 6 embryo transfer group in donor recipient cycles

What is known already: There are conflicting results regarding pregnancy impact on transferring delayed development of blastocyst (day 5 vs. Day 6)

Metanalysis in 2010 suggested similar on going pregnancy rates in both day 5 vs. day 6 embryo transfer group

In 2018 there is emergence of data suggesting lower on going pregnancy rats in day 6 blastocyst transfers

none of the studies have compared exclusive donor recipient cycles where there would be low expected chance of miscarriage due to younger age of donors, thus expecting similar results in day 5 vs. day 6 blastocyst transfers

Study design, size, duration: Retrospective observational study of three years

Four hundred and fourteen consecutive donor recipient programmed frozen thawed elective single blastocyst transfer cycles (FET)..All the embryos were non Preimplantation genetic tested (PGT) embryos.

Participants/materials, setting, methods: Highgrade blastocysts were frozen by vitrification on day 5 (n=335) or day 6 (n =79). Endometrial preparation was performed using estradiol valerate. Progesterone supplementation was commenced when the endometrial thickness had reached 7mm or more. Frozen blastocysts were thawed after 5 days of progesterone supplementation and assessed immediately after thawing

Thawed blastocyst clinical pregnancy and early pregnancy loss rates in fully expanded day 5 (Group A) versus day 6 FET donor recipient cycles (Group B).

Main results and the role of chance: Statistical analysis was done using chi square test**.** All parameters like average age of oocyte donor (25.03 years vs 24.97 years,p=0.986),endometrial thickness (8.06 mm vs 8.23 mm, p=0.961)and embryo survival (97% vs 98%,p=0.776) were comparable in both the groups respectively. The clinical pregnancy rate was similar between Group A vs. Group B (50.74% versus 49.36%, P =0.825, OR 1.05,CI 0.645-1.724). Clinical miscarriage rate in Group B was 33.33% as compared to 17.05% in Group A, p=0.022, OR 2.43, CI 1.12-5.28) which was statistically significant.

Robustness of this study is good sample size

All confounding factors like adenomyosis, untreated hydrosalpinx, intramural fibroids more than 3 cm, previous recurrent miscarriages in recipient were excluded.

Limitations, reasons for caution: none

Wider implications of the findings: In donor recipient cycles having younger age of donors with low expected pregnancy loss rates, from our study it raises an important question if these cycles would perform better if preimplantation genetic testing is offered when only day 6 embryos are available for transfer .

Trial registration number: NA

P-224 The 1st polar body transfer technique: it works despite the use of fresh or thawed oocytes

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Study question: Does the 1st polar body (PB) transfer technique using both fresh and thawed donor oocytes increase blastocyst yield in patients with poor response?

Summary answer: The transfer of the patient's 1st PB to donor oocyte cytoplasm using both fresh and thawed oocytes enhances success in IVF programs

What is known already: Patients with poor response require special methods in the infertility treatment. As shown by a previous study, the application of the 1st PB transfer technique is appropriate in doubling the number of oocytes suitable for further fertilization. This technique allows getting high quality blastocysts from modified oocytes in addition to embryos derived from maternal eggs. To increase the efficiency of these manipulations, many optimizations were performed at all stages of the procedure. One of them is the use of thawed donor oocytes instead of fresh.

Study design, size, duration: The study was performed in the Medical Centre IGR from March 2017 to December 2018. It involved 168 oocytes (group A) obtained from 39 patients and 162 oocytes (group B) that were received from 28 donors. The mean age of the oocyte donors and patients was 28.2±2.4 and 40.7±5.3 years, respectively. We evaluated the number of blastocysts from maternal and modified oocytes by the 1st PB transfer obtained from fresh and thawed donor oocytes.

Participants/materials, setting, methods: We used patients' oocytes obtained from 39 women with 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). Statistical analysis was carried out using Chi-square test, Spearman correlation and pair exponential regression.

Main results and the role of chance: The total number of oocytes in this study comprised 330 cells. The mean numbers of original oocytes per patient were 4.3±2.2 and modified oocytes were 4.2±2.1. Thereby, aggregated amount of oocytes suitable for the fertilization has grown up to 8.5±4.2 per patient. 50 (29.8%) high quality blastocysts developed from the oocytes of group A. In the group B there were 29 blastocysts (17.9%), that had statistically significant difference (SSD) compared to the group A. ($p<0.01$). Spearman correlation ($r=-0.827, p<0.05$) demonstrated strong and negative correlation between the patient's age and the number of blastocysts obtained from modified oocytes. The pair exponential regression confirmed that 13.96% of the variability in the number of embryos from modified oocytes is explained by patient's age. Further, the oocytes from group B were also divided into two subgroups: fresh (n=56) and thawed (n=106) donor oocytes used for modification. Embryos obtained from fresh oocytes comprised 14 blastocysts (25.0%), from thawed oocytes – 15 blastocysts (14.5%). SSD was found between fresh and thawed subgroups ($p<0.05$), it can be explained by the average age in groups, namely 39.6±4.6 and 41.2±5.4, respectively.

Limitations, reasons for caution: Importantly, oocytes from certain donors give a greater yield of blastocysts, regardless of whether they were fresh or thawed. Therefore, the success of the 1st PB transfer technique depends directly on the accuracy of donors' selection and the ability of their oocytes to overcome the stress after performed manipulations

Wider implications of the findings: Research shows that use of the1st PB transfer technique increases the yield of high qualitative blastocysts at least by 18%. The use of thawed oocytes will allow rational use of donor material, especially in cases with low patient's oocytes number, to avoid stimulation of a personal donor for oocytes doubling.

Trial registration number: N/A

P-225 In silico assessment of different embryo time-lapse algorithms suitability for an individual clinic

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Study question: Is it possible to test and develop embryo time-lapse morphokinetic algorithm(s) for use with the Geri time-lapse system using an *in silico* testing model?

Summary answer: Simplified testing based in Microsoft Excel allows clinics to assess and develop most suitable morphokinetic algorithm for its individual circumstances using the Geri time-lapse system.

What is known already: Different morphokinetic algorithms that may be used for assessing the developmental potential of an embryo have been published, but it has been shown that these algorithms are not necessarily applicable to all clinics due to different clinical practices. Hence any algorithm, published or customised, needs to be assessed against a clinic's own clinical outcomes data. Testing algorithms within a time-lapse system may be time-consuming, whereas *in silico* testing facilitates convenient analysis of several algorithms against a large amount of data. This model allows for customisation and development of algorithm with minimal effort.

Study design, size, duration: Four different algorithms, based on published studies, were adapted to the Geri Assess® 2.0 custom algorithm format. The algorithms were then applied to time-lapse data collected in clinical laboratories utilising Geri Assess® 2.0 automated annotations feature for capturing data for developmental time points. Clinical outcomes of the embryos were then analysed and algorithms assessed for their potential to predict clinical results.

Participants/materials, setting, methods: Only non-PGT embryos were included in the study. Algorithms were based on early developmental events (PN disappearance, 2 - 6-cell divisions) in different combinations. A maximum of three different conditional rules were applied to each algorithm, the final embryo grade dictated by different combinations of whether the rules were met or not. Clinical outcomes data was captured from BabySentry™ database management system. Microsoft Excel software was used to combine and analyse all the datasets.

Main results and the role of chance: Three clinical outcomes were analysed: 1) Embryo utilisation (transfer or cryopreservation) at Day 5/6, and 2) βHCG and 3) Fetal Heart pregnancy rates after transfer of fresh or vitrified embryos at Day 5/6. Total of 3,948 embryos were included into the study. In principle, an applicable predictive algorithm should show increasing utilisation and pregnancy rates from lowest to the highest scoring embryos. When excluding embryos where algorithms didn't return a grade, two algorithms

displayed a fair predictability for embryo utilisation, the other two exhibiting weaker predictive power. Only one algorithm showed some predictivity for pregnancy outcomes, however, number of pregnancies in some categories was low, making drawing conclusion difficult. The next phase of the work will be to modify algorithms to adjust them for differences between the clinic where they were developed and the clinic where they are to be used, to assists in finding the most suitable and predictive algorithm to be used with the Geri system.

Limitations, reasons for caution: The number of embryos in some grade categories was low, especially in βHCG and Fetal Heart pregnancy groups, allowing for disproportioned distribution which impacted algorithm performance assessment. Event annotations were collected via automatic annotation software and any limitations in their accuracies might have had impact on the outcomes.

Wider implications of the findings: The ability to test and modify algorithms *in silico* with retrospective data allows accurate selection and application of an algorithm(s) most suitable for an individual clinic in an efficient manner. Besides clinical practices, the time-lapse system that is used may also have an impact to the applicability of published algorithms.

Trial registration number: Not applicable

P-226 time-lapse analysis of the relationship between cytoplasmic wave location, pronuclei formation, and the developmental competence of single pronuclei (1PN) zygotes

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Study question: Is the location of the cytoplasmic wave related to the incidence rate of 1PN zygotes and developmental competence?

Summary answer: 1PN zygotes can be formed by sperm penetrating beneath the second polar body of the oocyte.

What is known already: The rate of diploidy in 1PN zygotes is higher for in vitro fertilization (IVF) than for intracytoplasmic sperm injection (ICSI) . Furthermore, blastocysts derived from 1PN zygotes exhibit a higher rate of diploidy than embryos that have become arrested in cleavage stage. One potential cause of 1PN is fusion of the maternal and paternal genomes fuse before the pronuclear envelope has formed. During fertilization, a cytoplasmic wave originates from the initial position go the male pronucleus (PN), while the female PN is formed close to the site where the second polar body (PB) is emitted.

Study design, size, duration: We retrospectively analyzed 927 zygotes (2PN:725, 1PN:202) derived from IVF or ICSI using time-lapse microscopy (TLM).These zygotes were collected from 427 patients (age range: 26 to 47 years) between 2016 and 2018. We used only zygotes for which the presence of a second PB was confirmed.

Participants/materials, setting, methods: Following insemination, all oocytes were cultured in a TLM system from day0 and the cytoplasmic wave was recorded. Each zygote, obtained from either IVF and ICSI, were divided into two groups according to the position of the cytoplasmic wave: near Abs (NPB) and distant from PBs or unclear (DPB). We then studied whether the position of the cytoplasmic wave affected the incidence of 1PN zygotes and their developmental competence.

Main results and the role of chance: The NPB rates for 1PN zygotes from IVF and ICSI were 70.4% and 26.4% respectively. These rates were significantly higher than that for 2PN (13.7% in IVF and 5.0% in ICSI, $P<0.05$). For both IVF and ICSI, the blastocyst developmental rates of 1PN embryos weresignificantlylower than for 2PN zygotes (55.3% vs. 68.6% in IVF and 21.2 % vs. 60.0% in ICSI). Blastocyst developmental rates for IVF and ICSI in the 1PN-NPB group were significantlyhigher than for the 1PN-DPB group (69.0% vs. 13.0% for IVF; 45.0% vs. 10.9% for ICSI). Blastocyst developmental rates in the 1PN-NPB group were comparable with that of 2PN zygotes, both for IVF and ICSI. None of the 1PN embryos, derived from IVF, developed to blastocysts; in these embryos the cytoplasmic wave was clearly distant from the PBs (0/12).

Limitations, reasons for caution: 1PN embryos in the NPB group had better developmental competence. However, it remains unknown as to whether these embryos were biparental or not.

Wider implications of the findings: In most of the 1PN embryos, especially from IVF, the cytoplasmic wave originated close to the PBs and had better developmental competence. We suggest that 1PN embryos could be formed incidentally due to the position of the sperm penetration site.

Trial registration number: not applicable

P-227 Embryo diameter and implantation potential after direct unequal cleavage of the blastocyst

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Study question: Is direct unequal cleavage (DUC) an additional parameter to assist in embryo selection for blastocyst transfer?

Summary answer: In blastocyst transfer, DUC has little significance as an indicator in embryo selection because it does not affect potential blastocyst expansion and implantation.

What is known already: DUC is defined as the abrupt cleavage of one blastomere into three daughter blastomeres or an interval of cell cycles less than five hours. The incidence of DUC is 10-30%. Embryos with DUC have lower rates of blastocyst formation and expanded blastocyst formation than embryos with a normal cleavage pattern. Furthermore, on day 3 of fresh embryo transfer, embryos with DUC have lower rates of implantation and live births than embryos with a normal cleavage pattern.

Study design, size, duration: This was a retrospective study of data acquired by time-lapse imaging of human embryos during in vitro growth. A total of 379 IVF cycles were cultured and observed in a time-lapse system between May and December of 2018. Of these, 232 embryos in 189 cycles from 184 patients were analyzed on which, single frozen blastocyst transfer was performed during December 2018.

Participants/materials, setting, methods: Embryos with DUC were recorded as DUC(+), and embryos with a normal cleavage pattern were recorded as DUC(-) using time-lapse monitoring. We compared the diameter of DUC (+) and DUC (-) blastocysts at the time of cryopreservation. In addition, we compared the implantation rates and miscarriage rates in frozen-thawed single embryo transfer cycles between the two groups. Statistical significance was determined by Chi-Square test.

Main results and the role of chance: A total of 232 frozen-thawed embryos were transferred. Of these, 68 (29.3%) were DUC(+). The mean patient age (mean±SD) in DUC(+) and DUC(-) were 35.1±5.1 (95% CI: 33.9-36.4) and 35.5±4.9 (95% CI: 34.7-36.3), respectively. The mean transfer counts were 2.3±1.9 (95% CI: 1.9-2.9) in DUC(+) vs. 2.2±2.1 (95% CI: 1.9-2.5) in DUC(-). No significant differences were found regarding patient age or transfer counts between the two groups (P=0.608 and P=0.684, respectively). The diameter of blastocysts were 174.9 μm (95%CI: 168.6-181.2) in DUC(+) versus 175.4 μm(95% CI: 171.3-179.5) in DUC(-), and no statistical differences were detected (OR=0.56, 95% CI: -7.0-8.08, P=0.883). The implantation rates were 41.2% (95% CI: 30.3-53.0) in DUC(+) vs. 40.9% (95% CI: 33.6-48.5) in DUC(-), and no significant differences were detected (OR=1.01, 95% CI: 0.57-1.80, P=0.934). No significant differences were found regarding miscarriage rates (14.3% in DUC(+) vs. 19.4% in DUC(-))(OR=0.69, 95% CI: 0.20-2.34, P=0.546). Furthermore, DUC(+) showed a similar implantation rate to DUC(-) when high quality blastocysts were transferred (48.4% vs. 48.7%) (OR=0.99, 95% CI: 0.45-2.20, P=0.979). No significant differences were found regarding miscarriage rates (6.7% vs. 20.4%) (OR=0.28, 95% CI: 0.03-2.36, P=0.177).

Limitations, reasons for caution: In this study, live birth or neonatal outcomes could not yet be analyzed. Because chromosomal analysis is strictly prohibited in Japan, this study had no data regarding the chromosomal integrity of the blastocysts.

Wider implications of the findings: Our results suggest that when an embryo with DUC reaches a blastocyst, it has a similar implantation potential as an embryo with a normal cleavage pattern.

Trial registration number: Not applicable

P-228 A multivariate logistic regression model for predicting live births improves clinical outcomes in frozen single blastocyst transfer cycles.

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Study question: Can a prediction model improve live birth rates (LBRs) in frozen-thawed embryo transfer (FET) compared to conventional embryo evaluation (CEE)?
Summary answer: The multivariate logistic regression model can predict the probability of live births better than CEE in single blastocyst transfers.
What is known already: Morphological parameters for the degree of blastocoele expansion, trophectoderm (TE), and the inner cell mass (ICM) have been used to select the best blastocysts for transfer, but it is difficult to weigh the importance of each parameter in embryo selection. Moreover, this process is subjective for some embryos and thus prone to human bias. As a result, morphological assessment results may vary extensively between embryologists and/or may fail to accurately predict embryo implantation and live birth potential. However, few studies have evaluated the effectiveness of a prediction model (PM) on live birth outcomes in frozen single blastocyst transfer cycles.
Study design, size, duration: We performed a retrospective cohort study for construction of a prediction model (PM) from a database of known live birth outcomes originating from 4717 blastocysts transferred between January 2009 and December 2015 in a single clinic in phase I. Data from 2092 FETs were collected between January 2016 and December 2017 for validation of the PM in phase II.
Participants/materials, setting, methods: The relationships between live birth outcomes and each factor included in the CEE were investigated. We constructed a model to predict live births using univariate and multivariate logistic regression analysis and the dataset. To validate the model, we compared the accuracy of prediction between when PM was applied and when CEE was applied. The area under the receiver-operating characteristic curve (AUC) was calculated, and model calibration was assessed.
Main results and the role of chance: In phase I, a total of 4717 frozen single blastocyst transfers were performed that resulted in 1490 live births (31.2% of the patients). The significant factors that showed no multicollinearity, indicating a state of high correlation among the independent variables, were selected for use in the multivariate analysis. The prediction model, constructed after multivariable logistic regression analysis, demonstrated that blastocyst diameter (odds ratio (OR) 678.7, 95% confidence interval (CI): 83.5–5517.3), embryo cryopreservation day (OR 26.9, 95% CI: 2.4–305.3), TE (OR 5.3, 95% CI: 1.5–19.3), and ICM (OR 4.1, 95% CI: 1.0–17.7) were predictive factors for live birth outcomes. The value of AUC for the prediction model for live births was 0.70 (95% CI: 0.67–0.72). The accuracy, sensitivity, specificity, positive predictive value, and negative predictive values for live births were 0.65, 0.63, 0.65, 0.41, and 0.82, respectively. In phase II, a validation study (n=2092) showed that use of this logistic model resulted in a significantly higher probability for predicting live births (0.71 vs. 0.69, p=0.0368). In addition, the values of the AUC revealed that the prediction model performed better than CEE in non-good blastocysts (0.69 vs. 0.61, p=0.007) compared to good blastocysts (0.65 vs. 0.64, p=0.18).
Limitations, reasons for caution: In this study, the morphokinetic variables provided by time-lapse monitoring could not be assessed, and embryo evaluation was limited to static observations. The interpretation of the findings of this study was limited by the retrospective nature of the analysis and the potential for unmeasured confounding.
Wider implications of the findings: Our results indicated that this logistic model could be useful for ranking embryos in frozen single blastocyst transfer. Improving the ability to select the embryos with the highest implantation potential would increase LBRs as well as shorten the time to pregnancy.
Trial registration number: not applicable

P-229 Impact of co-administration of cyclophosphamide with imatinib or dasatinib on in vitro mice preantral follicle development and oocyte acquisition

Abstract withdrawn by the authors

P-230 Embryoglue® as medium for embryo transfer: does it really improve the outcomes? A prospective randomized controlled trial.

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Study question: Can Embryoglue® (EG) culture medium improve clinical outcomes versus other conventional culture media?
Summary answer: EG offers the same clinical outcomes than other culture media for embryo transfer.
What is known already: Despite the advances in the IVF lab the embryo transfer (ET) success rate remains relatively low. One strategy to improve the results is the use of special culture media for ET.

In the past EG has been advocated for it but the evidence is scarcy and contradictory. In an attempt to elucidate this question we designed this RCT.
Study design, size, duration: Randomized controlled trial (RCT) carried out since March 2018 to January 2019, in which we randomized all the cycles with donated oocytes, performing a fresh transfer on day 5. The randomization included a control (GLOBAL® TOTAL®, LifeGlobal® or Continuous Single Culture® Complete, IrvineScientific®) and a study group (EG) and was done with a randomization program RNDSEQ V2011.09.09. Clinical parameters studied were implantation, clinical pregnancy and clinical miscarriage rates.
Participants/materials, setting, methods: Sixty one patients were included in the control group (GLOBAL® TOTAL®, LifeGlobal®) and 48 in the study group (EG). We performed a second randomization in which we included a total of 157 patients. Seventy six were included in the control group (Continuous Single Culture® Complete, IrvineScientific®) and 81 in the study group (EG). Patients that were diagnosed of recurrent implantation failure (RIF) were excluded.
Main results and the role of chance: In the first randomization we did not reach any statistically significant differences for implantation, clinical pregnancy and miscarriage rates between Global Total and EG, respectively (60.7% vs. 50.0%, p=0.628; 60.7% vs. 51.1%, p=0.335 and 1.7% vs. 8.5%, p=0.166).

In the second randomization we also had no statistically significant differences for implantation, clinical pregnancy and miscarriage rates between Continuous Single Culture and EG, respectively (35.4% vs. 38.8%, p=0.726; 44.4% vs. 53.4%, p=0.361 and 3.2% vs. 10.3%, p=0.346).
Limitations, reasons for caution: The study design is powered due to the fact that is a RCT with a sufficient number of patients. Notwithstanding, a higher number of cycles may vary these results. Also, we have to take into consideration that this study is focused only in recipients.
Wider implications of the findings: In our experience, hyaluronate enriched media, as EG did not improve the clinical outcomes. Even if statistically significant differences were not achieved in both randomizations, miscarriage rates should take into account in order to elucidate what implication could have EG in those outcomes.
Trial registration number: None

P-231 the challenges of temperature control in the IVF laboratory during necessary manipulations and assessments

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Study question: How do dishes, processes and durations on heated surface can all impact temperature within the IVF culture drops?
Summary answer: Detailed temperature mapping of IVF procedures highlights temperature variations caused by equipment and consumables in IVF laboratories and informs best practice.
What is known already: The recommended temperature range for human cells (embryos and oocytes) is 37-38 °C and pH of 7.3. During IVF processes, necessary manipulations and assessments, can cause changes in these optimal conditions. Time-lapse monitoring system (TMS) minimise the need for time outside of the culture environment, as assessments can be made using the imaging system, but many embryology procedures including oocyte retrieval and

insemination takes place outside the TMS, in ambient atmospheric conditions with the use of heated microscope stages.

Study design, size, duration: The study was designed to measure temperature changes within IVF culture dishes during routine procedures involving varied equipment and consumables. Small thermobuttons were placed inside culture medium microdrops to record temperature variation during IVF procedures. Each experiment was repeated at least twice and to confirm the temperature and timing data logged for analysis during October to December 2018 within a clinical IVF laboratory.

Participants/materials, setting, methods: Materials – Media (Supplier), Class II cabinets (Supplier), thermobuttons (Mintrone), thermocouple (Madgetech), incubators (Labotech, Cooper and Supplier), tube warmer (Supplier), Micromanipulator (Cooper), IVF dishes and tubes (Vitrolife).

Methods – Recording the media temperature variations for the mimicked IVF procedures with 1 minute intervals. Using the thermobuttons to trace the path of cells within the same dishes across equipment used for each stage of the process from oocyte recovery (OR) to insemination for a patient's treatment.

Main results and the role of chance: Temperature changes within the culture dishes during key processes in the IVF laboratory were evaluated.

The temperatures recorded for the whole oocyte retrieval (OR) to Fertilisation process pathway was maintained between 37°C and 39.5 °C. The mini incubator G85 was the most reliable for providing a constant and stable temperature within the required range (exactly 37.42 °C). The heated stages impacted the media temperature the most with the highest (39.64°C) and lowest temperatures 36.5°C recorded, these variation in temperature are outside of the required range and could impact embryo development.

Limitations, reasons for caution: These values are not transferable to every laboratory – as each different laboratory uses different equipment or consumables.

Wider implications of the findings: This is an effective procedure which enables mapping not only of the heated surfaces but temperature changes within culture dishes in order to protect gametes and embryos from unnecessary stress. This could have a positive impact on clinical results and arm the embryologists with information to avoid compromising temperature conditions.

Trial registration number: Not applicable

P-232 High levels of Follicular fluid oxidative stress are present in younger patients and fertile donors

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Study question: Is the information provided by the oxidative status of the follicular fluid related with fertility and maternal age?

Summary answer: A correlation was found among the follicular fluid oxidative status, its fertility status (donor/patient), woman age and number of retrieved oocytes.

What is known already: Oxidative stress has been reported in literature as a useful biomarker in male and female infertility. A novel technique detecting the oxidative status of different biological fluids, the Thermochemiluminescence (TCL) Analyzer™ (Carmel Diagnostics, Israel), has proved to be effective in different studies regarding seminal plasma, amniotic fluid and the spent embryo culture media. In this last one, we found a close relationship between the oxidative status of media and the subsequent clinical outcomes. Our present purpose is to examine the relationship between follicular fluid's oxidative status and relevant characteristics of an *in vitro* fertilization (IVF) treatment.

Study design, size, duration: This prospective observational study included 40 patients (from 31 to 45 years) undergoing an IVF cycle and 17 donors (from 20 to 34 years) enrolled in an egg donation program during the period from March 2018 to October 2018. The oxidative analysis was performed with the TCL Analyzer™ and embryos were cultured and evaluated in a time-lapse technology system.

Participants/materials, setting, methods: A total of 57 follicular fluid samples were collected during oocyte retrieval. 50 µl samples were assessed by the TCL Analyzer™, based on a heat-induced oxidation of biological fluids leading to the production of light energy, counted as photons emitted per second (cps), recorded after 55 seconds (H1), 155 seconds (H2) and 255 seconds (H3). A smoothing algorithm (sm) was used to normalize data. Data was statistically analyzed with Pearson chi-square test and one-way ANOVA.

Main results and the role of chance: Donor follicular fluid showed significantly higher values ($p<.05$) for the oxidative parameters than patients ones: **H1sm=** 290.53 ± 85.59 cps for patients vs. 361.88 ± 124.46 cps for donors, **H2sm=** 412.09 ± 116.92 cps for patients vs. 514.71 ± 133.20 cps for donors and **H3sm=** 628.37 ± 213.23 cps for patients vs. 783 ± 173.62 cps for donors. Regardless the follicular fluid origin (donor or patient), a significantly negative correlation was found between woman age and the level of oxidative stress in the sample ($p<.05$). The mean and standard deviation of the TCL parameters were as follows (cps): 555.39 ± 130.84 for ≤26 years, 506.11 ± 125.29 from 27 to 35 years, 429.10 ± 166.81 from 36 to 40 years and 424.37 ± 135.08 for ≥41 years. Congruent with the above results, significantly higher TCL parameters were found as number of retrieved oocytes increased ($p<.05$). For less than 20 oocytes retrieved: **H1sm** = 297.89 cps, **H2sm** = 418.53 cps and **H3sm** = 634.46 cps and for more than 20 oocytes retrieved: **H1sm** = 367.49 cps, **H2sm** = 534.69 cps and **H3sm** = 823.49 cps. However, no differences in TCL results were found regarding Body Mass Index (BMI).

Limitations, reasons for caution: The study of the follicular fluid can only give us information about the full oocyte and embryo cohort development, but never individual information of each embryo. This is a pilot study with a small sample size which must be increased in order to verify our conclusions.

Wider implications of the findings: An increase of the sample size would allow us to assess the impact of follicular fluid oxidative status over the embryo development and clinical outcomes. The measurement of follicular fluid oxidative status might help us designing improved strategies to better and more personalized IVF treatments.

Trial registration number: not applicable

P-233 Can apply Artificial Chemical Oocyte activation with Strontium Chloride for couples with severe male factor infertility or previous failed IVF cycles?

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Study question: Does activation with Strontium chloride($SrCl_2$) improve fertilization and embryo development in patients with severe male factor infertility or history of failed IVF cycles?

Summary answer: Application of AOA with $SrCl_2$ leads to an increased fertilization rate and blastocyst formation rate in patients with severe male factor or previous failed cycles..

What is known already: In patients with a history of previous fertilization failure with ICSI, artificial oocyte activation (AOA) after ICSI (ICSI-AOA) has been used to improve the fertilization and pregnancy rates. In addition,few studies recently reported that embryo development also improved with the ICSI-AOA method. However, these studies were done on women-based randomization and not on oocyte-based randomization. Studies on oocyte-based randomization still remain elusive. This study compares reproductive potential between ICSI-AOA and conventional ICSI on sibling oocytes from the same patients with severe male factor or previously failed IVF cycles.

Study design, size, duration: This prospective study was conducted in a private hospital between June 2016 and December 2018. In each case, the allocation of sibling mature oocytes (MII) to either the AOA (ICSI-AOA) or the control group (ICSI)was randomized. Fertilization and good blastocyst formation rates (≥ 4BB) in ICSI-AOA group were compared to conventional ICSI group with sibling oocytes. The best blastocysts formed were transferred.

Participants/materials, setting, methods: This study was conducted in patients (38.7 ± 4.6 years old) with male factor infertility (n=23) or a history of failed implantation following conventional ICSI (n=22). In the next treatment cycle, ICSI-AOA was performed on approximately half of the MII oocytes and conventional ICSI on the rest. For ICSI-AOA, the oocytes were exposed to 10 mM $SrCl_2$ for 60 min after performing ICSI. All fertilized embryos were cultured to blastocyst stage.

Main results and the role of chance: Fertilization and blastocyst utilization rates were higher in ICSI-AOA (77.9%, 116/149; 69.8%, 81/116) than

conventional ICSI group (50.3%, 72/143; 50.0%, 36/72) in patients with male factor infertility ($P< 0.05$). In the patients with previous failed pregnancy, fertilization rates did not differ (77.1%, 118/153 versus 74.8%, 107/143); however, significantly more ($P< 0.05$) good-quality blastocysts formed in the ICSI-AOA compared with the conventional ICSI group (57.6%, 68/118 versus 38.3%, 41/107, respectively). After transferring the embryos produced only from ICSI-AOA group, all babies born at the time of writing (9 babies) were healthy.

Limitations, reasons for caution: The sample size is low. More patients and a high number of mature oocytes available for injection per patient are needed.

Wider implications of the findings: Due to variable factors between patients, study on sibling oocytes might be the actual valid method to see usefulness of AOA. Our study implies that AOA treatment is an option to improve blastocyst formation rate for the couples who have severe male factor infertility or failed pregnancies in previous cycles

Trial registration number: Not applicable

P-234 Distribution of FSH Receptor polymorphism (FSHR) at position 680, in South Indian women and their responses to Controlled Ovarian Stimulation (COS) for ICSI cycles

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Study question: Does FSH receptor (FSHR) polymorphism at position 680 have any effect on controlled ovarian stimulation (COS) in ICSI cycles in South Indian population?

Summary answer: To determine the association of FSHR polymorphism at position 680 in predicting ovarian responses to COS in South Indian women and the distribution of polymorphism.

What is known already: The first SNP studied was the FSH receptor polymorphism Asn680Ser, which affects FSH level and increases gonadotrophin requirements during COH.Increasing the dose of FSH administration does not improve the oocyte development due to insufficient FSHR expression of the granulosa cells. In support of this,a SNP model at position 680 was reported to be associated with low response to exogenous FSH stimulation during COS .The expression of receptors differ in three genotypes (AA, AS, SS) which influences ligand/receptor interaction. Women of different ethnic population were studied and different distribution of allele pattern was noted .The response to COS were also assessed.

Study design, size, duration: Retrospective observational study conducted at Sri Ramachandra Medical College and Research Institute, Chennai, India from October 2013 to December 2016. Hundred and fifty normogonadotrophic women undergoing ICSI cycles were included after obtaining a written consent.

Participants/materials, setting, methods: Controlled Ovarian Stimulation (COS) was done using gonadotropins and antagonist protocol. Blood samples were collected in sterile EDTA vacutainers on the day of stimuation. DNA was extracted and were subjected to PCR amplification, Sequencing followed by elution. Final analysis was done using SeqScape genetic analyzer and the distribution of alleles were interpreted with specific color code signals for each allele AA, AS and SS SNP's.

Main results and the role of chance: FSH is essential for follicular growth and oocyte maturation. FSHR gene is essential for FSH for its action. A structural change in the FSHR gene changes the amino acid configuration which leads to altered ovarian response to gonadotropin stimulation. Our study showed 50% of women had AA polymorphism, 42% of women had AS and only 8% had SS polymorphism.The distribution of SS polymorphism was much lower in south Indian women, when compared to Caucasian and Chinese women which were 31.7% and 13% respectively. It was noted that women who had SS polymorphism were not poor responders when compared to women with AA and AS polymorphism. Women with SS polymorphism had higher MII oocytes (12.31±5.15) than the AA and AS group (7.27±4.65), (8.77±7.54) respectively and was statistically significant. There were no significant differences in duration of stimulation, fertilization rates, total dose of gonadotropins used between the three polymorphisms at position 680. Our study also showed that the cumulative pregnancy rates were slightly higher in patients with SS polymorphism which was 51% than AA (40%) and Asn/Ser (48%) and this may indirectly due to higher number of oocytes obtained during COS.

Limitations, reasons for caution: The sample was confined only to people proceeding for ART. Women who underwent COS in view of PCOS, endometriosis, Decreased Ovarian reserve, Previous ovarian surgeries for ovarian pathologies were excluded. Larger sample size may help to exactly find the distribution of polymorphism in Indian population.

Wider implications of the findings: Assessing polymorphism at position 680 will not improve the outcome of ART results by predicting the response in south Indian population. Women with SS polymorphism will have lesser chances of poor response and slight increase in MII oocytes number although this may not help in deciding the dose for COS.

Trial registration number: Not Applicable

P-235 The clinical outcome of ultra-low oxygen tension on post-thawed human blastocyst

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Study question: Does ultra low oxygen tension (2%) have an advantage over low oxygen tension (5%) for post-thawed human blastocyst?

Summary answer: Ultra-low oxygen tension (2%) did not show superiority over low oxygen tension (5%) regarding degree of blastocoel expansion and clinical pregnancy rate

What is known already: In the female reproductive tract, oxygen concentration fluctuates between 2-8%, which is considered to be at its highest level in the fallopian tube, while the lowest level is in the uterus. Pre-implantation embryo crosses the uterotubal junction after the time of compaction on Day 3, where it is exposed to a shift in oxygen tension to 2%. This variation may have a role in the metabolic reactions of the embryo, and in its preparation for implantation process. Some studies suggested that culturing embryos with oxygen tension below 5% might have an embryological advantage mimicking nature.

Study design, size, duration: A prospective randomized trail included 60 participants who underwent frozen embryo transfer cycles at Ganin Fertility Center from November 2018 to January 2019 and still recruiting patients. One day before embryo transfer, cases were randomized in to two equal arms; Control arm (Low oxygen tension 5% O2), and experimental arm (Ultra low oxygen tension 2% O2). Thawed embryos were cultured according to their assigned conditions until embryo transfer.

Participants/materials, setting, methods: Participants with female age below 40 years and had vitrified blastocysts on day 5/6 were enrolled in the study. Thawed blastocysts were cultured in pre-equilibrated media covered with oil in two different incubators: (5.7% CO2, 5% O2, and 89.3% N2 at 37°C) for the control arm, and (5.7% CO2, 2% O2, and 92.3% N2 at 37°C) for the experimental arm. Embryos were assessed for the degree of blastocoel expansion at the time of embryo transfer.

Main results and the role of chance: One hundred forty four embryos were included in this study. Control arm 5% O2 (81 embryos) and experimental arm 2% O2 (63 embryos). There were no significant differences in the female age (29.8 vs. 31.3 years), time till vitrification (132.6 vs. 129.1 hours), and time from embryo thawing till transfer (2.25 vs. 2.14 hours) between control arm (5%O2) and experimental arm (2%O2) respectively. Regarding embryological parameters, statistical analysis using SPSS showed no significance in degree of blastocoel expansion (48.6% vs. 55.7%) ($P> 0.05$) or clinical Pregnancy rate (46.6% vs. 53.3%) ($P> 0.05$) for control arm and experimental arm respectively. However, there is a higher tendency for blastocoel expansion and clinical pregnancy rate to the experimental arm (ultra-low oxygen tension 2%) than the control arm (low oxygen tension 5%).

Limitations, reasons for caution: The study was performed on small sample size, so more large well-conducted randomized controlled trials are needed.

Wider implications of the findings: The promising tendency towards more expanding blastocyst make 2% O2 need to be explored by more means of assessment of blastocyst viability.

Trial registration number: ClinicalTrails.gov ID: NCT03817671

P-236 BESST, a non-invasive computational tool for embryo selection using mass spectral profiling of embryo culture media

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Study question: Can a non-invasive mass spectrometry-based tool be used to identify embryos that result in ongoing pregnancy?

Summary answer: Algorithmic pattern scoring based analysis of embryo culture mass spectra provides a score of probability of implantation and ongoing pregnancies, identifying 76.9% of life birth.

What is known already: The current gold standard of embryo evaluation is based on morphology and morphokinetics, however there have been many efforts to design non-invasive tests to assess embryo viability, including imaging of embryo metabolism by Fluorescence Lifetime Imaging Microscopy (FLIM) and High-Resolution Nuclear Magnetic Resonance (H-NMR) for metabolic biomarker detection. Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-ToF) mass spectrometry offers an immediate, sensitive and straightforward analysis of spent embryo culture to give a confirmation of embryo viability within minutes. It is now possible to confirm pregnancy outcome using the retrospective analysis of embryo culture fluid.

Study design, size, duration: A retrospective cohort study, including 1190 spent media samples from embryo cultures collected from a single IVF clinic in USA. The samples were collected between March 2014 and March 2018. Outcomes subsequent implantation were intra uterine, negative, biochemical pregnancy and spontaneous abortion and preimplantation genetic screening outcomes of euploid and aneuploid. Only fresh single transfers embryos were used in the development of the tool.

Participants/materials, setting, methods: Upon receiving, embryo culture media was thawed, diluted and analysed using MALDI ToF mass spectrometry in a laboratory in the UK. Analysis was performed with CHCA matrix, spectra obtained in a mass range of 200 to 2000 m/z. Data files were subject to semi-quantitative computational analysis using Python programming language.

Main results and the role of chance: The Blastocyst and Embryo Screening and Selection Tool (BESST) was developed following the analysis of embryo culture media from a single, fresh transfer embryo culture media. The automated computational workflow was applied to generate a reference spectral pattern of ideal embryo profile with chosen samples. Criteria for sample selection included: highest quality scores as determined by Blastocyst Quality Score, a numerical blastocyst-morphology grading system, ongoing pregnancy outcome and euploids as tested by PGS.

Similarities to the reference pattern were computed, based on peak positions and intensity values, assigning a score for each embryo. The resulting complex, non-linear scores were subject to cluster analysis and subsequently mapped into five distinct classes of 0 to 5 with continuous numerical values, which can be interpreted linearly. With this method we were able to identify 76.9% of ongoing pregnancy cases for embryos that score >4, while score of <1.5 predicts that embryo have a 35.7% chance of ongoing pregnancy.

It is important to note that result of 100% or close cannot be achieved due to underlying confounding factor that is cases of unreceptive endometrium, regardless of how good the quality of the embryo. Hence the PPV of 76.9% shown here is a favourable result.

Limitations, reasons for caution: The study is limited in that the tool was developed on single fresh embryo samples only. Embryo freezing prior PGS testing requires a separate algorithmic tool. Furthermore, the study was based on data from one IVF centre and prospective validation studies should be carried out in different practices.

Wider implications of the findings: BESST (Blastocyst and Embryo Screening and Selection Tool) when applied prospectively, immediately prior to embryo transfer, could enable the objective, consistent and non-invasive analysis of the likelihood of achieving ongoing pregnancy and compared to morphology alone could be optimised to see improvement in pregnancy rates for any clinic using BESST.

Trial registration number: None

P-237 Evaluation of developmental competences of paired blastomeres of mouse 2-cell embryos and establishment of splitted monozygotic twin embryos model

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Study question: Are there inter-embryonic variations of developmental pattern and competence between paired blastomeres of mechanically splitted monozygotic twin embryos (SMTE) from a 2-cell embryo?

Summary answer: Each blastomere of a 2-cell embryo has no significant differences in embryo development. SMTE model might be valuable for subtle experiments for *in vitro* culture.

What is known already: Experiments with mouse pre-implantation embryos might have the inter-embryonic variations. Owing to these variations, exact differences of developmental competence and epigenetic alterations by culture conditions are hard to find during *in vitro* cultured preimplantation embryos. Several studies were reported that *in vitro* culture of splitting and isolating blastomeres of mouse and human cleavage-stage embryos. It has been applied to increase the number of embryos with same genetic backgrounds. However, the SMTE model has not been evaluated and established.

Study design, size, duration: We examined the developmental pattern and competence between paired blastomeres of SMTE from a 2-cell embryo. The developmental pattern was analyzed using the time lapse-lapse monitoring system from 1-cell to blastocyst stage. In vitro cultured blastocysts of paired SMTE were fixed and counted the number of nuclei. The paired SMTE were cultured and analyzed 17 times experiments.

Participants/materials, setting, methods: Mouse 2-cell embryos were collected and zona pellucida was removed by acid Tyrode's solution. The embryos were mechanically divided into a paired blastomere of SMTE-A and -A'. The developmental patterns and consequences of the paired SMTE were assessed with the time-lapse monitoring system. The developmental competences were analyzed by comparing the mean interval and cumulative time for next pre-implantation embryonic stage, and blastulation rate and the number of nuclei of blastocysts in the paired SMTE.

Main results and the role of chance: Blastomeres of 2-cell embryos were successfully splitted without any damage, and cultured individually. The interval times from 1-cell to 2-cell and 4-cell to morula, and the cumulative times from 1-cell to 4-cell were significantly different between paired SMTE. However, the times of further development to blastocyst stage were similar between paired SMTE. Overall development rate of paired SMTE-A and SMTE-A' were 71.2 ± 6.1% and 71.6 ± 5.8%, respectively. The numbers of nuclei of blastocysts were similar between paired SMTE-A and SMTE-A' as 48.5 ± 2.3 and 49.9 ± 2.1, respectively.

Limitations, reasons for caution: This study showed only morphological analysis of developmental competence in mouse SMTE. Lack of certain evidences which are able to evaluate SMTE model is valuable model in pre-implantation embryo research. Gene regulations, epigenetic modification, and other embryo developmental competence should be more clearly analyzed in the next study.

Wider implications of the findings: In order to minimize the number of sacrificed animals used in the various researches, SMTE model would be an alternative and efficient method. Also, the SMTE model could be utilized for comparing the epigenetic modification including DNA methylation, histone acetylation and others species including human in pre-implantation embryos.

Trial registration number: Not applicable

P-238 Should ICSI be the first line treatment for patients where low numbers of oocytes are retrieved during assisted fertility treatment?

Abstract withdrawn by the authors

P-239 Day 4 embryo vitrification: is it a valid option for IVF lab practice?

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Study question: To assess the efficiency of day 4 vitrified embryos after warming in terms of survival, development and pregnancy rate.
Summary answer: Vitrification of day 4 embryos is a valid alternative for embryo cryopreservation as it demonstrates high survival, and pregnancy rates.
What is known already: Vitrification has become the most commonly used method for embryo cryopreservation and has almost replaced slow freezing. The available data demonstrate that more than 90% of the vitrified cleavage embryos and blastocyst survive after warming and perform equally to the fresh. However, very little is known about the survival and overall performance after warming of embryos vitrified on day 4. In the absence of a common grading system for day 4 embryos, their morphological assessment may become a challenge; this may explain the preference for vitrification of cleavage embryos or blastocysts, where morphological assessment is more standardized and less operator-depended.
Study design, size, duration: This retrospective study was contacted from May 2015 to December 2018. During the study period, in total, 235 patients underwent frozen embryo replacement cycles (FERC) and received warmed embryos vitrified on day 4. For the statistical analysis the IBM SPSS program was used in combination with the X^2 Pearson test.
Participants/materials, setting, methods: Only embryos with clear signs of compaction were considered suitable for vitrification on day 4. The vitrified embryos were warmed on the 4th day after the onset of progesterone administration; and according to the number of the available vitrified embryos a day 4 transfer was scheduled for the patients with 1 to 2 vitrified embryos (Study Group 1) or a day 5 transfer (Study Group 2), for the patients with 3 or more vitrified embryos.
Main results and the role of chance: In total 674 vitrified embryos were warmed for the 235 patients and 650 were fully recovered (96,4%). For study group 1, 53 patients (mean age 36,8) received day 4 embryos (mean embryos transferred 1,64), 25 patients had a positive pregnancy test (47,2%) and 19 progressed to a clinical pregnancy (35,8%). For study group 2, 182 patients (mean age 35,9) received day 5 embryos (mean embryos transferred 1,98), 114 had a positive pregnancy test (62,6%) and 85 progressed to a clinical pregnancy (46,7%). Of the remaining embryos 97 reached blastocyst stage and were re-vitrified. As expected patients in study group 2 demonstrated significantly higher pregnancy rates compared to study group 1 ($p<0,005$). This finding could be explained by the fact that, a more advanced embryo selection was possible for study group 2 patients, as they had more available embryos, which were left to grow to blastocyst stage before 1 or 2 were selected for transfer.
Limitations, reasons for caution: There is not a commonly accepted grading system for day 4 embryos. Embryos on day 4 may present varying degrees of compaction, as a result, their assessment is subjective and operator depended. The data of this study, while promising, are still very limited due to the small number of patients.
Wider implications of the findings: Day 4 vitrification may become a new valuable treatment option for IVF practitioners by increasing flexibility for embryo vitrification and allowing tailor-made to patient needs, lab-approaches. For FERCs, vitrification of day 4 embryos in combination with day 5 transfer, may provide a more advanced embryo selection strategy for blastocysts.
Trial registration number: Not applicable

P-240 Role of major endocannabinoid-binding receptors during mouse oocyte maturation and spindle organization

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Study question: Are main endocannabinoids-binding receptors (CBRs) involved in mouse oocyte maturation and in proper spindle organization?
Summary answer: The different expression of CBRs during meiotic maturation reflects their role in the control of meiotic resumption (CB_1R, CB_2R) and in spindle organization (GPR55).
What is known already: Endocannabinoid system (ECS) includes lipid messengers termed endocannabinoids (eCBs), their receptor targets type-1 (CB_1R) and type-2 (CB_2R) cannabinoid receptors, G-protein coupled receptor 55 (GPR55), transient receptor potential vanilloid type 1 channel (TRPV1) and a number of metabolic enzymes. ECS has a key-role on virtually all steps of female reproduction. To date, among the main receptors, only 2 receptors have been extensively studied in the mammalian oocytes, i.e. CB_1R and CB_2R. In mouse oocytes, both receptors were modulated during meiotic maturation, and CB_1R signaling was linked to the activity of ERK and Akt.
Study design, size, duration: CBRs localization, mRNA and protein contents were determined in oocytes collected *in vivo* at germinal vesicle (GV), metaphase-I (MI) and -II (MII; n=180/group). CB_1R, CB_2R and GPR55 roles during meiotic resumption or maturation were assessed by incubating oocytes in the presence/absence of receptor antagonists SR1, SR2 and ML193 and a) by measuring intraoocyte cAMP concentration (n=600 oocytes/group); b) by evaluating spindle morphology at MI and MII (n=30 oocytes/group).
Participants/materials, setting, methods: Adult CD1 female mice were primed with 5 IU PMSG, and sacrificed: (a) 44 h later to obtain GV oocytes, (b) 8 or 12h after hCG (5 IU) injection to obtain MI or MII oocytes. CBRs mRNA and protein contents were assessed by qRT-PCR and Western blot; CBRs localization by confocal microscopy. cAMP concentration was assessed by EIA kit; MI/MII spindle morphology by appropriate immunofluorescent antibodies. Experiments were repeated at least 3 times.
Main results and the role of chance: Despite a significant decrease of CB_1R, CB_2R and GPR55 mRNAs (GV vs MI, MII: $P<0.05$), CB_2R and GPR55 protein contents increased from GV to MI and MII (vs GV: $P<0.05$). At GV, only CB_1R was localized in oolemma, although it disappeared at MI. TRPV1 (mRNA/protein) was always undetectable. When oocytes were *in vitro* matured with CB_1R and CB_2R but not GPR55 antagonists, a significant delay of GV breakdown occurred (Ctr vs SR1, SR2, $P<0.05$; vs ML193, $P>0.05$), sustained by higher intraoocyte cAMP concentration. Although CBRs antagonists did not affect polar body I emission nor chromosome alignment at metaphase plates (vs Ctr, $P>0.05$), ML193 impaired in about 75% of oocytes the formation of normal MI or MII spindles. Indeed, ML193-incubated oocytes showed a significant reduction of spindle length (MI=24,34±0,44 µm, MII=22.96±0.30 µm) as compared with Ctr (MI=35,20±0,13 µm, MII= 32,64±0,17 µm; $P<0.05$). These results suggest that while CB_1R and CB_2R are engaged in the control of meiotic resumption, GPR55 play a surprising and unexpected role in meiotic spindle formation.
Limitations, reasons for caution: The present study was performed in a mouse model, because it allows reproducible results. Any correlation with humans should be considered and adjusted accordingly.
Wider implications of the findings: In mouse oocytes all the major eCB-receptors are differentially expressed during meiotic maturation. CB_1R and CB_2R have prominent role in the control of meiosis resumption, GPR55 in MI and MII spindle organization. These findings open a new avenue to interrogate oocyte pathophysiology and offer potentially novel biomarkers for fertility problems.
Trial registration number: None

P-241 A non-invasive, rapid method to discriminate euploid and aneuploid embryo by MALDI Tof MS of culture media.

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Study question: Can a difference in mass spectral data from the culture media of embryos be used to discriminate between euploid and aneuploid genotypes without genetic testing?
Summary answer: It is possible to identify patterns of change in mass spectral data to discriminate between embryos with euploid and aneuploid phenotypes.
What is known already: PGS of biopsied blastocyst stage embryos is currently used to discriminate between euploid and aneuploid genotypes. However, randomized prospective clinical trials failed to show an increase in live birth rates after PGS selection of embryos. This could be assigned to the technical drawbacks and chromosomal mosaicism. Thus, the clinical cost benefits of PGS is questioned. Nevertheless, faster and more affordable means to screen for aneuploidies that persist to life birth are a needed. It has been shown that MALDI-ToF mass spectrometry of embryo culture media can detect differences between the phenotypes, offering rapid, sensitive analysis without requirement of vitrification.

Study design, size, duration: A retrospective cohort study, including 1190 spent media samples from embryo cultures collected from a single IVF clinic in USA. The samples were collected between March 2014 and March 2018. There were 149 euploid and 165 aneuploid embryos as analysed by PGS next-generation sequencing technique.
Participants/materials, setting, methods: Upon receiving, embryo culture media was thawed, diluted and analysed using MALDI-ToF mass spectrometry in a laboratory in the UK. The spectra were generated using CHCA matrix, obtained in a mass range of 200 to 2000 m/z to identify proteomic profile. Characteristic regions to discriminate euploids and aneuploids, enrichment analysis and intensity differences were obtained using automated computational workflow implemented in Python.
Main results and the role of chance: Different spectral patterns were found for two outcomes of euploid and aneuploid genotype in embryo culture media. Nine characteristic regions were identified that were statistically significant ($p < 0.001$). These regions had variable window size, ranging from 3 to 10 m/z, representing the peaks in the spectra. The peaks were localised in a mass range of 235 to 864 m/z. The obtained intensity differences between median values of peak intensity were substantially high going up to ten-fold. Enrichment analysis demonstrated between three to six-fold higher predominance of characteristic peaks for aneuploidies when compared to euploids.
Limitations, reasons for caution: Spectral pattern of aneuploidies is complicated to as there is such a wide range of chromosomal abnormalities observed. The spectral pattern is therefore identification of variance from Euploidy and not for specific aneuploidies such as T21.
Wider implications of the findings: PGS is invasive, expensive, time-consuming test that requires embryo vitrification. Embryo culture media analysis by MALDI offers a non-invasive method to assess euploidy of an embryo prior to transfer which could positively affect both clinics and patients.
Trial registration number: not applicable

P-242 Mitochondrial (mt) DNA copy number may affect blastulation timing

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Study question: Is the mtDNA content affecting embryo development ability?
Summary answer: Embryos carrying higher mtDNA copy number seem to need more time to achieve blastocyst stage, but it's not related with the expansion capacity after warming.
What is known already: Descriptive studies looking at the mtDNA load in blastocysts have shown that those blastocyst with higher quantities have more chances to be aneuploid, to have lower embryo quality as well as worst implantation potential. Few studies speaks about timings during embryo development, since cellular differentiation during blastulation have high energy demands, and mitochondria may supply the required ATP, we aimed to evaluate the relationship between mtDNA copy number and the time of blastulation and recovery after warming.
Study design, size, duration: Retrospective study comprising a total of 229 embryos from a total of 134 patients undergoing PGT-A between 2017 and 2018. Embryos were divided in two study groups depending of the values of mtDNA relative quantities. Group A: 102 embryos carrying values above the median and Group B: 127 with values below or equal to the median value.
Participants/materials, setting, methods: Embryos were cultured under embryoscope and biopsied on day 5 or 6. The calculation the mtDNA score was done by NGS. The number of reads mapping to the mitochondrial genome was divided by the number of reads mapping to the nuclear genome T-student was used for statistical comparisons
Main results and the role of chance: The relative mtDNA content in the analyzed blastocysts ranged from 4 to 135. When comparing the two groups, we observed that blastocysts with higher mtDNA values, group A, needed more time to commence blastulation (101.64 h) and to expand (108.54 h) that the group B, 97. 78 h and 104.16 h respectively ($p < 0.0001$). Patient with embryos belonging to group B were 38.7+ 3.7 years old compared to patients of the group B 37.8 + 3.9 ($p > 0.05$). Significant lower quality embryos were found in the group A (51%) compared with Group B (32%).Thirty three embryos were warmed and left in culture up to 3.6 h before transfer. In these cases the mtDNA content did not affect to the ability of embryos to re-expand.
Limitations, reasons for caution: Higher mtDNA quantity negatively affected blastulation and expansion timing, however worse embryo quality was observed as well. Body mass index or stimulation parameters can be also contribution factors of the results. In order to confirm these results, other variables and bigger sample size need to be considered.
Wider implications of the findings: Higher mtDNA may be indicative of cellular stress and mitochondrial dysfunction, under this situation embryos not only may encounter more
Trial registration number: Not applicalbe

P-243 Retrospective study of changes in mitochondrial levels of blastocysts in a single-step culture medium

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Study question: Does a single-step culture system affect the mitochondrial level in the blastocyst?
Summary answer: The mitochondria level of the blastocysts is independent of the energy substrates composition and concentration in the medium.
What is known already: Mitochondria is the powerhouse of various cellular functions and in the recent years, its potential as a biomarker for embryo viability has been widely explored. With the introduction of time-lapse technology, single-step medium was developed to allow non-disturbed extended culture from fertilization to blastocyst stage. In order to do this, single-step medium needs to have the energy substrate composition and concentration that supports the embryos development up to blastocysts stage. Therefore, this research aims to study if there is a shift in the mitochondria level of these blastocysts in accordance to the different medium substrates composition and concentration.
Study design, size, duration: Retrospective analysis of 292 biopsied blastocysts cultured for clinical IVF procedures with PGT option between July 2017 to January 2019.
Participants/materials, setting, methods: From July 2017 to August 2018, sequential medium (Vitrolife, G1-Plus & G2-Plus) was used for blastocysts culture (n=142) according to the manufacturer protocol i.e. fresh medium change on Day 1, Day 3 and Day 5. From September 2018 to January 2019, the blastocysts (n=150) were cultured in single-step medium (Vitrolife, G-TL) using the same volume. The blastocysts were biopsied on Day 5/6 and mtDNA was analysed using the Ion Torrent NGS platform.
Main results and the role of chance: There was no significant difference in the mtDNA level of the blastocysts in the sequential medium (0.0012±0.0007) compared to the single-step medium (0.0013±0.0008), p=0.253. It was also found that there was no significant difference in the mtDNA level of euploid blastocysts vs aneuploid blastocysts in both groups (0.0013±0.0006 vs 0.0014±0.0009), p=0.314. Similar results were also obtained for euploid blastocysts vs mosaic blastocysts (0.0013±0.0006 vs 0.0012±0.0008), p=0.69.
Limitations, reasons for caution: The mitochondrial content can also differ depending on the site of biopsy and sampling handling time before amplification. Hence, in order to use this indicator as a biomarker of implantation potential, it has to be complemented with other evidence such as morphological findings and PGT status.
Wider implications of the findings: The change in media composition from sequential to single-step does not affect the energy level in the embryos. The use of single step medium can help to reduce the workload of embryologists and reduces the cost of consumables in an IVF cycle.
Trial registration number: Not Applicable

P-244 Does the thaw to transfer interval (TTI) affect the outcome of a frozen embryo transfer (FET) cycle?

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Study question: Does the time between embryo thawing and transfer affect the positive test (POS), clinical pregnancy (CP) or live birth rates (LB) of a FET cycle?

Summary answer: TTI does not affect POS or CP. However, a TTI of >4 hours does significantly increase the LB.

What is known already: Literature is limited regarding TTI and outcome. However, a study by Rato et al (2012) suggests an inverse correlation between TTI and implantation rates (IR) and LB for cleavage stage embryos. A previous retrospective data analysis by Woodhead et al (2019), suggests no significant difference in POS, CP or LB for blastocyst FETs with varying TTIs (ranging from <2 to >6 hours).

Study design, size, duration: 4374 standard FET cycles from January 2014 to March 2018 were retrospectively analysed. The data was collated from 4 UK clinics using the same protocols. Results were statistically analysed using ANOVA and subsequent t-test.

Participants/materials, setting, methods: Data included patients of all ages that had a single blastocyst FET. Failed thaws were excluded to avoid the impact of freezing or warming complications. The data was grouped into 6 main groups of TTI (<2, 2-3, 3-4, 4-5, 5-6, >6 hours). All outcomes (POS, CP, LB) were measured per embryo transfer.

Main results and the role of chance: The overall POS, CP and LB were 53.1%, 39.2% and 20.7% respectively (n=4374). Relatively equal sizes were observed between the TTI groups (from short to long, n=885, 750, 588, 998, 812 and 341 respectively). The POS for each TTI group, from shortest (<2 hours) to longest (>6 hours), were 53.8%, 51.9%, 54.3%, 51.8%, 55.2% and 51.0% respectively. The CP for each TTI group, from shortest to longest, were 39.8%, 37.9%, 39.3%, 38.6%, 41.5% and 37.2% respectively. ANOVA testing showed no significant differences between either of these results (p=0.89 and 0.86 respectively). The LB for each group, from shortest to longest, were 18.4%, 16.1%, 19.6%, 22.1%, 23.8% and 27.0%. ANNOVA testing showed significant differences between the groups (p<0.001). Subsequent t-tests showed a significant increase in the LB of 4-5 hours TTI compared with <2 and 2-3 hours TTI (p<0.05, p<0.01). A TTI of 5-6 hours showed and significant increase in LB when compared to <2, 2-3 and 3-4 hours TTI (p<0.01, p<0.001, p<0.05). A TTI of >6 hours showed a significant increase in LB when compared to <2, 2-3 and 3-4 hours TTI (p<0.01, p<0.001 and p<0.05).

Limitations, reasons for caution: Factors such as patient age, cause of infertility and blastocyst age or quality were not accounted for. Further analysis may be needed to ensure the differences in LB are solely due to TTI, rather than these additional factors.

Wider implications of the findings: Immediately thawing and replacing of cryopreserved blastocysts may not be the optimal way to perform FETs. Post thaw assessments could be implemented to aid the embryologist in determining blastocyst survival and this extended TTI would maximise the take home baby rate.

Trial registration number: N/A

P-245 Efficacy of embryo culture in dry and wet system: a prospective randomized sibling-oocyte study

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Study question: To compare the impact of different Embryos Culture System (ECS) of sibling-oocytes cultured in wet and dry incubators, on embryo development and clinical outcomes.

Summary answer: The humidified and dry atmosphere has no impact on efficacy of ECS.No significant differences were found in blastocyst formation rate in those two culture systems.

What is known already: The incubator plays a critical role in providing a stable culture environment to support embryo development.With technological advances a "dry culture system" (DCS) which has no tray of water in the incubator has been designed for embryos culture and the DCS is replacing the wet one. A comparative study showed that the blastocyst formation, implantation, and clinical pregnancy rate was lower in DCS compared to humidified culture, however there is no universal agreement among scientist and the choice of the best incubator is still controversial. To date, most studies not compare the sibling embryos development

Study design, size, duration: Prospective randomized study of 350 sibling oocytes from 40 women (aged ≤ 39 years) undergoing oocyte retrieval procedure for intracytoplasmic sperm injection (ICSI) at Nuova Villa Claudia, from July to December 2018

Participants/materials, setting, methods: A total of 350 injected oocytes were randomly cultured in two different incubators: the conventional humid incubator containing a water tray (ASTEC; Group A) and the bench-top dry incubator (MIRI; Group B). Culture was performed up to blastocyst at 37°C, 5% O2, 6% CO2. The single oocyte is placed in a single drop covered with mineral oil. Logistic regression analysis was used to control for confounding factors

Main results and the role of chance: The fertilization rate was similar between the two groups [group A 77,65% (139/179) vs group B 75.88% (129/170) *p*=NS]. Blastocyst formation rate was not significantly different between group A 51,79% (72/139) and group B 62,79% (81/129) (*p*=NS). The clinical pregnancy rate of group A and B [50% (N=18) vs 47.61% (N=21) *p*=NS] respectively was similar as well.

Limitations, reasons for caution: The number of oocytes must be increased to have more evidence about the culture systems efficiency. These data should be confirmed by careful multi-center studies and by extend the clinical analysis to live birth rate

Wider implications of the findings: Although *in vitro* culture system under conventional humidification conditions simulates the *in vivo* situation and appears to be physiologically sound, our results suggest that the dry culture system is as efficient as the wet one. Moreover, the dry incubation system greatly reduces costs, space and the risk of microbial contamination

Trial registration number: NA.

P-246 High culture media oxidative profile as a biomarker of good quality embryos: a non-invasive tool to select the embryo to transfer.

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Study question: Is the spent´s embryo culture media oxidative profile, provided by Thermochemiluminescence (TCL) Analyzer™, a good biomarker of embryo quality?

Summary answer: Although each culture media shows different oxidative status, good quality embryos remain having higher oxidative parameters, which indicate more extensive oxidative metabolism.

What is known already: Novel non-invasive strategy TCL (Carmel Diagnostics, Israel), based on spent culture media analysis, proved to provide additional valuable data to morphology and morphokinetic analysis in the selection of the best embryo for transfer. In particular, the assessment of the embryo's oxidative profile with the Thermochemiluminescence (TCL) Analyzer™ suggests a new approach in determining embryo's quality or viability and subsequent implantation potential.

Study design, size, duration: A total of 683 spent embryo culture media from 174 *in vitro* fertilization (IVF) cycles, incubated and monitored with the time-lapse incubator Embryoscope®, were collected for analysis from May 2017 to December 2018.

Participants/materials, setting, methods: Oxidative status of 15 µl/embryo of culture media was measured with the use of the TCL assay, as photons emitted per second (**cps**) amplitude after 55 seconds (**H1**), 155 seconds (**H2**) and 255 (**H3**), in a 300-second period. The **Ratio**, as the slope of the three parameters, and the **Average** were also calculated. Different culture media were assessed. Oxidative data was normalized with a smoothing algorithm (**sm**) and analyzed by the statistical test ANOVA.

Main results and the role of chance: Different oxidative profile was noticed among the media culture included (p<.001). The average of the TCL parameters (**AVEHsm**) were (cps): 81.38 for Cook® (n=367), 114.29 for Genea Biomedx®, (n=228), 68.20 cps for Irvine Scientific® (n=42) and 95.79 cps for Life Global® (n=39). Despite using different culture media, transferred and vitrified embryos (V) remain showing higher values for the oxidative parameters than no viable embryos (NV): **H1sm**= 85.88 for NV vs. 88.70

for V, **H2sm**= 87.82 for NV vs. 91.03 for V and **H3sm**= 93.78 for NV vs. 98.17 for V. Regarding day 5 quality embryos, according to ASEBIR classification criteria, oxidative stress level decreased as the embryo quality got worse: AVEHsm=**95.56 cps** for embryos of type A (n=47); **94.04 cps** for embryos of type B (n=269); **92.70 cps** for embryos of type C (n=125) and **72.52 cps** for embryos of type D (n=26). This therefore implies high quality embryos have a more extensive oxidative metabolism exerting an oxidative load on their surrounding media.

Limitations, reasons for caution: No balanced sample size was assessed in different media culture niether in embryo quality. Oxidative status database will increase while using TCL to pursue an accurate optimal range for oxidative parameters. Additionally, present study would require a prospective validation for its routine clinical use.

Wider implications of the findings: The fair correlation between TCL oxidative results, embryo quality proves its application as a clinical biomarker. A more accurate selection of the best embryo, especially in good-quality embryo cohorts, would determine IVF success.

Trial registration number: None

P-247 Prospective, multi-center, randomized, open, 2-arm, non-inferiority trial comparing a semi-automated, closed vitrification system with a manual, open vitrification system

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Study question: Does the semi-automated, closed vitrification system Gavi™ provide as good survival rates after thawing zygotes as the standard procedure with manual open vitrification (Cryotop®)?

Summary answer: The survival rate of 2PN-oocytes is similar when using the semi-automated, closed Gavi™ system or the manual, open Cryotop® system for vitrification.

What is known already: Standardization is expected to play an ever-increasing role in clinical laboratory technology. For cryopreservation of oocytes and embryos, the manual, open Cryotop® vitrification method offers extremely high cooling and warming rates and survival rates of >90% have consistently been reported. The semi-automated Gavi™ unit automates key steps of the vitrification procedure (microfluid exchange by robotic handling unit, equilibration times) in a stable temperature environment Oocytes or embryos are kept in a pod (automated heat-sealing following equilibration) and manually plunged into liquid nitrogen for storage. Gavi™ has become commercially available; however, comprehensive testing in a clinical setting has not been performed.

Study design, size, duration: Prospective, randomized, open, non-inferiority trial conducted at three German IVF centers (NCT03287479; EC Luebeck: 17-093; 10/2017 to 12/2018). Randomization 1:1, stratified by center and by freshET/surplus2PN or freeze-all/2PN, cryopreservation of 2PN-oocytes by Gavi™ or Cryotop®. Taking attrition into account (mostly pregnancies from fresh ET), it was planned to randomize 180 patients to generate minimally one warming procedure with all subsequent events up to live-birth in minimally 122 patients within the study period.

Participants/materials, setting, methods: Eligibility: women, 18-40 years, COS, IVF or ICSI, hCG or GnRH-agonist trigger, cryopreservation of surplus 2PN-oocytes or freeze-all of 2PN-oocytes. Exclusion: PGD-AT/PGD cycles, uterus malformation and low responders. Primary outcome: survival rate of 2PN-oocytes after warming (assessment 2hrs after warming: intact oolemma, regular cytoplasm, later cleavage). Secondary outcomes: number and quality of embryos, pregnancy (PR) and live birth rates, procedure duration and staff convenience (questionnaire). All investigators received Gavi™ training before embarking on the study.

Main results and the role of chance: Overall, 149 patients were randomized to the Gavi™ (n=75) and Cryotop® (n=74) group. The mean female age (33.4±3.7 *vs.* 33.6±4.0; *p*=0.87) and the mean number of vitrified 2PN-oocytes (9.0±5.9 *vs.* 7.6±4.9, *p=0.25*) was similar between the Gavi™ and Cryotop® groups. At the time of writing, 50/75 (67%) patients in the Gavi™ and 60/74 (81%) patients in the Cryotop® group had started a frozen-thawed embryo transfer (FET) cycle and 4.9 ±2.2 and 4.4 ±1.8 2PN-oocytes, respectively, were warmed for transfer (*p=0.21*). The mean survival rate of 2PN-oocytes after warming was 89.0% (±21.4) in the Gavi™ and 95.2% (±13.5) in the Cryotop® group (adjusted difference: -2.11%, CI: -5.11 – 0.89; *p*=0.17). The incidence of a 100% survival rate was 35/50 (70%, 95%CI: 56.2-80.9) and 51/60 (85%, 95%CI: 73.9-91.9) in the Gavi™ and Cryotop® group, respectively (difference -15%, 95% CI: -30.4 – 0.6%; *p*=0.057). The mean number of transferred embryos was 1.5±0.6 and 1.6±0.5 (*p*=0.64) in the two groups. The clinical PR per FET was 12/50 (24.0%, 95%CI: 14.3-37.4) in the Gavi™ group vs. 12/60 (20.0%, 95%CI: 11.8 – 31.8) in the Cryotop® group (difference: 4%, 95%CI: -11.3 – 19.7; *p*= 0.61). Data are presented as mean±SD or numbers (percentage).

Limitations, reasons for caution: At the time of writing (30-Jan-2019), the sample size is still increasing as not all patients have utilized their cryopreserved 2PN-oocytes. Some patients will undergo more than one FET cycle and cumulative outcomes are still missing.

Wider implications of the findings: A semi-automated vitrification system appears well suited for clinical use. Further investigations need to be done on issues of practicability, procedure duration and financial costs.

Trial registration number: NCT03287479

P-248 Effect of culturing human embryos in group on the composition of spent culture media

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Study question: Does culturing human embryos in group improve their development and the culture molecular profiles compared to individual culture?

Summary answer: In IVF technologies the number of human embryos per culture drop will affect the molecular signature of spent culture and the embryo development until blastocyst.

What is known already: Embryos modify the culture media in which they are suspended as they grow, establishing autocrine and paracrine communication. Paracrine signals from adjacent embryos may replace similar paracrine interactions occurring in the reproductive tract, so it is hypothesized that group culture of embryos may enhance their development by this positive interaction. Up to 35 % of IVF laboratories perform group embryo culture, in which developing embryos share the same culture media. However, a standard approach to this technique is currently lacking, with each centre applying different protocols, namely in terms of physical contact and embryo density.

Study design, size, duration: Prospective sibling study of oocytes derived from the same women following Assisted Reproduction Treatment (ART). A total of 349 zygotes from 45 patients were included in the study from May 2017 to January 2018 in a single private IVF setting. Part of them were made spectroscopic analysis of the spent culture medium. Only IVF/ICSI cycles in which the whole cohort of embryos was cultured to blastocyst stage were included.

Participants/materials, setting, methods: Embryos were cultured in 30µL medium, covered by oil at 37°C, 6% CO_2, 5% $O_2$and 90% humidity, till day 3 on G1Plus being transferred on day 3 to G2Plus, been maintained till blastocyst achievement between day 5 and 6. Embryos were cultured individually or in group of 2 or 3 embryos per drop.Disposable medium at the day 3 and day 5 or day 6, was analysed by FTIR spectroscopy.

Main results and the role of chance: In the group culture (GC) arm, the average group size in Day 1-3 was 2.54 embryos per 30 µL drop, and 2.41 on Day 3-6. Day 3 good quality embryo rates were 0.83 vs. 0.72 (p=0.019) per zygote in group GC vs. individual culture (IC) respectively. The usable blastocyst rate was 0.60 in GC vs. 0.51 in IC (p=0.102) per total Day 3 embryos, and

0.72 in vs. 0.71 in IC (p=0.785) per good quality embryo. Of 45 cycles, 27 underwent fresh embryo transfers, with 16 (59%) achieving positive biochemical pregnancy. Of 38 embryos with known implantation data, 17 (44%) produced a gestational sac.

Differences between the molecular composition of culture media at day 3 and day 5 or 6 were observed by principal component analysis of the second derivative spectra, independently of the number of embryos cultures per drop.

Differences in the culture molecular composition were identified, based on analysis of ratios of spectral bands, at 10% of significance, between ICandGC, andbetween cultures with 2 and 3 embryos per drop. These results highlight the high impact of the number of embryos perculture drop, in the culture media composition and consequently on the embryo metabolism.

Limitations, reasons for caution: Zygotes from good prognosis patients were included. The analysis was conducted with embryos obtained by different procedures and with oocytes from different origins. The potential confounding factor of variables that can influence the analysis outcome, such as media type, volume of the culture drops, culture conditions, has to be identified.

Wider implications of the findings: Despite a tendency towards improvement in the total usable blastocyst rates per cultured zygote, it is unclear whether GC in the described conditions is superior to IC. The informative MIR spectra shows potential to be used as a predictor of embryo development to blastocyst stage.

Trial registration number: not applicable

P-249 Utilization of hyaluronic acid-alginate hydrogel for encapsulation and culture of mouse preantral follicles

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Study question: Does hyaluronic acid-alginate (HAA) hydrogel improve *in vitro* growth and development of mouse preantral follicles in comparison with alginate (ALG) hydrogel?

Summary answer: HAA hydrogel is a promising hydrogel for follicle culture and could relatively ameliorate the culture condition when compared to ALG hydrogel.

What is known already: *In vitro* culture of ovarian follicles is one of the potential approaches for fertility preservation in patients with cancer. Recently, ALG hydrogel has been used for encapsulation and culture of isolated follicles and yielded desirable results; however, it has some disadvantages such as slow biodegradability and poor bioactivity. Hence, the combination of ALG with more degradable and bioactive compounds is suggested. Hyaluronic acid can combine with ALG to make a composite hydrogel suitable for follicle culture. Although HAA has been used for the culture of different cell types, it has not been yet applied for follicle culture.

Study design, size, duration: Preantral follicles (n=188) were isolated from mice ovaries, encapsulated in ALG and HAA hydrogels and cultured for 13 days. The follicles' diameter, survival and antrum formation rates were evaluated during culture. After hormonal induction, the oocytes' maturation was assessed on day 14. The next step, follicles were cultured and on day 13, survived follicles (75 follicles/group) were collected to evaluate their genes expression and their conditioned media also were gathered to measure follicles hormonal secretion.

Participants/materials, setting, methods: Preantral follicles (100-130 µm in diameter) were mechanically isolated from the ovaries of 2-week-old NMRI mice and individually encapsulated and cultured in freshly prepared ALG (0.5% w/v) and HAA (0.5% ALG+5 mg/ml hyaluronic acid) hydrogels. The follicles diameters were determined as the mean of two perpendicular measurements. Gene expressions were analyzed using qRT-PCR. Hormonal secretions were measured by ELISA. All statistical analyses were conducted in SAS version 9.4. Differences were considered significant at $P<0.05$.

Main results and the role of chance: The data revealed that there was no significant difference in the diameter, survival and antrum formation rates of ALG- and HAA-cultured follicles. Nevertheless, evaluation of obtained oocytes after hormonal induction showed that a higher percentage of HAA-developed oocytes resume meiosis up to GVBD/MII stages in comparison with ALG-developed ones (74.50 vs. 55.55%, respectively; $P<0.05$). Moreover, HAA-cultured follicles expressed *Gdf9*, *Bmp15*, and *Gja4* genes more marked than ALG-cultured ones ($P<0.05$); while the expressions of *Gja1*, *Bax*, and *Bcl2* genes did not significantly differ between groups. Finally, data of hormonal secretion indicated that HAA-cultured follicles secreted higher levels of estradiol compared to ALG-cultured ones (28.27±1.67 vs. 10.08±0.79 ng/ml, respectively; $P<0.05$); however, no significant differences were found in the levels of progesterone and androstenedione between groups.

Limitations, reasons for caution: None.

Wider implications of the findings: Despite the better results obtained from HAA-cultured follicles compared to ALG-cultured ones, further optimization of the hydrogel characteristics may be necessary to enhance survival and maturation rates. Also, future studies should determine the functional competence of *in vitro* matured oocytes, their ability to become fertilized and form blastocysts.

Trial registration number: Not applicable.

P-250 The result obtained in the first transfer of an IVF cycle could predict the success probability in the second transfer

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Study question: The result of the first embryo transfer of one IVF cycle can be predictive of the probability of success in the second one?

Summary answer: Achievement of a delivery after the first embryo transfer is of good prognosis for the success of the second transfer from the same oocyte retrieval.

What is known already: IVF cycles often led to the production of several morphologically good quality embryos. More than one embryo transfer is performed in some of those cycles. IVF cycles with several good morphology embryos are obviously more likely to end up in a delivery or even in more than one delivery. On the other hand, it is believed that only a few of the total oocytes retrieved after ovarian hyper-stimulation led to truly viable embryos. The role of embryologists would then be to accurately identify those viable embryos to reach pregnancy in as few embryo transfers as possible.

Study design, size, duration: Retrospective analysis of 301 good morphological quality- frozen-embryo replacements. Oocyte retrievals performed after 2004 to women <40 y/o. Embryos were morphologically evaluated for the first transfer of each cycle either fresh of after cryopreservation ("Freeze-all" cycles). We analysed each of the next frozen-embryo replacement cycles after the completion of that first transfer. The transfers included in this analysis were the second transfer of each IVF cycle and were all performed before 2018.

Participants/materials, setting, methods: We compared two groups of IVF cases: Those with (Group A) or without (Group B) live birth after the first transfer of every cycle. In both groups, the result of the following cycle of (good quality) embryo thawing was studied. This was the first thawing of the cycle for cases with fresh embryo transfer and the second thawing for freeze-all cycles. Main endpoint was delivery rate per thawing. Chi-square was used for statistic significance evaluation.

Main results and the role of chance: The percentage of "very good" (57% / 54%) and "good" (43% / 46%) morphological quality embryos was similar in the thawed embryos included in the analysis for Group A / Group B.

In Group A (second transfer after delivery in the previous one) 117 embryos were thawed in 67 cycles and in Group B (second transfer after a previous one without successful delivery), 427 embryos in 234 cycles; 1,8 embryos per thawing in both groups. Survival rate and transfer cancellation rate were similar: 92,3% and 1,5% in Group A and 95,1% and 0,9% in Group B. The percentage of intact embryos after thawing was 70,1% in Group A and 72,4% in Group B. The clinical pregnancy rate (FHB+) per thawing was 53,7% in Group A and 37,2% in Group B (p<0,03). The delivery rate per thawing was 46,3% in Group A and 30,8% in Group B (p<0,02). Twin delivery rate was lower in Group A (3,2%) than in Group B (22,2%)(p<0,02). Clinical pregnancy and delivery rate per thawing was higher in Group A than in Group B.

Limitations, reasons for caution: This is a retrospective study that spans several years. The number of cases is different in both groups and not very high

although the differences both in pregnancy rate and delivery rate per thawing cycle were statistically significant.
Wider implications of the findings: In IVF cases with a cohort of embryos that allows for more than one good-quality embryo transfer; achievement of a successful implantation with delivery after the first transfer predicts higher probability of delivery after the following transfer of frozen embryos originated in the same oocyte retrieval.
Trial registration number: Not applicable

P-251 Comprehensive examination of the ICSI-related procedural timings and their effect on mean blastulation rates per cycle

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Study question: Do the ICSI-related procedural timings affect the mean blastulation rate (m-BR) per cycle?
Summary answer: A model entailing maternal age, sperm concentration, time from ovulation-induction (OI) to oocyte-denudation (OD) and number of same-day ICSI procedures partially explains m-BR per cycle.
What is known already: In IVF, specific timings must be complied with to preserve gamete viability/competence. During IVF procedures, oocyte exposure to suboptimal environmental conditions should be minimized. Nevertheless, mainly old-fashioned studies examined the putative impact deriving from daily schedules, number/timings of procedures, as well as operators' skills and experience. A careful inspection of the performance levels of each operator/laboratory is although critical to outline guidelines and key-performance-indicators. ICSI represented a ground-breaking advancement in IVF that has been quickly and widely implemented. In this study, we attempted to define whether and to what extent the ICSI-related procedural timings might impact m-BR per cycle.
Study design, size, duration: Observational study conducted at a private IVF clinic (January-2016 to January-2018). All ICSI cycles (n=1444) with ≤12 fresh autologous oocytes (n=7999) inseminated with ejaculated sperm were included. All operators and critical procedural timings (OI-to-OD, OD and ICSI) were automatically-recorded through an electronic-witnessing-system (RI Witness^{TM}). The primary outcome was the m-BR among inseminated oocytes per cycle. All confounders were prospectively registered in a relational database (Fertilab Manager) and used as corrective measures in regression analyses.
Participants/materials, setting, methods: Controlled ovarian stimulation was performed with recombinant-gonadotrophins in an antagonist protocol. Fourteen and twelve operators were involved in denudation and ICSI procedures, respectively. Beyond procedural timings and operators, the other confounders registered were: maternal/paternal age and karyotype, history of recurrent-implantation-failure and/or miscarriage, categorized sperm concentration (>15mil/ml, 6-15mil/ml, 1-5mil/ml, <1mil/ml), main cause of infertility, number of same-day ICSI procedures, culture media, incubator, number of inseminated oocytes.
Main results and the role of chance: The mean female age and number of inseminated oocytes were 38.6±3.9yr (range:21-45) and 5.5±3.2 (range:1-12), respectively. The sperm concentration was >15mil/ml,6-15mil/ml,1-5mil/ml,<1mil/ml in 1037 (71.8%), 224 (15.5%), 129 (8.9%) and 54 (3.7%) ICSI-cycles, respectively. The mean OI-to-OD time was 39.3±1.3hr (36-42). The mean procedural timings for OD and ICSI were 8.1±3.8min (range:2-20) and 12.6±6.4min (range:2-36); both these variables were significantly ($p<0.01$) dependent on the number of inseminated oocytes (η^2:0.38 and 0.71;power=100%) and the operator (η^2:0.15 and 0.25;power=100%). The overall m-BR per inseminated oocyte per cycle was 34.0%±27.9% (range:0-100). Among all the confounders under investigation, maternal age was the variable showing *per se* the highest association with the m-BR per cycle (η^2:0.03;$p<0.01$ and power=100%). When adding sperm concentration, OI-to-OD time and number of same-day ICSI procedures performed to the model, a significant ($p<0.01$ and power=100%) adjusted-r^2=0.06 could be achieved. Of note, OD and ICSI procedural timings and operators instead did not show any association with the m-BR per cycle. When the primary outcome under investigation was inspected versus OI-to-OD timings, the highest values were reported for ICSI procedures performed earlier than 38hr (n=223 ICSI-cycles, m-BR:40%±28%) and were then stably-lower (m-BR:33%±28%; $p<0.01$) for ICSI procedures (n=1221 ICSI-cycles) started later than 38hr.
Limitations, reasons for caution: This is a single private IVF center study. The limited number of cycles cultured in an undisturbed incubator (n=415, 28.7%) and with a sequential culture media (n=279, 19.3%) might have hindered their potential beneficial/detrimental effect. Lastly, an investigation of ICSI-related procedural timings effect on blastocyst implantation potential is also needed.
Wider implications of the findings: Maternal age and sperm concentration represent the main variables affecting the m-BR during ICSI-cycles. Nevertheless, to efficiently schedule the daily workload considering time of OI, egg-retrieval and insemination to comply with the number of same-day procedures might result in better outcomes in the hands of well-trained and constantly-monitored operators.
Trial registration number: None

P-252 High male birth rate in the patients with higher blastocyst grades

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Study question: Differences in sex ratio at birth depending on the stages and grades of transplant embryos were studied.
Summary answer: The male birth rate is markedly higher in patients with higher inner cell mass (ICM) grades and in those with higher trophectoderm (TE) grades.
What is known already: In recent years, there have been several studies on children born with assisted reproduction technologies, including a higher male birth rate with blastocyst transfer, and higher birth weight in children associated with frozen-thawed embryo transfer (FET) than in those associated with fresh embryo transfer (ET).
Study design, size, duration: This is a retrospective study, including 568 patients (age, 34.8 ± 4.16 years) who underwent single ET at the author's clinic between May 2007 and 2017, and the patients were later reported to deliver a full-term infant.
Participants/materials, setting, methods: The male birth rates among 109 fresh ET cases (ET group) and 459 FET cases (FET group) were compared by transplant embryo stages [cryopreserved embryo transfer (CET subgroup) and blastocyst embryo transfer (BET subgroup)] and by grades for blastocyst embryo transfer cases (Grade A and Grade B subgroups for ICM, and Grade A and Grade B subgroups for TE based on Gardner's grading criteria).
Main results and the role of chance: No significant difference was found in the male birth rate between the ET and FET groups (47.7% vs. 51.4%), and by stages, between the CET and BET subgroups in the ET group (42.9% vs. 52.8%) and between the CET and BET subgroups in the FET group (50% vs. 52.6%). By grades, the male birth rate was significantly higher ($p < 0.05$) in the subgroups of Grade A for ICM and TE: 66.7% vs. 26.7% between Grade A and B subgroups for ICM and 73.7% vs. 35.0% between Grade A and B subgroups for TE in the ET group; and 62.7% vs. 47.2% between Grade A and B subgroups for ICM and 69.4% vs. 49.1% for TE in the FET group.
Limitations, reasons for caution: Effects of the embryo culture solution used at the author's clinic could not be eliminated. Cases of *in vitro* fertilization and intracytoplasmic sperm injection were not separately considered.
Wider implications of the findings: Selection of desired sex may be possible without damaging embryos.
Trial registration number: None.

P-253 C-kit signaling promotes pre-implantation embryonic development and blastocyst formation

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Study question: To determine whether c-kit is expressed in human pre-implantation embryos, and to investigate the possible regulatory mechanism of c-kit signaling in the process of embryonic development.

Summary answer: C-kit signaling might promote human pre-implantation embryonic development by up-regulating ETV5 transcription factor via MEK/ERK pathway.

What is known already: Although the in vitro culture system has been optimized in the past few decades, few or no high quality embryos to transfer caused by abnormal embryonic development from in vitro culture has been still not completely solved. Accordingly, fully understanding the regulatory mechanism of pre-implantation embryonic development would be beneficial to further optimize the in vitro embryo culture system. Recent studies have found the expression of c-kit in mouse embryo and its promotion effects on mouse embryonic development. However, it is unclear the expression, the role and the related molecular regulatory mechanism of c-kit in human pre-implantation embryo development.

Study design, size, duration: 457 women (age: 22-37 years) who underwent IVF therapy were enrolled. 100 human immature oocytes (GV: 50 and MI: 50) and 660 human 3PN embryos resulting from one oocyte fertilized by two spermatozoa simultaneously were collected. 600 mouse embryos from 50 ICR female mice were used.

Participants/materials, setting, methods: Samples were distributed randomly into three different experimental groups: SCF group: G-1TM or G-2TM+HAS solution+rhSCF; SCF+imanitib group: G-1TM or G-2TM+HAS solution+rhSCF+imanitib; Control group: G-1TM or G-2TM+HAS solution+PBS; The rate of good quality embryos at day 3, blastulation at day 6 and good quality blastulation at day 6 were analysis. RT-PCR, western blot and immunofluorescence staining were applied to detect the target genes and proteins in samples collected from human or mice, respectively.

Main results and the role of chance: c-kit mRNA and protein were expressed unexceptionally in all human immature oocytes, 3PN embryos (day 3) and 3PN blastocysts (day 6). In the experiment of human 3PN embryos, compared with other groups, SCF group showed obviously higher rate of good quality at day 3, better rate of blastocyst formation at day 6 and higher rate of good quality blastocyst formation at day 6. There was no difference between SCF+imatinib group and PBS group. Furthermore, we observed a higher ETV5 expression in SCF group than that in other groups. Similar results were also found in animal experiment. Interestingly, we also found a higher phosphorylation level of MEK/ERK signal molecule in mice embryos from SCF group than those from other groups.

Limitations, reasons for caution: The present results were obtained from human abnormal fertilization embryos and mouse embryos, which might not be representative of the true human normal embryonic development.

Wider implications of the findings: The findings of this study are valuable for further understanding the regulatory mechanism of human pre-implantation embryonic development. Furthermore, our results provide a new idea for optimizing the *in vitro* embryo culture system during ART program, which is beneficial to obtain high quality embryos for infertile patients.

Trial registration number: non-clinical trials

P-254 The Effect of Reduced Glutathione and Ulinastatin on Improving the Xenotransplantation Efficiency of Frozen-thawed of Human Ovarian Tissue

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Study question: Do reduced glutathione (GSH) and ulinastatin (UTI) can improve follicular pool in human ovarian xenografts by intramuscular injection into hosts?

Summary answer: The follicular survival rate can be increased in patient ovarian xenografts as the administration of GSH, UTI and GSH+UTI into hosts.

What is known already: In decade, an increasing number of medical institutions can help patients prepare cryopreserved ovarian tissue prior to ovary operation or chemo- or radiation therapy, as a means of preservation of fertility potential, because those cryopreserved ovarian tissues can be autotransplanted for natural pregnancy

Dominant follicle was formed 10 weeks after subcutaneous transplantation of ovarian tissue which was sustained at least 21 months. Another study proven that oocytes originated from subcutaneous transplantat of cryopreserved ovarian tissue can be fertilized by intracytoplasmatic sperm injection (ICSI), in which hCG can be measured from the resulting embryo.

Study design, size, duration: Cryopreserved human ovarian tissue was xenografted into NOD-SCID mice (total number: 96), which were intramuscular injected GSH, UTI and GSH+UTI. The ovarian grafts were collected after xenotransplantation. The ovarian grafts were collected at the 1st, 3rd, 7th, 14th, 28th, 56th, 85th days after xenotransplantation.

Participants/materials, setting, methods: Observation of follicle survival rate was performed by H&E staining. Angiogenic activity was evidenced by immunohistochemical staining with anti-VEGF, anti-CD31 mice and anti-CD34 human antibodies. Macrophage recruitment during the ischemia reperfusion period was evaluated by immunohistochemical staining. The transcript levels of antioxidant enzymes and inflammatory cytokines in ovarian grafts were measured by real-time PCR. Malondialdehyde (MDA) levels in ovarian grafts were test by microplate assay.

Main results and the role of chance: 1. SCID mice with the treatment of GSH, UTI, and GSH + UTI show a higher survival rate than control group at the same period. GSH + UTI group presented an obviously high survival rate from the 1st day to 85th day after xenotransplantation.

2. Hosts with the treatment of GSH, UTI and GSH+UTI had a higher expression level of VEGF in the ovarian grafts.

3. Human ovarian grafts were underwent the loss of its microvessels, at the same time the SCID mice's microvessels were reconstructed, especially during the IR period. In addition, hosts with the treatment of GSH, UTI and GSH+UTI showed higher MVD of mice microvessels than control group

4. During the IR period, GSH, UTI, and GSH+UTI could suppress the recruiting response of macrophages and inflammatory cytokine, while GSH+UTI showed the best effective.

5.Hosts with the treatment of GSH, UTI, and GSH+UTI could eliminate oxidative stress in ovarian grafts by increasing the level of SOD1 and SOD2. GSH+UTI showed the best effects among all groups.

In summary, hosts with the treatments of GSH, UTI and GSH+UTI accelerated angiogenesis and increase the follicular survival rate in human ovarian xenografts by suppressing oxidative stress and inflammations during ischemia reperfusion periods.

Limitations, reasons for caution: We should knock-down VEGF expression to confirm the role of VEGF

Wider implications of the findings: Therapeutic strategies employing GSH+UTI have become a promising method to increase the survival rate of follicles in the human ovarian xenografts.

Trial registration number: 23455

P-255 assessment of the relationship between artificial oocyte activation and early embryo development

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Study question: Does artificial oocyte activation (AOA) influence in development patterns of early embryos?

Summary answer: The cell cycle of early embryos activated with calcium ionophore is significantly faster in old age group (38yr≤).

What is known already: AOA is beneficial method for patients with a history of fertilization failure. In the previous study, we found AOA with calcium ionophore can have a positive effect on both fertilization and pregnancy rates for patients who failed fertilization or showed less 50% fertilization in their previous ICSI cycles.

However, the relationship between morphokinetics of embryo development and AOA following ICSI is not well known.

Study design, size, duration: A retrospective study was conducted on 488 patients from January 2017 to December 2018. All embryos obtained after ICSI conducted in a time-lapse system were included (n = 1,946).

To examine the effect of AOA on early embryo development, we compared the time-points of morphokinetic events between ICSI and ICSI-AOA according

to age (≤37yr; ICSI, n = 737 versus ICSI-AOA, n = 256 and 38yr≤; ICSI, n = 700 versus ICSI-AOA, n = 253, respectively).

Participants/materials, setting, methods: Oocytes were exposed to 10 µmol/L calcium ionophore (A23187) for 30 min after ICSI for AOA.

Both age groups, younger (≤37yr) and older (38yr≤), were divided into ICSI and ICSI-AOA and all embryos were assessed in EmbryoScope™. We compared time-points of each morphokinetic events and morphokinetic variables (time of cell division, interval of cell cycle, multinucleation or uneven division at 2cell, direct division (<5h) and irregular division).

Main results and the role of chance: Student's t-test was used to compare the timing between the groups and Chi-square test was used to compare properties. The timing of the early cell division between ICSI and ICSI-AOA did not differ statistically in younger group. Additionally, no statistically significant difference was observed for multinucleation, uneven division, direct division and irregular division.

On the other hand, the embryos of ICSI-AOA in older group showed a significant faster timing in tPNf (24.05 ± 3.56, 24.67 ± 4.21, p = 0.026), t2 (26.55 ± 3.73, 27.19 ± 4.40, p = 0.026), t3 (35.74 ± 5.88, 36.71 ± 5.76, p = 0.025), t4 (38.12 ± 5.77, 39.14 ± 6.24, p = 0.018), t5 (48.16 ± 8.60, 49.89 ± 8.64, p = 0.006) and t6 (51.59 ± 7.70, 52.85 ± 7.54, p = 0.033) compared to the embryos of the ICSI. Whereas, the abnormal development was not significantly different between ICSI and ICSI-AOA.

Limitations, reasons for caution: The study was retrospective and more data will be collected in future prospective studies.

Wider implications of the findings: The present study shows an impact of AOA in early embryo development for the old age group. Therefore, it is necessary to set the proper time-lapse score of the activated embryos for transfer by morphokinetic analysis to improve the success rate for old age group.

Trial registration number: not applicabke

P-256 effect of women's age on cleavage time and abnormal cleavage event prior to 3rd cleavage: a retrospective study time-lapse imaging

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Study question: Does female age have an impact on timing of embryo development and abnormal events before the 8-cell stage?

Summary answer: Female age was found to affect events of uneven cleavage and multinucleation prior to 3rd cleavage, even though no significant difference appeared in cleavage timing.

What is known already: It was well known that increasing female age has been associated with anomalies of oocytes and poor embryo quality.

But knowledge of morphokinetic developmental parameters as female age is still limited.

Moreover, there is no information on the comparison to abnormal cleavage events using time-lapse according to female age.

Study design, size, duration: This retrospective study examined 1191 embryos resulting from normal fertilization in 608 patients between January 2014 and June 2018. Embryonic events were analysed in three age categories, less than 35 years old (Group A); 35–38 years old (Group B), over 39 years old (Group C).

Participants/materials, setting, methods: All embryos were cultured and assessed in the EmbryoScope™ time lapse incubator until day 3. Statistical analysis was performed with SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). Student's t-test was used for statistical analyses. Average hour±SD from ICSI insemination are reported for all stages.

Main results and the role of chance: We analyzed 12 parameters and abnormal cleavage event. Regarding the mean timings of the tPB2, tPNa, tPNf, t2, t3, t4, t5, t6, t7, t8, s2, cc2, there were no significant differences between the three groups.

We compared abnormal cleavage events in embryonic development. There were no differences between the three groups in the direct cleavage from 1 to 3-cell (19.4% vs. 21.0% vs. 20.3%, P = 0.062).

Regarding the rate of multinucleation and uneven blastomere size at the 2-cell stage, there was no significant difference between group A and B or group B and C.

However, the rate of multinucleation at the 2-cell stage showed statistically significant difference between group A and C (44.4% vs. 37.8%, P < 0.05).

In addition, significant difference was observed between group A and C for the uneven blastomere size at the 2-cell stage (14.8% vs. 16.8%, P < 0.05).

Limitations, reasons for caution: This study was limited to the analysis of embryos from Maria fertility hospotal. Developmental speed of embryos can also be impacted by the culture environment, therefore these findings apply only to this patient group.

Wider implications of the findings: Patient age seems to be an important variable to consider when scoring embryo development events through time-lapse technologies.

Trial registration number: None

P-257 Zygote state parameters assessed by time-lapse monitoring correlate with morphological good quality embryo

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Study question: Can zygote state parameters such as pronuclear time points, pronuclear morphology and presence of halo predict the best embryo?

Summary answer: The disappearance time of pronuclear (tPNf) and orientation of polar bodies (PBs) are related to embryo quality.

What is known already: It is important to select and transplant good quality embryos during the ART procedure. Zygote state parameter is the first assessment of the serial embryo quality evaluation. Pronuclear morpho-kinetics such as the timing of the appearance(tPNa) and disappearance(tPNf) of pronuclear are known to be related to the embryo quality. However, other parameters of zygote state such as pronuclear morphology or halo has been limited to observation, so there are still rare known. Recently, time-lapse monitoring has been used to monitor and analyze early stage of embryonic development. So it is effective to get dynamic information about the zygote state.

Study design, size, duration: This was a retrospective study involving a total of 502 embryos cultured in the time-lapse system (Embryoscope®, Vitrolife) from January 2017 to November 2018. Monopronuclear (1PN), multipronuclear and those not clearly captured were excluded. The embryo quality was evaluated on day 3, and blastocyst formation was observed on day 5. Statistical analysis was performed by SPSS 23.0 using a t-test, chi-square test or logistic regression analysis. P-values<0.05 were considered significant.

Participants/materials, setting, methods: The tPNa, tPNf, pronuclear morphology and halo were observed through a time-lapse system. Pronuclear morphology was based on Scottet al., 2000, and simply modified into type A and B so that any evaluator can make a clear reading; pronuclear position: (A)juxtaposed, (B)non-juxtaposed, different size or fragmented, nucleolar morphology: (A)large size in both pronuclei; (B)small size in at least one pronucleus-scattered, the orientation of PBs: (A)the longitudinal or perpendicular axis of pronuclei and PBs; (B)different positions.

Main results and the role of chance: The results showed a statistically significant association between tPNa/tPNf and and good-quality embryos at day 3. In good-quality embryos, tPNa and tPNf were quicker than poor-quality embryos (9.46h vs 10.5h, p=0.002; 24.48h vs 27.61h, p<0.001, respectively). The PBs alignment of type A was related to good-quality embryos (66.7% vs 53.3%, p=0.002). However, the pronuclear position and distribution of NPBs were not related to embryo quality (63.2% vs 53.8%, p=0.066; 62.3% vs 56.9%, p=0.290, respectively). Also, the presence of halo is not correlated with embryo quality (61.1% vs 60.6%, p=0.904). Multivariate analysis showed that tPNf and orientation of PBs were associated with good quality embryo(OR=0.89, 95% CI=0.85-0.92, p<0.001, OR=0.56, 95% CI=0.38-0.82, p=0.003, respectively).

Furthermore, the blastocyst formation rate (BFR) showed the same result. The blastocyst formed on day 5 had a quicker tPNf (23.78h vs 26.49h, p<0.05). Also, the PB alignment of type A had a higher BFR (44.3% vs 32.0%, p=0.032). But, the tPNa, pronuclear position, distribution of NPBs and halo were not related to BFR (8.72h vs 9.38h, p=0.67; 40.9% vs33.8 p=0.296; 41.6% vs

32.5%, p=0.156, respectively). The tPNf and orientation of PBs remained significant after controlling for confounding factors (OR=0.86, 95%, CI=0.81-0.93, p<0.05; OR=0.55, 95%, CI=0.33-0.91, p=0.091, respectively).
Limitations, reasons for caution: While observing the nucleus form for 16-20 hours, its type is prone to misleading due to dynamic changes. Most of the parameters were from a single observation at a static time point. Thus, the evaluation of parameters based on the dynamic approach of the time-lapse system remains a challenge.
Wider implications of the findings: Using the time-lapse system, we have observed the dynamics of zygote state in detail. We found a relationship between zygote status and embryo quality, suggesting that tPNf and orientation of the PB could be one of the predictor. This may help when selecting the best embryo for embryo transfer.
Trial registration number: None.

P-258 FOR ABSOLUTE ASTHENOZOOSPERMIA IN IVF/ICSI: IS LASER-ASSISTED IMMOTILE SPERM SELECTION (LAISS) EFFECTIVE AND SAFE?

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Study question: Laser-assisted immotile sperm selection (LAISS) to collect viable spermatozoa for Intra-cytoplasmic sperm injection (ICSI) in complete asthenozoospermia cases: is it effective and safe?
Summary answer: Five out of eight absolute asthenozoospermia couples achieve pregnancy ranging from 10 to 33 weeks without any detected abnormalities for both mothers and fetuses.
What is known already: Absolute asthenozoospermia (100% spermatozoa are immotile) has been reported to happen once in every 5000 men, in which the percentage of "alive" sperms ranges from 0% to 100%. To identify viable spermatozoa in complete immotile samples for ICSI remains a challenge for embryologists. Despite the fact that the use of laser for cases with total immotile sperms was firstly reported in 2004, the feasibility, effectiveness and safety of this procedure requires to be well investigated.
Study design, size, duration: In 2018, nine absolute asthenozoospermia couples were enrolled in the study. After IVF and embryo transfer cycles, mothers' and fetuses' health is ongoing monitored. This study also includes verbal interview on reproductive medical history and continuous communication between staff and patients.
Participants/materials, setting, methods: The absolute asthenozoospermia couples underwent IVF/ICSI cycles with controlled ovarian stimulation. Sperms either from ejaculated semens or surgical procedures were LAISS-applied prior to ICSI: A single laser beam of 1.48 µm diode was shot at the far end of the tail. Viable sperms were collected if they had positive reaction to the laser beam: tail curling or a single immediate tail wagging movement. Frozen or fresh embryos were transferred day 3 or day 5.
Main results and the role of chance: All nine couples had fertilization; the average number of MII occytes retrieved and average fertilization rate was 14.3 and 66.8%, respectively, resulting in 90 day-2 embryoes in total. As on 31/01/2019, eight couples have had their embryo transfered, in which five couples are currently pregnant (33, 27, 16, 14 and 10 weeks respectively): four singleton and one twin pregnancy. From verbal interview data, the reproductive medical history of these five couples was revealed as followed: the duration of infertility was ranging from 3.5 to 7 years; all five couples had undergone andrology treatments using various Western and traditional herbal medicines without any improvement in sperm motility. Currently, the twin pregnant woman has been indicated a cervical cerclage at week 13. Otherwise, the mothers' health is in normal condition, no fetal anormaly has been detected.
Limitations, reasons for caution: To prove the safety of LAISS, besides monitoring mothers', fetuses' and take-home-babies' health, it is required to develop a method to confirm that LAISS does not affect DNA integrity and the centriole-containing neck of sperm. We currently investigate the cellular mechanisms of absolute asthenozoospermia and propose genetic tests for embryos.
Wider implications of the findings: LAISS together with IVF/ICSI appears to be a feasible and effective solution for infertile couples with 100% immotile spermatozoa. The safety of this procedure has been partly proved by normal condition of the fetuses. LAISS would propose great clinical value in asthenospermia treatment.
Trial registration number: Not applicable

P-259 The greater the number of oocytes, the worse the embryo quality in young donors

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Study question: Is the number of oocytes retrieved in an egg -donor cycle related to the embryo quality?
Summary answer: The number of oocytes retrieved in an egg-donor cycle is directly associated with a decrease in embryo quality and in the number of useful embryos.
What is known already: There is a strong correlation between the number of oocytes collected after pick up and the IVF outcomes. The objective of this study was to analyze if there was an association between the number of oocytes retrieved in egg donors and the quality and number of useful embryos obtained after IVF/ICSI.
Study design, size, duration: This is a retrospective study including 1131 cycles of fresh oocyte donation carried out during the period 2015-2017.
Participants/materials, setting, methods: We only included fresh egg donors cycles with normozoospermic samples. In all cycles day five not biopsied blastocyst were transfered. We evaluated the quality and the number of embryos that were able to transfer and/or freeze according to number of eggs retrieved. We also checked our clinical results.

Univariate comparisons were performed using Pearson's Chi-square test for categorical data and the T-student test for continuous measures. All of the multivariate analysis were adjusted by confounding factors.
Main results and the role of chance: An increase in the number of oocytes retrieved was correlated with a decrease in the percentage of good-quality embryos (p=0.0002) and with a smaller number of useful embryos (p=0.0002). However, we did not find any significant difference according to our clinical outcomes.

Finally, we analyzed our data according to the technique we used (conventional IVF or ICSI). We observed that the blastocyst quality and the number of useful embryos were significantly higher with conventional IVF rather than with ICSI (p=0.000) and (p=0.007), respectively.
Limitations, reasons for caution: This is a retrospective study. All oocytes were used for a single recipient, rarely we shared the donor or freeze some oocytes.
Wider implications of the findings: It has been proven that more retrieved ooyctes are not the best. We should adjust the donor's dose of gonadotrophins to avoid collecting a large number of oocytes, which affects the embryo quality at the blastocyst stage. The use of conventional IVF in normozoospermic samples generated more good quality blastocysts.
Trial registration number: .

P-260 Embryo culture conditions under high humidity significantly enhances blastocysts formation and quality according to an automatic time-lapse algorithm

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Study question: Is blastulation and quality rate conditioned by morphokinetics at early stages of embryo development?Is the humidity in an incubator chamber affecting morphokinetics and blastocyst rates?

Summary answer: There is a correlation between blastulation rates and quality with the Xtend-classification and a higher proportion of embryos with better Xtend scores in high humidity.

What is known already: Blastulation may be predicted by early stages of embryo development although controversial results still are present in the literature. The optimal incubator humidity is still a controversial topic in IVF laboratories. Even though the in vivo condition is humid, the possible growth of microorganisms under these conditions are higher than with dry incubators.

However recent studies have shown better results in humidified chambers probably due to more stable culture conditions regardless oil overlays in culture dishes (Fawzy et al Fert Ster 2017).

Study design, size, duration: University-affiliated infertility clinic. Retrospective cohort study in ovum donation program from January 2018 to October 2018. In the first part of the study, we analyzed 3001 embryos from 361 patients. In the second part, 116 patients with 1016 embryos were randomly distributed under high or low humidity in a continuous embryo monitoring incubator.

Participants/materials, setting, methods: Embryos were generated by ICSI and incubated in a Time-lapse incubator (Geri, Genea, Australia) that used an automatic cell-tracking software. An improved version of this algorithm was developed taking into the values of P2 and P3, egg age, number of cells on day3 and a texture image analysis correlated with fragmentation.

In the second part, the embryos were randomly distributed in chambers with or without humidity.

Main results and the role of chance: The Xtend classification was directly correlated with higher blastocyst rates and better good quality embryos. With an average blastulation rate of 73.6%, we studied rates in each category (1:91%; 2:86.30%; 3:79.7%; 4:63%; 5:38.3%). We studied the percentage of good-quality blastocysts (A/B ASEBIR morphological classification) in each Xtend category (1:57%; 2:47%; 3:36%; 4:25%; 5:11%)($p<0.001$).

A total of 1016 embryos were incubated in the Geri system under different humidity conditions, 561 in chambers with humidity and 455 in chambers without humidity. All of them were classified according to the Xtend algorithm. In the humidity chamber, 23.5% were classified as 1, 22.1% as 2, 17.6% as 3, 18.2% as 4 and 18.5% as 5.

In the dry chambers 26.2% were classified as 1; 18.9% as 2, 12.7% as 3, 17.6% as 4 and 24.6% as 5. We assembled the Xtend categories in two groups(1-2-3 vs 4-5)and compared the proportion of embryos in each group (humid vs dry incubation) showing more embryos classified as 1-3 in humid (63.30%) compared with dry incubation (57.26%), even though differences were not statistically significant($p=0.075$).

Moreover, blastocyst rate in humidity chambers was 77.2% versus 70.9% in dry chambers and percentage of good quality blastocysts 42.8% (humidity) vs 35.8% (dry)($p=0.022$).

Limitations, reasons for caution: The retrospective nature of this study may be a reason for caution. The classification system itself has some errors due to difficulties in cell tracking generating "none result", however we only included cases where classifications were provided. A further analysis could include different humidity measurements to establish the optimal value.

Wider implications of the findings: This study correlates Xtend categories with blastulation rates, demonstrating a direct link with the diagnostic test. According to humidity,results obtained show a higher proportion of good-quality embryos(classified as 1-2-3)as well as higher blastocyst rates under humidity conditions.

Therefore, incubation under high humidity might improve the final outcome of the cycle.

Trial registration number: Not applicable.

P-261 What is the implantation potential of vitrified average quality blastocysts?

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Study question: The aim of this study was to determine whether the survival rate and implantation potential of frozen average quality blastocysts justified its clinical application.

Summary answer: The survival rates of vitrified-warmed average quality blastocysts (AQB) are comparable to vitrified-warmed good quality blastocysts (GQB), and implantation rate is comparable to fresh AQBs.

What is known already: Average quality blastocysts have a lower implantation potential than good quality blastocysts. On this basis, it may not be routine practice in many IVF clinics to freeze embryos of this quality due to their lower potential. However, the potential of vitrified average quality blastocysts is still relatively unclear.

Study design, size, duration: This is a retrospective study of 1905 blastocysts, either fresh or frozen, that were transferred between July 2015 and December 2018. The data was divided into three groups, frozen average quality blastocysts, frozen good quality blastocysts and fresh average quality blastocysts. Survival and implantation rates were compared between groups. Patients with a blastocyst grade $\leq$ Cc, as per the Gardner blastocyst grading scheme and those without known implantation data were excluded.

Participants/materials, setting, methods: The data was initially divided into good (Aa, Ab, Ba, Bb; n=1121) and average quality (Ac, Bc, Cb, Ca; n=403) vitrified blastocysts, as per the Gardner blastocyst grading scheme, and the survival and implantation rates (IR) were compared. The data was also divided into fresh (n=381) and frozen AQBs (n=403) and IR were compared. Chi-squared analysis was performed on the data and a p value <0.05 was considered to be statistically significant.

Main results and the role of chance: This study shows that there was no significant difference in survival rates between vitrified-warmed AQB and GQB (96% vs 97%, respectively, $p=0.2656$). However, vitrified-warmed AQBs had a significantly lower IR compared with GQBs (23.3% vs 38.7%, respectively, $p<0.05$). When comparing the IR of vitrified-warmed AQB to fresh AQB, there was no significant difference (23.3% vs 23.1%, respectively, $p= 0.9398$). Blastocysts were vitrified and warmed using the same cryopreservation media (Cook Blastocyst vitrification kit/ warming kit), therefore reducing a possible effect of cryopreservation media on survival and success rates.

Limitations, reasons for caution: Despite a large sample size, the study groups are not evenly distributed; with a larger number in the GQB group. Our vitrification protocol was modified prior to this study to include laser-induced collapsing; however some of the warmed embryos in this study may have been vitrified prior to this modification.

Wider implications of the findings: Our research suggests that it is advisable to vitrify AQBs, particularly for patients that have no GQBs available after transfer. This provides these patients with the opportunity to freeze embryos and offers them a further treatment attempt without undergoing the higher costs and strenuous stimulation required for a fresh cycle.

Trial registration number: Not applicable

P-262 Single frozen-thawed embryo transfer after time-lapse culture is associated with higher implantation rates and lower miscarriage rates in young patients

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Study question: Does time-lapse embryo selection have preferential results for embryo quality evaluation compared with conventional assessment?

Summary answer: Better clinical outcomes in IVF cycles are achieved using time-lapse embryo selection than during conventional embryo selection.

What is known already: Optimal conditions for embryo culture and possibility of proper assessment are key factors in assisted reproductive technology. Time-lapse follow-up of embryo development has become popular worldwide. The most significant benefits of this method include the possibility to maintain undisturbed culture and the morphokinetic evaluation of embryo development. Nevertheless, there are a lot of debates about the effectiveness of the time-lapse culture compared with conventional culture. There is still lack of evidences to support one of these approaches, so the question remains open.

Study design, size, duration: In this retrospective study implantation rates and early pregnancy loss were analyzed after 456 embryo transfers in the five-year period (2013-2017). Oocyte donation and array comparative genomic hybridization (aCGH) cycles were excluded from the study. Only the first Day 5 single frozen-thawed embryo transfers for each patient were included.

Participants/materials, setting, methods: Two randomized groups of patients under the age of 36 (Mean age was 31±3.31) were compared. Embryos of the first group (group A: n = 232) were cultured with the application of time-lapse monitoring. Embryos of the second cohort (group B: n = 224) were

assessed by conventional method. ICSI as well as vitrification were performed for all cycles. Embryos were cultured under stable conditions (5.3% CO2, 5.0% O2, 37°C) within the same culture medium.

Main results and the role of chance: The analysis showed that the application of time-lapse monitoring was associated with a higher implantation rate in the group A compared with the group B (59% versus 48%; OR: 1.55; P<0.05). The level of miscarriages was lower in the group A (13.9% versus 26.9%; OR: 0.44; P<0.05).

Limitations, reasons for caution: There was no control group for assessing the time-lapse incubator itself. Male factor infertility cause wasn't excluded from both groups of this investigation. However, all ongoing pregnancies resulted in births, stillbirths were not evaluated.

Wider implications of the findings: Time-lapse monitoring could serve as a platform for more detailed researches in future. It worth mentioning that according to our previous investigation the miscarriage rates in aCGH cycles with time-lapse culture were dramatically lower (2.7%) even for the patients of advanced maternal age.

Trial registration number: None.

P-263 cryopreservation in very low number sperm count

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Study question: Can we determine the minimum amount spermatozoa to be cryopreserved but still had a good quality to be used and assess motility and viability after being cryopreserved?

Summary answer: Sperm in any amount could be cryopreserved with a good result.

What is known already: Cryopreservation of sperm today is the most widely procedure because by using cryopreservation it means that the necessary biological material can be available at the time it is needed and can be stored for a certain period of time.

Conventional cryopreservation is an obstacle to achieve sufficient harvest in cases of severe oligospermia.

Sometimes cryopreservation can not be performed because too little sperm count and it means that the sperm can not pass the freeze-thaw procedure.

Until now there is no marker or cut off value that can predict how much sperm can be carried out by cryopreservation.

Study design, size, duration: This was an experimental pretest post test group design study.

This study was conducted at Medical Biology Laboratory Faculty of Medicine Airlangga University. Sample of the study was ejaculate obtained from patients attending Ferina Center for Reproductive Medicine. Thirteen patients were included in this study. Inclution criteria are men 21-40 years old with sperm oligoastenoteratozoospermia sperm analysis with concentrations range 7-9 million / ml.

Hematospermia and leucospermia were excluded from this study.

Participants/materials, setting, methods: Sperm preparation by using simple washing technique followed by side migration technique.

We devided the subgroups into 4 group: group of 71-100 sperm, group of 31-70 sperm, group 11-30 sperm and group 1-10 sperm.

Moving spermatozoa were aspirated into pippete in certain amount and put into 2 µl microdrops of freezing medium. The drops were put in the tip of Cryologic® and left in liquid nitrogen vapour for 1 min prior to be plunged in it.

Main results and the role of chance: This study showed there was a significant differences of motility rate between groups (p<0,05, Confidence Interval 95%), The highest result obtained from group 1-10. There was no significant differences of viability between groups (p>0,504, CI 95%). We also count living motile spermatozoa, living immotile spermatozoa and non living spermatozoa. In some immotile spermatozoa did not mean they are non viable, there was still possibility that some on them were viable, only need time to make a progressive movement.

Limitations, reasons for caution: This study was not a real situation, this was just a model of a very low sperm obtained from oligozoospermic patients.

We should use hypoosmotic swelling test to assess viability instead of Eosin test

We did not have enough time to assess motility because the drop could dry immediately.

Wider implications of the findings: By using this technique, sperm in any amount even one or two sperm could be cryopreserved with a good result.

Trial registration number: Not applicable

P-264 Correlation between number of oocytes retrieved during a single IVF/ICSI cycle and cumulative live birth rate in different age groups

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Study question: What is the association between number of oocytes collected from different age categories and the cumulative live birth rate (CLBR) in a single IVF/ICSI cycle?

Summary answer: Cumulative live birth rate continues to increase with oocyte number

What is known already: Controlled ovarian stimulation (COS) aims to create surplus embryos. Assessment of its effectiveness is most valid per full cycle, including fresh and frozen embryo transfers (ETs). It has been reported there is no benefit to live birth rate (LBR) if >15 oocytes are created, with 13-15 oocytes suggested to be optimum for the generation of an adequate number of embryos for fresh and frozen ETs. However, such studies primarily looked at oocyte number and LBR in relation to fresh ETs. Other studies have discussed CLBR over a number of stimulation cycles, but few reported results from one complete COS.

Study design, size, duration: This study is a large cohort analysis of retrospective data from January 2009 to December 2015 in a tertiary centre - Guy's and St Thomas' Hospital, Assisted Conception Unit

Participants/materials, setting, methods: 754 patients, aged 19-46 years, planning a single embryo transfer (sET) following IVF/ICSI were selected. Only patients with one completed cycle (fresh and frozen embryos) were included in the analysis (n=470). Slow freezing was the method of cryopreservation used for storing surplus embryos. To evaluate the impact of oocyte yield on CLBR, patients were divided into 5 groups based on the oocyte number for their cycle.

Main results and the role of chance: The average age of the patients included was 35.3+/- 4.3, whilst the average number of eggs and 2pn embryos was 12+/- 6.8 and 6.6+/ 4.2 respectively. In addition, the mean number of embryo transfers resulting from one stimulation was 1.7+/- 1.1. The overall CLBR from one complete cycle, taking into account all oocyte numbers and age groups, was found to be 42%. It was found that in patients <35 years old the CLBR was 85% over 4 transfers, when >20 oocytes were collected, compared to 40% over the same number of transfers, when <6 oocytes were available. For those patients in the >40 years old category the CLBR over 4 transfer was 38% when >20 oocytes and 18% when <6 oocytes,. Finally, the regression analysis shows that overall there is a significant increase in CLBR when compared to the oocyte yield (P<0.032).

Limitations, reasons for caution: This is a cohort analysis using retrospective data collection based on relatively limited figures. Despite the use of a statistical analysis, the presence of biases due to retrospective data cannot be excluded. Furthermore, these results need to be supported with the generation of larger, prospective investigations.

Wider implications of the findings: Our findings contradict previous studies that discuss an 'optimal' oocyte number for high LBR. With increasing oocyte number, there is increasing CLBR thus patient's expectations can be more adequately managed depending on oocyte yield following a single COS.

Trial registration number: Not applicable

P-265 Total mitochondrial DNA (mtDNA) content decreases along embryo development, insights of mtDNA turnover in human preimplantation development.

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Study question: To assess the mtDNA content along the human embryo development.
Summary answer: This study demonstrates that not only the mtDNA content per cell decreases but also the total mtDNA copy number experiences a significant reduction before implantation.
What is known already: It has been shown that mtDNA has a half-life in culture of 2-4 days. In animals, mtDNA copy number has been revealed to change depending on the species. Hashimoto et al., (2017) observed that total and per cell mtDNA copy number decreased along human embryo development, however there was an increase of total mtDNA copy number at the blastocysts stage. mtDNA content along embryo development has not been widely studied in humans but this is an important topic to address in order to better understand the mitochondrial dynamics in preimplantation embryos under in vitro culture conditions.
Study design, size, duration: An observational prospective study was performed with a total of 89 samples. 36 fresh unfertilized oocytes, 21 vitrified day 3 embryos and 32 vitrified aneuploid blastocysts were warmed and immediately collected in PCR tubes with 2,5 µl of PBS. Finally, a Q-PCR was performed to determine total and per cell mt DNA copy number.
Participants/materials, setting, methods: Q-PCR was performed with SurePlex DNA Amplification System (Illumina) using specific primers for the ATP8 and β-Actin genes to assess the total and per cell mtDNA copy number. Data was statistically analysed by ANOVA test with Scheffé multiple comparison for categorical variables and linear regression for numerical variables.
Main results and the role of chance: Fresh oocytes have significantly more total and per cell mtDNA copy number (Log_2 ATP8= 20.32, Log_2 ATP8/β-Actin=7.49) than embryos on day 3 of development (Log_2 ATP8= 18.26, Log_2 ATP8/β-Actin=5.46; $P<0.05$). At the same time, day 3 embryos have more total and per cell mtDNA copy number than blastocysts (Log_2 ATP8= 16.39, Log_2 ATP8/β-Actin=3.15; $P<0.05$). Interestingly, mtDNA content increased significantly ($P<0.05$) with age in oocytes (18-34 years) and embryos on day 3 of development (25-37 years) but not statistically significant ($P>0.05$) in blastocysts (24-44 years). Once fertilization takes place, mtDNA content in embryos on day 3 of development decreases along cleavage achieving minimal levels at blastocyst stage. No differences on mtDNA content were found when grouping blastocysts by quality or by day of development ($P>0.05$). This study shows that mtDNA content diminishes along embryo development and no mitochondrial biogenesis takes place at blastocyst stage. Also we have found a relation with the patient age in oocytes and embryos on day 3 of development within the age ranges of the study.
Limitations, reasons for caution: All the analyzed blastocyst were aneuploid, so we need to check whether euploid human blastocysts will have similar behavior. Moreover, some of the embryos included were vitrified however, since they were tubed immediately after warming, the impact should be minimal.
Wider implications of the findings: According to previous studies in animals, during preimplantation development human embryos seem to experience a significant decrease on mtDNA content that support the embryo quiet hypothesis. Therefore any situation resulting in mtDNA content increase should be understood as a stress condition. This findings could be used to improve culture conditions.
Trial registration number: Not applicable

P-266 Can we use blastocyst collapsing as a marker of embryo implantation?

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Study question: Can blastocyst collapses provide information about embryo quality?
Summary answer: According to our results, spontaneous blastocyst collapses are related to implantation rate and embryo morphokinetics.
What is known already: In order to increase results in assisted reproduction techniques, embryo selection criteria are continuously improving. Blastocyst collapses is one of the studied parameters to be included or not as a tool for embryo selection. Some groups have shown that weak collapses help the subsequent hatching of the embryo, however, strong collapses can affect implantation capacity. Studies in mouse blastocysts have shown that collapse is associated with the Na + / K + -ATP pump, which implies an extra energy expenditure. This suggests that repeated strong collapses in human blastocysts, could lead to a lack of energy and subsequent embryo blocking.
Study design, size, duration: We retrospectively analyzed data obtained from 113 blastocysts from 96 patients. All patients carried out an in vitro fertilization treatment, and embryo transfers (ET) were performed on day 5. The treatments were recorded between January of 2015 and December of 2018.
Participants/materials, setting, methods: Embryos were cultured in a MIRI-TL (ESCO-Medical ©) time-lapse incubator in microdroplets of single culture media (CSCC©, Irvine Scientific) covered with mineral oil (Irvine Scientific) until ET. The amount and type of collapses, that defined groups (strong, weak and mixed collapses) were recorded. Morphokinetic characteristics of each embryo were collected too. Statistical analysis was carried out with the SPSS program (V22.0, IBM Statistics), using the t-student test to find differences between groups.
Main results and the role of chance: Observed collapses were classified into three groups: strong, weak and mixed collapses. Number ranged from 0 to 4. Blastocysts that had strong collapses showed a significant negative impact on the implantation rate, observing, in addition, a zero-implantation rate in mixed type collapses. We observed that weak collapses were related to embryo implantation. Regarding number of collapses, those which did not perform any type of collapse did not show significant differences in the pregnancy rate. However, as the number of collapses progressed, there was a notable tendency towards less implantation.

Regarding morphokinetic data, those blastocysts with strong collapse exhibited shorter cell cycles during their development and less synchrony between divisions, as opposed to weak collapses. No significant statistical differences were found, but a trend was observed.
Limitations, reasons for caution: One of the limitations of the study is the number of embryos included. We will go on collecting data to increase sample size in every single group.

In order to visualize all the collapses, time-lapse technology is required, which is not yet available in all laboratories.
Wider implications of the findings: Our results show a correlation between the collapses at the blastocyst stage and the implantation rate probably due to the waste of ATP in expansions and the fast mitotic division.

This information could help us to better select embryos with the highest implantation potential.
Trial registration number: Not applicable.

P-267 Morphokinetic assessment of embryos in carriers with structural chromosome rearrangements undergoing preimplantation genetic testing: a longitudinal cohort study

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Study question: Whether unbalanced embryos, arising in meiosis during the gamete formation, in the carriers of structural rearrangements (SR) undergoing preimplantation genetic testing, can be detectable through non-invasive, morphokinetic assessment.
Summary answer: Multivariable analysis of time-lapse morphokinetic parameters at first cleavages and blastocyst formation cannot be used as a predictive factor for selecting balanced embryos.
What is known already: Using morphokinetic data to predict balanced embryos is intriguing since it is non-invasive and could easily be implemented in most IVF laboratories. However, results from studies trying to identify any predictive value of morphokinetic analysis for embryonic chromosomal status are contradictory.
Study design, size, duration: Longitudinal cohort analysis of time-lapse imaging, to measure timing, synchronicity and cleavage patterns of the first embryonic divisions. The frequency of direct cleavage events was introduced

as a dynamic score These parameters were correlated with the chromosome complement of the embryos obtained from 101 couples undergoing 120 IVF cycles of preimplantation testing for structural rearrangements and aneuploidy testing, between 2014 and 2018. All procedures and protocols were carried out in Embryogenesis, Athens, Greece.

Participants/materials, setting, methods: 579 fertilized embryos were monitored using the Embryoviewer™. Trophectoderm and day 3 biopsy was performed in 111 and 468 embryos respectively. Biopsied samples were analyzed for the specific structural rearrangement and for 24 chromosome aneuploidy testing and classified as (a) balanced/euploid, (b) balanced/aneuploid, (c) unbalanced/euploid and (d) unbalanced/aneuploid.

Main results and the role of chance: Statistically significant differences were not observed between groups (a) and (b) for t2 (p=0.55), t3 (p=0.72), t5(p=0.67) and t6 (p=0.8). The time intervals between t5-t2; t5-t3; t5-t4; t3-t2 (p<0,001) and synchrony from 2-4 cells (t4-t3, p=0,03) were significantly different between groups (c) and (d) but not between groups (a) and (b) [t5-t2; t5-t3; t5-t4; t3-t2 (p=0,144) (t4-t3, p=0,12)]. The occurrence of dynamic score was 10%, 12%, 23% & 62% for Groups a, b, c & d respectively.

Multi variable logistic regression analysis identified the time interval t4-t3 (Odds Ratio=0.80;95% Confidence Interval,0.70-0.94) in combination with direct cleavage frequency (OR=0,33;95%CI,0.18-0.61) as the most relevant variables in selecting unbalanced/aneuploid embryos. t3-t2 time interval along with direct cleavage frequency, has also high predictive value when comparing groups (c) versus and (d). In contrast, none of the above parameters have high predictive value when comparing groups (a) and (b).

Limitations, reasons for caution: When biopsy and chromosome analysis is performed on day 3 the high degree of embryonic mosaicism could affect the results and lead to misdiagnosis. Several variables such as ovarian stimulation, maternal age, culture media, culture conditions and BMI can affect time-dependent measurements and create a bias in the morphokinetics analysis.

Wider implications of the findings: Although a relationship between morphokinetic factors and ploidy status has been reported, the predictive values of these markers cannot replace the preimplantation genetic testing for structural rearrangements

Trial registration number: not-applicable

P-268 Reproductive Outcomes of Testicular versus Ejaculated Sperm for Intracytoplasmic Sperm Injection into Sibling Oocytes

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Study question: Is testicular sperm (TS) a better option in infertile couples who failed to achieve clinical pregnancy after intracytoplasmic sperm injection (ICSI) with ejaculated sperm (ES)?

Summary answer: TS-ICSI and ES-ICSI revealed similar fertilization rate(FR), blastocyte development rate(BDR), clinical pregnancy rate(CPR) and live birth rates(LBR) when they are performed on sibling oocytes.

What is known already: The use of TS instead of ES for ICSI has gained popularity in the recent years, because of studies reporting better CPR and LBR especially in cases with severe oligoasthenoteratozoospermia and high sperm DNA fragmentation. However, there is scarce of data in the literature comparing TS and ES in cases of recurrent poor embryo development. Considering the significant role of oocyte quality in the success of assisted reproductive techniques, the impact of sperm retrieval technique on reproductive outcomes can be better demonstrated among sibling oocytes whose qualities are similar.

Study design, size, duration: The records of 51 couples with at least 2 unsuccessful ES-ICSI attempts between January 2014 and April 2017 have been retrospectively evaluated.

Participants/materials, setting, methods: The demographic data, clinical findings and sperm/oocyte characteristics of 51 couples were recorded. Sibling oocytes were fertilized with ES-ICSI and TS-ICSI. FR, BDR, CPR and LBR obtained with ES-ICSI and TS-ICSI were compared.

Main results and the role of chance: The mean age of the female and male patients were 33.49±5.30 (23-44) and 37.35±5.60 (25-47), respectively. The couples had mean 2.82±1.55 unsuccessful previous ES-ICSI attempts. Of the couples 22 and 25 were suffering from female factor and male factors respectively whereas no etiological factors could be identified in 4 couples (unexplained infertility). Mean sibling oocyte number collected for the ES-ICSI and TS-ICSI was 16.75±7.94 (6-40). TS cells have been harvested via testicular sperm aspiration (TESA), testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA) in 29, 18 and 4 patients respectively. Of the 327 MII oocytes, which underwent ES-ICSI, 241 fertilized normally (2PN) whereas 334 MII oocytes which underwent TS-ICSI 218 were fertilized normally (73.7% vs. 65.3%, p=0.214).Of the oocytes fertilized with ES-ICSI and TS-ICSI, 63 and 58 blastocyst developed (26.14% vs. 26.61%, p=0.716). Of the couples, 31 and 36 achieved clinical pregnancy with ES-ICSI and TS-ICSI respectively (50.00% vs. 65.79%, p=0.186).Finally LBRs were 41.18% vs. 60.53% (p=0.065)in ES-ICSI and TS-ICSI groups, respectively.

Limitations, reasons for caution: The sample size was small and embryologists were not blinded to the medical history of the patients which may be prone to operator bias.

Wider implications of the findings: ES-ICSI and TS-ICSI have similar FR, BDR, CPR and LBR among couples with at unsuccessful ES-ICSI attempts. Considering the higher (but statistically insignificant) CPR and LBR in sibling oocytes, TS-ICSI may be recommended to couples with previous unsuccessful IVF treatments with ES-ICSI

Trial registration number: N/A

P-269 2 hours post-thaw survival assessment of warmed blastocysts predicts clinical pregnancy

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Study question: To determine the shortest relevant recovery time after warming of vitrified blastocyst studied in SET (single embryo transfer) cycles.

Summary answer: A minimum recovery time of two hours before assessing embryo survival is beneficial to clinical outcome and avoids the transfer of fairly survived blastocysts.

What is known already: Several studies have reported on the relevance of blastocoele expansion after thawing as a predictor of implantation in single/-double transfer cycles. In SET frozen/thawed cycles the degree of reexpansion has been shown to be the best predictor for live birth rate.

Manufacturers advise to let the embryos recover from 2 to 4 hours after warming before the transfer. Some studies have reported various recovery delays like as short as 30 minutes post warming before the transfer. The shortest relevant post thaw culture time remains stil unclearly defined.

Study design, size, duration: Prospective observational study during 2017 to compare pregnancy outcome after ≤1 hour post thaw assessment of blastocyst survival and 2 hours post thaw assessment. Two clinicians conducted the study in order to avoid practice variations. Both practitioners performed vitrified/warmed blastocyst transfers either after less than 1 hour post thaw or 2 hours post thaw. Only SET cycles were included to control for embryo competency.

Participants/materials, setting, methods: 251 cycles included: group A (n=76) transferred ≤1 hour post-thawing (8.30AM) and group B (n=175) transferred 2 hours post-thawing (10.30AM). Blastocyst survival was assessed before the catheter load to discard damaged/non-reexpanded ones.

258 thawed blastocysts (Vit Kit® -Thaw, IrvineScientific®) recovered in Sage 1-Step™ (Origio). Catheters were loaded with EmbryoGlue® (Vitrolife) for ultrasound-guided transfers in natural cycle at 6-8 days after LH surge.

Survival and clinical pregnancy rates were compared between groups by t-test and Chi-square test.

Main results and the role of chance: Female age was similar between groups (A : 36.5+/-5.4, B : 35.7+/-3.1, p=0.47).

78 blastocysts were warmed in group A and 180 in group B. Survival rates were similar between groups (A: 97.3%, B: 96.0%, p=0.59).

Clinical pregnancy rates were significantly higher in group B compared to group A (36.3% vs 18.9%, p=0.048).

Limitations, reasons for caution: The study would benefit from a larger data size and from comparison of SET warming cycles in a PGT-A population were the embryo quality bias would be most limited.
Wider implications of the findings: Assessing blastocyst survival before transfer is mandatory to discard embryos with low implantation potential. In our center a minimum recovery time of two hours before survival evaluation enhanced clinical outcome by avoiding damaged blastocysts. Therefore all frozen/thawed transfers shifted after 9.30 AM to allow minimum two hours recovery after warming.
Trial registration number: Not Applicable

P-270 Mechanically disconnected mouse preantal follicles lead to developmentally competent oocytes following follicle culture, likely due to the reestablishment of functional transzonal projections

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Study question: Are mechanically disconnected mouse preantral follicles able to re-establish transzonal projections (TZPs) connections and acquire competence comparable to oocytes derived from intact cultured follicles?
Summary answer: Reconnection of disrupted mouse preantral follicles, mediated by TZPs reestablishment, leads to embryo formation rates and gene expression comparable to oocytes from intact cultured follicles.
What is known already: Impaired oocyte-granulosa connections can be restored in mouse preantral follicles during *in vitro* follicle culture. Increased TZPs density and function has been shown following 2 days of 3D in vitro culture of disconnected follicles. Oocyte secreted factors support granulosa cells to generate new TZPs to ensure normal follicle growth. The impact of temporary communication disruption between the two follicular compartments, somatic and germ cell, has not been assessed widely with regard to the oocyte developmental capacity.
Study design, size, duration: Mouse secondary follicle culture was performed for a period of 10 days. On day 5 of culture, in half of the follicles, the oocyte was mechanically disconnected from its granulosa cells and placed back in culture, whereas the other half of follicles remained intact. We compared the culture outcome of the disconnected versus the intact follicle group. Two independent experiments, comprising a minimum of 6 culture plates with 10 follicles for each condition, were performed.
Participants/materials, setting, methods: Developmental capacity of mouse (C57BL/6J x CBA/Ca) MII oocytes from intact and disconnected follicles after *in vitro* culture was tested by IVF. Gene expression analysis was performed in MII oocytes and corresponding cumulus cells for oocyte quality markers and early embryo development (Gdf9, Bmp15, Zar1, Mater, Nmp2, Stella, Oct4) and cumulus expansion (Has2, Ptgs2, Ptx3). Two cell embryo and blastocysts rates were assessed. TZPs formation in disconnected follicles was identified by confocal microscopy.
Main results and the role of chance: The disconnected group attained the same morphological features as the control, by the end of the follicle culture period. Presence of TZPs was identified in the experimental group 2 days after mechanical disconnection of the oocyte. By day 9 of culture, an increased density of these connections was notable, presumably due to de novo formation. F actin immunofluorescence staining and confocal imaging were used for TZPs visualization.

The oocyte maturation rates were not significantly different (p=0,5511): 75% for intact follicles and 70% for disconnected ones.

The 2 cell embryo rate did not reach statistical significance (p=0,4604) between the experimental group (58%, n=77) and control (52%, n= 59). The blastocysts formation was comparable in the intact (68%, n=40) and disconnected follicles (71%, n=55).

The qPCR analysis showed a tendency for lower expression levels for all of the selected oocyte quality and early embryo development markers in the experimental group. However, these values did not reach significant differences.
Limitations, reasons for caution: In this study, the oocyte disconnection from the follicles was mechanically induced. In case of spontaneous oocyte release due to abnormal follicle physiology, re-establishing the TZPs might require a different approach.
Wider implications of the findings: Our data expands previous published results. These findings propose a possible strategy for rescuing the competence of partially denuded human COCs undergoing IVM. Oocyte cumulus connections are critical for meiosis inhibition during pre-IVM. Improving these could allow proper oocyte pre-maturation leading to an increased developmental capacity.
Trial registration number: not applicable

P-271 Does the day of blastocyst biopsy alone affect PGD clinical outcome?

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Study question: Does the day of trophectoderm biopsy affect PGD clinical outcome?
Summary answer: Trophectoderm biopsy (TEBx) of day 5 (D5) blastocysts is associated with a significantly improved clinical outcome compared to that of day 6 (D6) blastocysts.
What is known already: Increased clinical pregnancy rates (CPR) have been reported following D5 versus D6 transfer in frozen embryo transfer (FET) cycles (Haas *et al*, 2016). Similarly, higher ongoing pregnancy rates were reported following the transfer of D5 versus D6 embryos for PGS (Liebermann *et al*, 2017). However, the impact of blastocyst quality on D5 or D6, followed by TEBx and vitrification, on CPR has not been adequately explored. In addition, data investigating the clinical outcome of biopsied and vitrified blastocysts, selected for FET based on the day of biopsy and/or quality, are limited (Yang *et al* 2016; Wang *et al* 2016).
Study design, size, duration: This retrospective study included 1058 single FET cycles following TEBx on D5 and D6 blastocysts. In those 1058 cycles, 1069 blastocysts were warmed and 1031 were transferred between December 2013 and October 2018. Transferred embryos were divided according to the day of biopsy into D5 and D6 groups. Each group was subdivided based on embryo quality into good (>BB), average (AC, CA, BC, CB) and poor (CC). The CPR was compared between the different groups.
Participants/materials, setting, methods: Blastocysts subjected to laser-assisted TEBx and vitrification on D5 (n=726), and D6 (n=305) were warmed and transferred individually. Blastocyst grading was carried out on the day of TEBx using the Gardner grading system. CPRs were reported according to the day of biopsy and embryo quality. Chi-squared test was used to compare the CPR. The association between the day of biopsy, embryo quality and CPR was evaluated by multivariable logistic regression.
Main results and the role of chance: There was no significant difference in patient age between the two groups (32.7±3.85 vs 32.9, ±3.85; *P*=0.44). The overall blastocyst survival rate (SR) per embryo warmed was 1031/1069=96.4%. SR was significantly higher in D5 compared to D6 (726/742=96.5% and 305/327=91.1%, *p=0.003*). CPR per transfer was also significantly higher in D5 compared to D6 (CPR: 384/726=52.9% and 96/305=31.5%, *P*<0.05). Logistic regression analysis identified the day of biopsy and embryo quality as significant predictors of pregnancy in PGD-FET cycles (*P*<0.001). Our results showed that D5 poor quality embryos had similar CPR to good quality D6 embryos (D5: 20/58=34.5% *vs* D6: 54/143=37.8%, P>0.05). The CPR of the 6 sub-categories was the following: D5-good 314/565=56%, D5-average 41/103=40%, D5-poor 20/58=35%; D6-good 54/143=38%, D6-average 27/88=31%, D6-poor 13/74=18%.
Limitations, reasons for caution: Blastocyst grading, was based on subjective assessment, by different scientists performing the biopsy (practitioners) and the possible effect of other confounding variables.
Wider implications of the findings: This is the first study investigating CPR in relation to day of TEBx and blastocyst quality. Preference should be prioritised to D5 biopsied blastocysts for PGD-FET cycles to achieve higher CPR regardless of blastocyst quality. Continuous D5 embryo monitoring will maximize the number of D5 biopsied blastocysts compared to D6.
Trial registration number: N/A

P-272 Implantation rate of vitrified-warmed blastocysts according to their expanding blastulation time : assessment by time-lapse technology.

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Study question: Is expanding blastulation (B3 degree) time efficient, in addition to morphologic criteria, to select blastocyst in frozen embryo transfer (FET) cycles?
Summary answer: There is a strong likelyhood for a better implantation when embryo reach B3 expansion degree earlier.
What is known already: Routinely, day 5 (D5) and day 6 (D6) blastocysts morphology is evaluated at 116+/- 2 and 140+/-2 hours after fertilization (hAF) respectively (ESHRE 2011 consortium). There is a consensus indicating that fresh transfer of late blastulating embryos leads to lower implantation rates (IR). However a debate persist regarding implantation chances of D6 embryos transferred after cryopreservation. Time-lapse allows continuous follow-up of embryo development and therefore could serve to identify this embryo-specific timing, whether it has been vitrified on D5 or D6. In this context, we queried whether exact expanding blastulation time (tB3ED) is more predictive than the day of vitrification.
Study design, size, duration: A single center retrospective cohort analysis realized on 143 frozen blastocysts with known outcome. Including all single embryo transfers and double embryo transfers with either 2 or no implanting embryos that have been cultured in time-lapse system during 2 years. These included 102 D5 and 41 D6 blastocysts cryopreserved from autologous IVF cycles (137 FET) without any age or other clinical criteria exclusion.
Participants/materials, setting, methods: Blastocysts achieving a B3 expansion degree (B3ED), at 118 or 142 hAF, with at least one B grade for inner cellular mass and/or trophectoderm were frozen on D5 or on D6 respectively. We compared 3 groups of frozen embryos: D5 embryos reaching B3ED before 118 hAF (group 1), D6 embryos reaching B3ED between 118 and 122 hAF (group 2) and slower ones (group 3). Primary outcomes were tB3ED and IR. A Mann-Whitney test was used.
Main results and the role of chance: Regarding transfer outcomes, we observed that embryos leading to pregnancy reach a B3ED earlier than those with no implantation (median 106.7h [94.5-129.3] and 110.8h [90.4-138.3] respectively). Amongst our studied blastocysts, 75.5% (108/143) reached B3ED before 118 hAF and 24.5% (35/143) after. Furthermore, we observed a tendency for a higher IR for embryos that reached B3ED before 118h compared to the others: 31.5% (34/108) *vs* 14.3% (5/35) respectively. This difference was though no statistically different *(p=0.058)*. More precisely, in group 1, the IR was higher when embryos reached B3ED before 114 hAF (32.3% *vs* 26.7%). For groups 2 and 3, IR was 27.3 % (3/11) and 8.3 % (2/24) respectively. When we look at the day of vitrification: third of frozen D6 embryos (31.4%) had actually reached B3ED later on D5 and could have been frozen routinely on D5 instead of D6. These embryos seems to have a better outcome than those with a delayed development (B3ED after 122h): 27.3% (3/11) *vs* 8.3% (2/24) respectively, *p=0.15*.
Limitations, reasons for caution: The main limitation was the size of the sample; due to the availability of only one time-lapse incubator, the exclusion of single pregnancies after DET and our thawing strategy. Indeed, D5 blastocysts are firstly warmed for FET cycles leading to several D6 still frozen at the time of the study.
Wider implications of the findings: Our study is preliminary : if the tendency becomes significant with a higher number of embryos included, it could be interesting to define a B3ED time to optimize blastocyst selection in addition to morphologic criteria.
Trial registration number: None

P-273 Current laboratory standards for assessing vitrified-warmed blastocyst viability fail to accurately predict their implantation potential.

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Study question: Do current standard assessments of vitrified-warmed embryo survival and cellular viability accurately predict blastocyst functionality post-transfer? Do all embryos deemed viable at 3hr sustain development?
Summary answer: Blastocysts appearing viable at +180min may not continue development 24hr later, confirming that standard laboratory yiability assessments are visual not functional determinants of cellular health.
What is known already: Vitrification has revolutionized cryopreservation in the field of reproductive medicine by achieving >95% complete blastocyst survival rates. A variety of open and closed device vitrification techniques use mixed solutions of cryoprotectants and concentrated macromolecules to form a supercooled vitreous solid. These methods rely on faster warming rates than cooling rates to avoid damaging ice formation and optimize post-warming/dilution survival. Pre-transfer embryo survival evaluation typically occurs between 0 and 3hr post-warming. Although high live rate rates are associated with vitrified embryo transfer cycles, it is unknown if current survival assessments at 3hrs accurately identify viability.
Study design, size, duration: Research consented vitrified blastocysts (n=60), from four Ovation laboratories, were warmed using standardized, published protocols. Survivability and blastocoele expansion measurements were assessed at +1min, +30min, +60min, +120min, +180min, and +24hr post-warming. Initial survival was determined at +30min, while two viability assessments were made, the first at +3hr post-warming using standard transfer viability criteria. At +24hr, a second viability determination was based on complete hatching or positive growth from +180min to +24hr.
Participants/materials, setting, methods: Blastocysts were warmed using standardized, published protocols based on the vitrification system used: Cryolock, Cryotech, or µicroSecure devices. Post-thawing and dilution, all blastocysts were incubated in low O2, humidified, tri-gas environments at 37°C. At designated time intervals, blastocysts were analyzed for continued development and documented by photography (200X). Subsequently, measurements of blastocoele diameter were estimated along the longest axis of the trophectoderm. Differences in survival and viability were determined using a χ^2test ($p<0.05$).
Main results and the role of chance: Initial survival was 97% (58/60) at +30min post-thaw. The first viability assessment at +3hr post-warming determined no change in developmental status, with a non-viable rate of 3.33% (2/60) maintained. After +24hr, the second viability assessment resulted in a higher ($p<0.05$) non-viable rate of 13.8% (8/58). The latter 8 blastocysts failed to exhibited blastocoele growth past the +3hr mark and were deemed non-viable. Growth rates assessing increases in blastocoele diameter between time intervals and laboratories were significant between vitrification methods. Not surprisingly, notable blastocoele recovery and trophectodermal expansion was evident between +30min to +180min, with non-DMSO exposed, microSecure-treated blastocysts tending ($p<0.10$) to be more expanded up to +120min. Overall, our preliminary data reveals that viability assessments between laboratories (non-DMSO, closed device, n=20; D
Limitations, reasons for caution: Survival and viability are two separate assessments that are commonly performed simultaneously to determine blastocyst implantation potential. Current embryo viability evaluations at 0-3hr post-warming can be inaccurate predictors of implantation potential. In turn, alternative methods, perhaps integrating short-term time lapse imaging, are needed to improve our estimation of embryo viability.
Wider implications of the findings: The viability of embryos post-warming is difficult to assess as most laboratories artificially collapse or biopsy the trophectoderm of blastocysts prior to vitrification. Robust expansion may take several hours. Time-lapse imagery may prove beneficial to identify key growth rates to more accurately identify viability.
Trial registration number: None

P-274 Suboptimal oocyte cohort maturity impairs the implantation potential of euploid blastocysts

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Study question: Can oocyte cohort maturity predict the implantation potential of euploid blastocysts? Do stimulation protocols influence aneuploidy and the ability of euploid blastocysts to implant?

Summary answer: Ovarian hyperstimulation protocols yielding reduced oocyte maturity rates(<70%) are associated with lower (P<0.05) blastocyst development, euploidy and implantation rates, likely correlated to incomplete cytoplasmic maturation.

What is known already: Controlled ovarian hyperstimulation in IVF has greatly improved the success of assisted reproduction. Stimulation protocols, medications, duration and LH trigger timing are used by physicians to optimize mature oocyte production, and have been studied rigorously to identify their effect on oocyte quality and pregnancy. Oocyte quality is difficult to independently quantify, while nuclear maturation of oocyte cohorts indexes overall stimulation effectiveness. Applying PGT-A technology, euploid blastocysts are transferred with the expectation of a constant implantation potential across age groups. However, it is unknown what role oocyte cytoplasmic preparedness (i.e., maturation) could play on the implantation potential of euploid embryos.

Study design, size, duration: A 5-year (2014-2018) retrospective analysis of 1358 autologous and donor stimulation cycles using PGT-A and 1002 vitrified-thawed euploid embryo transfers was examined. Nuclear maturity was defined by the presence of a polar body at ICSI and cycle cohort comparisons were separated by Group 1: ≥70%(n=1124) and Group 2: below 70% (n=234). Data comparisons using t-test and chi-square were performed for cycles failing to produce blastocysts, cycles resulting in normal embryos, aneuploidy rates and implantation success.

Participants/materials, setting, methods: Single physician clinic stimulated 1138 patients for 1358 cycles having a cohort of oocytes retrieved. All ICSI-derived zygotes were grown to the blastocysts stage for PGT-A and vitrification. Only the first transfer attempt was counted in our analysis. Stimulation protocols varied but were predominantly antagonist based. Oocytes were retrieved 35-36 hours post-hCG trigger and cumulus complexes denuded 2-3 hours post-retrieval. Patient's percent mature oocytes were calculated based on Metaphase II status upon denuding.

Main results and the role of chance: Average patient age was 37 and 38 for groups 1 and 2, respectively. Oocyte cohort maturity of 70% or greater (Group 1) occurred in 83% of the cycles,with 17% considered suboptimal at less than 70% maturity (Group 2). Embryo development comparisons focusing on blastocyst yield, aneuploidy and whether a normal embryo was produced/cycle exhibited a lower (p<0.01) blastocyst yield (mean=2.7) in Group 2 compared to Group 1 (mean=5.7 blastocysts). Furthermore, Group 2 had a statistically higher aneuploidy rate of 61% and more cycles (41%) failing to result a normal embryo, in contrast to Group 1 having an aneuploidy rate of 54% and 23% of its cycles not resulting in a normal embryo. Most importantly, the implant ability of euploid embryos was lower (p<0.05) for Group 2 at 61% compared to 70% in Group 1. The reduced cytoplasmic preparedness of Group 2 oocytes led to developmental incompetence as indicated by 18% more cycles failing to develop a euploid blastocyst and significantly more aneuploidy. The issue of reduced cytoplasmic maturation of Group 2 oocytes was further manifested when they produced top quality euploid blastocysts with a lower(p<0.05) implantation potential upon transfer.

Limitations, reasons for caution: Multiple factors influence ovarian stimulation responsiveness and oocyte cohort maturity, nonetheless 5 years of data has identified a group of cycles that increased the risk of aneuploidy and produce euploid embryos with reduced implantation potential. These significant trends suggest possible adverse developmental events associated to related incomplete cytoplasmic maturation.

Wider implications of the findings: Many factors can influence controlled ovarian stimulation, oocyte maturity and subsequent negative cycle outcomes. Further investigations and evidence of oocyte-cellular cytoplasmic proteomics, metabolomics and gene activation/regulation is needed to better identify how sub-optimal maturity can adversely influence subsequent embryo viability and healthy, term live birth success.

Trial registration number: none

P-275 Histone methyltransferase Setd2 is required for mouse oocyte early development

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Study question: Does histone methyltransferase *SET domain containing 2 (Setd2)* play an important role in mouse oocyte early development and how it works?

Summary answer: Setd2 is essential for oocyte early development, mediating H3K36me3 in maintaining dynamic DNA methylation and normal DNA damage response.

What is known already: *Setd2* is responsible for lysine-36 of histone H3 trimethylation (H3K36me3), which is associate with actively transcription, transcriptional elongation, DNA damage response and DNA methylation dynamic. Primordial oocytes arrest from embryonic day 18.5 till day 10 post-partum to build *de novo* DNA methylation and restart transcription, which might accumulate DNA damage triggering cell cycle checkpoint. Products of the oocyte genome support oocyte growth and development till silencing, when oocyte grow to Graafian follicles, and are stored for use during the silencing supporting oocyte maturation. *Setd2* defect results in embryonic lethality while conditionally knockout in sperm could cause aberrant spermatogenesis.

Study design, size, duration: We generate $Setd2^{fl/fl}$; *Zp3*+ conditional knockout mouse line, and set mice with $Setd2^{fl/fl}$ genotype as control group. Each experiment included at least 3 independent samples and was repeated 3 times. Morphology phenotype were observed in at least 15 oocytes each. Growing oocytes were digested from 10, 15, 18 day-old mice while GV oocytes were super-ovulated from 21-23-day-old mice.

Participants/materials, setting, methods: We collected surrounding nucleoli germinal vehicle (SN-GV) oocyte and the growing oocytes. The efficiency of knockout was verified by WesternBlot using 150 oocytes each. Germinal vehicle break down (GVBD) rate, MII rate and fertilization rate were calculated in 150 oocytes each. We performed Chromatin Spreading and Immunocytochemistry staining of β-tubulin, 5-Methylcytosine, H3K36me3, H4K20me3, phosphor-histone H2A.X (γH2AX), etc. We also performed RNA sequencing from single oocyte using Smart-seq2.

Main results and the role of chance: We cross female mice with wild type C57 male mice, *Setd2*-deficient mice presented totally infertility for 6 months while control female mice had produced the expected range of pup number. While total number of observed oocytes were comparable, *Setd2*-deficient oocytes display significantly lower GVBD rate (16% vs 81%), fertilization rate (9% vs 78%). After 12.5h of in-vitro maturation, 90% of *Setd2*-deficient oocytes still contain GV (90% vs 5%).Immunocytochemistrystaining of β-tubulin shows *Setd2*-deficient oocytes present a rim of Hoechst-positive chromatin that surrounds the nucleolus and a thread-like chromatin organization while control oocytes present barrel-shaped bipolar spindle with all the chromosomes aligned at the equator plate. The result of Chromatin Spreading matches Immunocytochemistryphenotype above. *Setd2*-deficient oocytes shows lower signal and different DNA methylation pattern revealed by Immunocytochemistrystaining of 5-Methylcytosine.Immunocytochemistrystaining of γH2AX shows accumulated DNA damage in *Setd2*-deficient oocytes during its early development, 10 dpp, 15 dpp, 18 dpp, 21dpp. Single cell RNA sequencing finds 192 genes with increased RNA copy (fold>2) and 231 genes with decreased copy. The different expressed genes are enriched in GO biological pathways of DNA repair, Nuclear division, DNA metabolic process, cell cycle transition, etc.

Limitations, reasons for caution: The clinical significance of *SETD2* in human oocyte development is not explored yet.

Wider implications of the findings: This study might identify *Setd2* as a key regulator in oocyte, demonstrating essential role of *Setd2*-mediated H3K36me3 in maintaining dynamic DNA methylation and DNA damage response in post-proliferative cells. Deficiency of *SETD2* could be a hallmarker of human infertility and even the gene therapy target in the future.

Trial registration number: Not applicable.

P-276 Effect of different commercial media on spontaneous in vitro maturation and kinetics of germinal-vesicle human oocytes

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Study question: To assess the maturation in vitro rates and dynamics of germinal-vesicle (GV) oocytes recovered from stimulated donors' cycles, depending on different commercial culture media

Summary answer: G2 PLUS medium is the best option to in-vitro maturing germinal-vesicle oocytes. In their absence, continuous media or Sequential Cleav could be used equally.

What is known already: Following controlled ovarian stimulation, nearly 15% retrieved oocytes are immature and usually reproductively discarded. Recently, Escrich et al (2018) reported that nearly 70% GV oocytes, retrieved from stimulated cycles and cultured in CCM progressed spontaneously in vitro to the MII stage and were able to be used for reproductive endings. Now, Vitrolife has removed the CCM medium from the market. Thus, it is needed to test different commercial media in order to determine an efficient medium in terms of allowing the nuclear oocyte maturation, at first.

Study design, size, duration: A prospective study including 1260 cumulus-free GV oocytes, recovered from donors, who underwent standardized ovarian stimulation protocols between February 2017 to October 2018.

Participants/materials, setting, methods: 4 hours after aspiration, GVs were cultured in 10 different media in a time-lapse system at 37°C, 6% CO2 and 5% O2 for 24h. Denudation was considered as time point zero, and the first polar body (1PB) extrusion were assessed. We calculated also the MI duration by subtraction of these timings (1stPB – GVBD). Nuclear maturation rate was calculated as a percentage of MII oocytes per cultured GV.

Main results and the role of chance: Concerning the spontaneous nuclear maturation, the highest rates were observed for the Sequential Cleav- Origio (55.3%), G2 PLUS - Vitrolife (56.7%), IVM - Origio supplemented with hormones (45.5%), GEMS - Genea Biomedx and GLOBAL media - Life Global (52.1% and 46.0%, respectively; $p>0.05$). Moreover, these rates were significantly higher (51.9% in average) than

those observed for GV oocytes cultured in phase 0 media (LAG medium - Origio, LAG supplemented with hormones, Sydney IVF Fertilization Medium – Cook and IVM media non-supplemented with hormones) and CSCM – IrvineScientific, all ranged from 16.4% to 40.2%, showing HLAG medium the lowest nuclear maturation rates (16.4%; $p<0.05$).

According to the kinetics, there were significant differences amongst media for all analyzing timings. Regarding the 1PB extrusion, it occurred at comparable timing in all experimental groups, exception made for the LAG and G2 medium ($p<0.05$). However, there were significant differences in the timing of occurrence of the GVBD and therefore, in the MI duration. Thus, regarding the latter, and taking as a MI duration reference that achieved by oocytes cultured in G2 medium, there were no significant differences between G2 and all media, with the exception of the Sequential Cleav, and all three tested one-step media.

Limitations, reasons for caution: Despite the ability of the analyzed media to sustain the GV progression on meiosis, there were no tests on the cytoplasmic competence of in vitro rescued GV oocytes.

Wider implications of the findings: Our results suggested that G2-PLUS (Vitrolife), Sequential Cleav (Origio), GEMS (Genea Biomedx) and GLOBAL TOTAL (Life Global) are efficient media in terms of nuclear maturation rates and dynamics to be used in a GV rescue scenario.

Trial registration number: 1301-C-111-MJE

P-277 Spontaneous ovulation versus human chorionic gonadotropin (hcg) triggering for intrauterine insemination(IUI): a retrospective study with meta-analysis.

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Study question: To evaluate whether IUI syncronization with spontaneous serum LH surge or r-hCG trigger in women undergoing gonadothropin-stimulated IUI may provide different chances for pregnancy

Summary answer: hCG administration improves clinical pregnancy rates and ongoing live birth rate in women undergoing gonadotropins-stimulated IUI cycles.

What is known already: There are different factors that may influence IUI outcome, of which one of the most important is the timing of IUI. Nevertheless, there is still no consensus whether timing IUI with spontaneous ovulation (i.e. by detecting LH-surge) or by administering hCG may provide higher pregnancy rates. Recent studies on women undergoing gonadotropins-stimulated IUI cycles found controversial evidence on the effectiveness of each single strategy for IUI timing, concluding that further studies were needed.

Study design, size, duration: We performed a single-center observational study on 391 consecutive women undergone IUI after ovarian stimulation with gonadotropins from 2012 to 2017. 104 women were included in Group_A (LH surge) and 287 in Group_B(r-hCG trigger). Our data was pooled with that from other studies in a meta-analysis.

Participants/materials, setting, methods: Participants were young normo-ovulatory women (18-37 years) undergoing COS before IUI. Gonadotropin injections were administered starting on day 3 of the menstrual cycle and continued until ovulation. Patients were monitored with ultrasound and serum estradiol, LH, and progesterone until the time of either serum LH surge or hCG administration. IUI was performed approximately 24 hr after either observation of a spontaneous serum LH surge or 32 hr after r-hCG administration.

Main results and the role of chance: Group_B experienced a significantly higher clinical pregnancy rate in comparison to Group_A (21.3% vs 12.5%; p=0.032). Similarly, ongoing pregnancies were considerably higher in Group_B (13.94% vs 5.77%; p=0.032); no difference was observed in the miscarriage rates (p=ns). A subgroup analysis was performed in Group B, splitting patients in two subgroups according to the presence (Subgroup B_1) or absence (Subgroup B_2) of LH surge at the time of hcg administration. No significant difference was found between subgroups in terms of clinical pregnancy rate (19.4% vs 21.8%), ongoing pregnancy rate (12.9% vs 14.2%) and miscarriage rate (33.3% vs 34.7%). Our results were pooled in a meta-analysis of five studies (n=1532 IUI cycles). In line with our results, meta-analysis found a significantly higher pregnancy rate in patients receiving hcg administration before IUI compared to those inseminated after spontaneous LH surge (OR 2.28, 95% CI 1.06–4.92, I2=29%, p=0.03). No difference was found between women receiving hcg after LH surge and those in which LH surge did not occur (OR 1.03, 95% CI 0.63–1.69, I2=35%, p=0.90).

Limitations, reasons for caution: Some limitations must be considered; first, the design of our study was retrospective, potentially limiting the reliability of our findings. Secondly, the size of LH surge group was limited, potentially introducing bias due to imprecision.

Wider implications of the findings: This present study found a significant increase in the clinical pregnancy rate in gonadotropins-stimulated IUI cycles after hcg administration. Our findings may suggest that hcg may positively act on the mechanisms involved in follicular rupture, eggs maturation or embryo implantation in women undergoing IUI after ovarian stimulation with gonadotropins.

Trial registration number: Not applicable

P-278 Is it ethical to perform ICSI in all assisted reproductive treatment cycles: Does the method of fertilisation confer an additional benefit?

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Study question: Does the method of fertilisation impact the live birth outcome in the absence of a clinical indication?

Summary answer: Intracytoplasmic sperm injection (ICSI) has a lower failed fertilisation rate and higher clinical pregnancy rate, but this is not reflected in the live birth outcome.

What is known already: ICSI plays a central role in the management of male factor subfertility, with some advocating the use of ICSI for all types of infertility to reduce the risk of failed fertilisation. However, given the complex and more invasive nature of ICSI, adoption of such a policy would have wide implications for the future of assisted reproductive treatments (ART), with a major effect on the use of resources and safety. Currently, little evidence exists regarding the optimal method of fertilisation in cases of non-male factor subfertility, with the two main RCTs not identifying benefit conferred by ICSI in these patients.

Study design, size, duration: Retrospective cohort analysis of ART cycles between 1991-2016 from the Human Fertilisation and Embryology Authority (HFEA) database. 453,847 fresh cycles excluding male factor subfertility were undertaken. Of these, 340,773 and 113,114 underwent IVF and ICSI, respectively. Assuming a 30% live birth rate for IVF and 40% for ICSI, a power calculation demonstrated that 712 cycles would need to be analysed for 80% power and a 5% significance level to detect 10% difference.

Participants/materials, setting, methods: The singleton live birth rate (SLBR) was stratified by maternal age and adjusted by the type of treatment cycle (fresh IVF versus fresh ICSI). Cycles complicated by male factor subfertility were excluded from the analysis as the method of fertilisation is predetermined in these cases. Statistical significance was determined using Multiple Logistic Regression and Chi-square; $p<0.05$ was considered statistically significant.

Main results and the role of chance: The overall IVF to ICSI ratio in 1991, is solely dominated by IVF (100:0), with a continuous rise seen in ICSI cycles from 1999, to 66:34 in 2016, with an associated rise in ICSI cycles undertaken in the absence of male factor subfertility.

The clinical pregnancy rate is statistically lower with IVF cycles (n=81322; 30.0%) compared with ICSI cycles (n=30653;31.2%) (odds ratio [OR] 0.89, 95% confidence intervals [CI] 0.88-0.92, $p<0.0001$).

The overall live birth rate per embryo transferred is lower in the IVF group (n=86970;17.5%) compared with ICSI cycles (n=37003;20.8%), however, this did not reach statistical significance (OR 1.01, 95% CI 0.99-1.03, p=0.251) when adjusted for female age, number of previous IVF cycles attempted, previous live births through IVF and stage of embryo transfer.

The failed fertilisation rate was statistically higher for IVF cycles (n=13638; 4.00%) compared with ICSI (n=3925;3.47%) (relative risk [RR] 1.17, 95% CI 1.13-1.21, $p<0.0001$). Based on this, 189 ICSI cycles would need to be performed, to avoid one failed fertilisation through IVF.

Limitations, reasons for caution: The accuracy of the database is dependent on the information submitted to the HFEA. Until 2007, this data was manually captured adding the risk of data entry error. Furthermore, information on embryo quality is not available including the inability to account for cumulative pregnancy rates in women with previous attempts.

Wider implications of the findings: ICSI does not confer additional benefit in achieving a live birth in cases of non-male factor subfertility when adjusted for compounding variables. The additional complexity of ICSI including unknown long-term medical outcomes of the conceived children and costs to the patient are not supported by the findings of this study.

Trial registration number: Not applicable

P-279 Is there a relationship between time-lapse parameters and neonate gender? An analysis of 102 live births conceived through single fresh embryo transfer

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Study question: Is there a relationship between morphokinetic time-lapse parameters and embryo gender?

Summary answer: Although time-lapse morphokinetic parameters do not differ between genders, a high known implantation data (KID) score was more prevalent among male embryos.

What is known already: Previous studies that focused on morphologic embryo assessment are controversial regarding the existence of a correlation between embryo development in-vitro and embryo gender. Only few studies have investigated the association between time-lapse morphokinetic parameters and embryo gender, and in only three gender determination was based on live births. The results of these studies are controversial. While one study showed similar cell division kinetics in male and female embryos, others suggested different morphokinetic parameters that may be associated with embryo gender.

Study design, size, duration: A retrospective cohort study reviewing files of all live births from successfully treated infertile patients, who underwent ovarian stimulation, IVF, embryo culture in time-lapse monitoring incubators and fresh single embryo transfer in a tertiary medical center between 2013 and 2017. The study group consisted of 102 single embryos transfers that resulted in 102 live births; 57 females (56%) and 44 males (43%). One case was excluded as a result of missing data regarding fetal sex.

Participants/materials, setting, methods: Sixty-four embryos were transferred on day 3 (26 male/37 female) and 38 embryos (18 male/20 female) were transferred on day 5. Embryos selection was based on locally established annotation criteria and the KID score provided by the Embryoscope^{tm}. Morphokinetic parameters of male and female embryos were compared. Main outcome measures were early morphokinetic parameters and day 3 KID score, and secondary outcome measurements were late morphokinetic parameters of the embryos cultured to day 5.

Main results and the role of chance: Maternal baseline and treatment characteristics were similar in both groups except for older maternal age in the female embryos group (31.7 years vs. 29.3 in the male embryos group, p=0.016). High KID score ($\geq$4) was more prevalent in male compared to female embryos (100% of males vs. 74.1% of females, p=0.04). Early morphokinetic time-lapse parameters of male and female embryos including pronucleus fading, cleavage timings (t2-t8), second cell cycle duration (CC2= T3-T2) synchrony (S2=T4-T3) and interval between 8 and 5 cells (S3=T8-T5) were similar. Late morphokinetic parameters including full compaction at morula stage (tM), start of blastulation (tSB), full blastocyst (tB), and hatching (tHB) were similar between the groups as well.

Limitations, reasons for caution: Retrospective design and sample size. Nevertheless, our findings are statistically and clinically significant.

Wider implications of the findings: Although morphokinetic parameters were not associated with embryo gender, a high day 3 KID score was more prevalent among male embryos. This suggests that the KID score might favor male embryos and that it's relevancy to female embryos might be lower.

Trial registration number: not applicable

P-280 Can oocyte DNA repair mechanisms counteract impaired sperm genomic integrity?

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Study question: Can proportional oocyte maturity at retrieval shed light on an oocyte's ability to repair fragmented sperm chromatin and support embryo implantation?

Summary answer: When the proportion of mature oocytes was below a certain threshold, implantation rates of couples with compromised sperm chromatin fell drastically, regardless of maternal age.

What is known already: Abnormally elevated sperm chromatin fragmentation (SCF) has been linked to a reduced ability to achieve a pregnancy and to an increased risk of pregnancy loss. Oocytes have DNA repair mechanisms that play a role in repairing damaged sperm chromatin during early embryonic development. These repair mechanisms are known to be less effective as an oocyte ages. The ability to obtain mature oocytes is contingent on the female partner's response to a superovulation protocol, which may affect cytoplasmic maturity.

Study design, size, duration: This study included couples treated by intracytoplasmic sperm injection (ICSI) using ejaculated spermatozoa between 2006 and 2017. Female partners were limited to $\leq$35 years of age to control for

female-related aneuploidy. Nuclear maturity was assessed after the patient's own oocytes were denuded.

Cycles were allocated into two groups classified by the SCF of the male partner, normal and abnormal, and then stratified by the proportion of mature oocytes obtained at retrieval.

Participants/materials, setting, methods: All treatments took place at our center. Female patients ≤35 years old underwent ovarian superovulation by pituitary suppression utilizing either gonadotropin-releasing hormone agonist or antagonist, were treated with daily gonadotropins, and were triggered with human chorionic gonadotropin. ICSI was performed in the standard fashion.

SCF was assessed by terminal deoxynucleotidyl dUTP nick-end labeling (TUNEL) on ejaculated spermatozoa using a commercially available kit. At least 500 spermatozoa were assessed with a normal threshold of ≤15% SCF.

Main results and the role of chance: A total of 84 couples where the male partner had a normal TUNEL underwent 124 ICSI cycles, while 43 couples where the male partner had a compromised SCF underwent 67 ICSI cycles. The average SCF in the normal and abnormal groups was 9.8±3% and 24.1±11%, respectively ($P < 0.0001$). When oocyte maturity was greater than 80% at the time of retrieval, the groups had comparable implantation and clinical pregnancy rates (CPRs); the normal SCF group had 21.5% implantation and 27.0% clinical pregnancy rates, and the abnormal SCF group had 23.4% implantation and 30.0% clinical pregnancy rates, respectively, supporting the ability of the oocyte to correct the male genome.

However, once the proportional oocyte maturity fell below 80%, cycles with abnormal ejaculate SCF (n=27) appeared to lose ability to achieve a pregnancy when compared to cycles with intact sperm chromatin (n=62). The CPR trended lower in the group with compromised sperm genomic integrity (14.8%) when compared to the group with normal SCF (32.2%), and the implantation rate drastically fell to 8.3% while it was 23.6% ($P = 0.02$) in the normal SCF group.

Limitations, reasons for caution: This is a preliminary study on a relatively small study group. Based on the longevity of the study, it is not possible to remove all biases and variables that may affect the results, despite controlling for maternal age, body mass index, smoking and drinking habits, and ethnicity.

Wider implications of the findings: These findings provide further support of the existence of oocyte repair mechanisms as well as their relationship to oocyte maturity. This may help providers obtain superior ICSI outcomes despite aberrant SCF by tailoring superovulation protocols that yield higher proportional oocyte maturity.

Trial registration number: N/A

P-281 Trophectoderm biopsy may be associated with increased risk of anatomic and vascular placental pathology

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Study question: Does biopsy of the trophectoderm for pre-implantation genetic testing (PGT) impact placentation?

Summary answer: Pregnancies from blastocysts that underwent trophectoderm biopsy may have an increased risk of marginal cord insertion, membranous placental vessels, accessory lobes, and intervillous/subchorionic/septal placental thrombi.

What is known already: Increases in placental pathologies, including cord insertion anomalies have been associated with IVF compared to spontaneously conceived pregnancies. Trophectoderm biopsy is now a commonly performed procedure on blastocysts for pre-genetic testing (PGT) to diagnose monogenic disorders or aneuploidies. Despite its increasing use and relative safety in terms of blastocyst survival, its potential effects on placentation are unknown.

Study design, size, duration: A cohort of live births with placental pathology arising from autologous IVF/ICSI cycles between 2004 and 2017 was retrospectively reviewed.

Participants/materials, setting, methods: We compared placental pathology reports from IVF pregnancies who conceived with fresh transfers, cryothaw transfers, and transfers of cryopreserved embryos after blastocyst biopsy at a large academic fertility center. We used Pearson's chi-squared test for analysis. Placental pathology was categorized by an experienced placental pathologist as: anatomic (e.g. cord insertion), infectious (e.g. acute chorioamnionitis), inflammatory (e.g. villitis of unknown etiology (VUE) or chronic plasma cell deciduitis), and vascular (e.g. maternal or fetal vascular malperfusion).

Main results and the role of chance: Pathology was available for 933 fresh transfers, 192 cryothaw transfers, and 22 cryothaw transfers after PGT. There was no difference in mean age, BMI, or Day 3 FSH between women undergoing fresh and cryothaw transfers. The mean number of embryos transferred was 2.0 (fresh) and 1.6 (cryothaw). No differences were observed in infectious and inflammatory pathology. Compared to fresh transfers, there was a significantly increased incidence of anatomic pathology in the cryothaw and cryothaw with PGT groups, including marginal cord insertion (10.9% for fresh vs. 17.2% vs. 22.7% for cryo and cryo with PGT, respectively, $p = 0.02$); accessory placental lobes (5.1% vs. 9.9% vs. 13.6%, respectively, $p = 0.01$), and membranous vessels in absence of a membranous cord (3.2% vs. 5.2% vs. 18.2%, respectively, $p < 0.001$). Placental thrombi were also found more frequently with cryothaw and cryothaw with PGT transfers (intervillous thrombi, 16.7% vs. 24.5% vs. 18.2%, respectively, $p = 0.04$; subchorionic thrombi, 2.3% vs. 6.8% vs. 18.2%, respectively, $p < 0.001$; septal thrombi: 0.1% for fresh vs. 2.6% for cryo, respectively, $p < 0.001$).

Limitations, reasons for caution: The relatively small number of PGT cycles performed limits the power of our database, and thus we were unable to control for potentially confounding variables in our analysis. The retrospective design of this study is also a limitation.

Wider implications of the findings: Our findings suggest that other anatomic and vascular pathologies may occur more frequently in cryopreserved embryos that have undergone trophectoderm biopsy. Further research with a larger cohort is needed to better understand the effects of trophectoderm biopsy, particularly given its rise in use, while controlling for potential confounding variables.

Trial registration number: Not Applicable

P-282 To biopsy or not to biopsy? Laboratory and clinical outcome after embryo biopsy, cryopreservation and warming of previously vitrified-warmed human blastocysts

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Study question: Does subsequent biopsy, vitrification and warming have any effect on the laboratory & clinical outcome of previously vitrified-warmed human blastocysts during PGT-A?

Summary answer: Subsequent embryo biopsy, vitrification and warming of previously vitrified-warmed human blastocyst can result in increased embryonic loss and lower clinical pregnancy rate.

What is known already: Successful cryopreservation of biopsied human blastocysts is a prerequisite in PGT-A cycles. Currently, PGT-A with comprehensive chromosomal screening involves embryo biopsy, subsequent vitrification & warming (where available) of chromosomally normal day 5 or day 6 human blastocysts for embryo transfer. In some cases, previously-vitrified human blastocysts are preferentially considered for PGT-A through warming and a second vitrification-warming cycle after embryo biopsy. However, data in the literature regarding if this strategy brings any harm/benefit on the laboratory & clinical outcome are scarce and controversial.

Study design, size, duration: This retrospective study included PGT-A cycles performed as blastocyst biopsies on day 5 and day 6 of embryo development between January 2013 and October 2018. Based on the availability of a euploid embryo after comprehensive chromosomal screening (CCS), a subsequent frozen embryo transfer was performed after warming. Embryo survival after warming, embryo euploidy rate after biopsy and clinical pregnancy rate (Presence of a sac under ultrasound) were primary outcome measures.

Participants/materials, setting, methods: A total of 4216 PGT-A cycles were started and 2850 could reach the blastocyst biopsy stage (67.6%). During

the same period, 122 previously frozen blastocysts of 33 patients were subsequently warmed, biopsied and re-vitrified again for the purpose of PGT-A. Laboratory and clinical outcome were documented and compared with general PGT-A cases.

Main results and the role of chance: Out of 122 previously vitrified blastocysts of 33 patients, 107 (87.2%) survived after warming, 71 (58.2%) biopsied and re-vitrified on either day 5 afternoon or day 6. In 5 patients (15 embryos), no embryos showed enough expansion for embryo biopsy after warming hence the cycle was cancelled (15.2%). Out of 71 embryos were biopsied, 68 (95.7%) of them could be successfully analyzed and 50.7% of the analyzed embryos were found to be euploid. Eleven patients could not reach the embryo transfer stage due to the lack of a euploid embryo (39.2%). Twenty euploid embryo transfers with re-vitrified embryos in 17 patients were performed, resulting in 45% (9/20) clinical pregnancy rate. In the same time frame, out of 4216 PGT-A cycles started, in 1366 cycles embryo biopsy could not be performed due to the lack of blastocyst development (32.4%). A total of 7842 embryos were biopsied, 6785 of them were sent for CCS analysis, 6696 embryos (98.6%) were successfully analyzed and 2586 embryos were found to be euploid (38.6%). At least one euploid embryo was found in 1538 cycles (53.9%). One thousand four hundred twenty-three vitrified euploid embryo transfers resulted in 70.7% clinical pregnancy rate in the general PGT-A population.

Limitations, reasons for caution: Main limitation of the study is its retrospective nature. Secondly, the number of cases and embryos in the study group needs to be increased in order to draw a firm conclusion.

Wider implications of the findings: Our findings show that the approach involving subsequent warming, biopsy and re-vitrification of human blastocysts brings increased embryonic loss and lower clinical pregnancy when compared with the results of PGT-A cycles involving single vitrification & warming approach. Possible effect of embryo storage duration remains to be explored.

Trial registration number: None.

P-283 Evaluation of protein supplementation in culture medium after blastocyst thawing in frozen-thawed embryo transfer cycles.

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Study question: To evaluate if protein supplementation in culture media after blastocyst thawing improves clinical outcomes in single frozen-thawed embryo transfer cycles.

Summary answer: Protein supplementation in culture medium after blastocyst thawing seems to improve clinical and ongoing pregnancy rates.

What is known already: To date, few studies have investigated the cellular effects of embryo vitrification and its impact on the ability for the thawed embryos to implant after transfer. The cellular dehydration induced by the cryoprotectants would be responsible of a protein aggregation and a denaturation leading to a precipitation causing a qualitative and quantitative protein depletion. Thus, some authors recommend supplementing the embryo culture medium with proteins after embryo thawing. However, this strategy is still under controversy due to a lack of scientific evaluation of protein supplementation in biological and clinical outcomes in frozen-thawed embryo cycles.

Study design, size, duration: This was an observational cohort study including all the frozen-thawed blastocyst transfer cycles performed in our center between November 2017 and May 2018. Vitrified blastocysts at day 5 (D5) or day 6 (D6) derived from frozen oocytes or obtained after prolonged culture of frozen zygotes or cleaved embryos were excluded from the study. Only D5 or D6 vitrified blastocysts derived from fresh oocytes and scheduled for an elective single thawed embryo transfer (eSET) were included.

Participants/materials, setting, methods: Frozen-thawed blastocysts were allocated between two groups: (i) those cultured during 2-3h after thawing in a culture media already containing 10% HSA and (ii) blastocysts cultured in the same culture media supplemented with 20% HAS. Clinical outcomes including clinical and ongoing pregnancy rates and miscarriage rate were evaluated in the two groups. Embryo survival rate after thawing was also studied. Statistical analysis of the data was carried out using univariate and multivariate logistic regression models.

Main results and the role of chance: A total of 785 vitrified blastocysts were thawed for an eSET (441 in group 1 with 10% HSA and 344 in group 2 with 20% HAS). After univariate analysis, women age, fertility background, fertilization method, D5 and D6 blastocysts allocation and good quality blastocyst distribution were comparable between the 2 groups. The clinical pregnancy rate was significantly higher in group 2 (32,4% vs 39,2%; p = 0,048). The ongoing pregnancy rate was not significantly higher in group 2 but a tendency emerged (22,0% vs 27,9% ; p = 0,056). The miscarriage rate was similar in the two groups (32,2% vs 28,9% ; p = 0,553). The embryo survival rate after thawing was also comparable between group 1 and 2 (96 .4% vs 95.1% ; p = 0,363).After a multivariate analysis, female age > 35 years old, blastocyst expansion at D6 and a fair embryo quality at D5 or D6 were independently associated with a significantly lower ongoing pregnancy. A tendency emerged concerning 20% HAS supplementation (OR = 1.339; IC= 0.956-1.876; p = 0,09).

Limitations, reasons for caution: This study was not prospective and randomized. Nevertheless, a larger series should be analyzed in order to confirm these results with the evaluation of the live birth rate as the main outcome of this evaluation.

Wider implications of the findings: Our study findings seem to describe an improvement of the intrinsic embryo quality thanks to a protein supplementation in the culture media after thawing (higher pregnancy rate associated to an unchanged miscarriage rate). These findings could lead to a systematic protein supplementation after embryo thawing.

Trial registration number: Not applicable

P-284 Effect of zona pellucida drilling time on blastocyst development in preimplantation genetic testing for aneuploidy cycles

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Study question: Is the day of zona pellucida (ZP) drilling affect blastocyst development in preimplantation genetic testing for aneuploidy (PGT-A) cycles?

Summary answer: ZP drilling on day3 has a better blastocyst formation rate than on day5.

What is known already: ZP drilling is routinely adopted in blastocyst biopsy cycles for hatching trophectoderm. Drilling on Day3 and Day5 both can be selected, but it is not known which time is better.

Study design, size, duration: This is a retrospective cohort study analyzing 1644 Day3 drilling cycles and 1577 Day5 drilling cycles between May 2016 and May 2018.

Participants/materials, setting, methods: In July 2017, a significant change was made on the time of ZP drilling, which was performed on Day3 a.m. before July 2017, and on Day5 a.m. after July 2017. In all PGT-A cycles, the blastocysts were scored and biopsied on Day6 a.m. The overall blastocyst formation rate and high-quallity blastocyst (Gardner score $\geqq$ 3BB) formation rate on Day6 were compared in two groups.

Main results and the role of chance: There was no significant difference in maternal age (38.74±4.66 vs 38.52±4.52), the mean number of antral follicles (12.05±9.11 vs 12.36±9.06), the mean AMH (2.69±2.6 vs 2.78±2.72), the mean number of retrieved oocytes (7.68±5.58 vs 7.71±5.62) and the mean number of normal fertilized oocytes (5.24±4.02 vs 5.21±3.94) between Day3 and Day5 drilling groups. The overall blastocyst formation rate (46.3% vs 44.1%) and high-quallity blastocyst formation rate (27.0% vs 23.7%) on Day6 were both significant higher in Day3 drilling group than in Day5 drilling group.

Limitations, reasons for caution: This study is retrospective and some bias cannot be excluded. The embryo implantation outcome must be tracked.

Wider implications of the findings: This study suggested an appropriate time for ZP drilling.

Trial registration number: None

POSTER VIEWING

ENDOMETRIOSIS, ENDOMETRIUM AND FALLOPIAN TUBE, AND BENIGN DISORDERS OF THE ENDOMETRIUM AND FALLOPIAN TUBE

P-285 Metabolomics shows no impairment of the microenvironment of the oocyte-cumulus complex in the follicular fluid of women with isolated endometriosis

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Study question: Is there any metabolomic evidence of oocyte-cumulus complex microenvironment impairment in the follicular fluid (FF) of women with isolated endometriosis?

Summary answer: Metabolomics shows no impairment of the microenvironment of the oocyte-cumulus complex in the FF of women with isolated endometriosis.

What is known already: Although discriminant metabolomic signatures have been detected in the endometrium, plasma and peritoneal fluid of women affected by endometriosis, very few studies have investigated the metabolomic profiles of endometriosis in the FF. FF reflects the exchanges occurring between the oocyte and its microenvironment during the acquisition of gametic competence. The aim of our study was to compare the metabolomic profiles of the FF in patients undergoing IVF for isolated forms of endometriosis to those of patients free from endometriosis to evaluate the impact of the disease on the microenvironment of the oocyte-cumulus complex with potentially deleterious consequences on oocyte quality.

Study design, size, duration: We performed a prospective observational study on the FF retrieved from 79 women undergoing IVF from January to July 2018 at the Angers University Hospital, France.

Participants/materials, setting, methods: The patients were divided into two groups: the study group and the control group. The study group comprised 39 patients with isolated endometriosis, i.e. without any other diagnosis of infertility. The control group included 40 patients free from endometriosis. A targeted quantitative metabolomic and lipidomic analysis of the FF was performed using high-performance liquid chromatography coupled with tandem mass spectrometry. The FF levels of 188 metabolites were assessed by univariate and multivariate analyses.

Main results and the role of chance: The patients' characteristics, including age, body mass index, tobacco usage, hormonal profile, antral follicle count, were similar in the endometriosis group and the control group. Fifty-four percent of the patients (21/39) in the endometriosis group received an antagonist protocol compared to 98% of the patients in the control group (39/40) ($p<0.001$). There was no significant difference in the cumulative FSH dose used for stimulation between the endometriosis and the control groups (2732 *vs.* 2257 IU, p=0.09, respectively). Twenty patients in the endometriosis group (51%) had intracytoplasmic sperm injection (ICSI) compared to 24 (60%) in the control group (p=0.43). The oocyte maturation rates in ICSI were similar in the two groups (72.2% *vs* 77.7%, respectively, p=0.6). The fertilization rates in IVF and ICSI did not differ significantly between the two groups (49.4% *vs.* 50.2%, p = 0.9 and 76.4% *vs.* 68.8%, p = 0.53 respectively). Among the 188 metabolites analysed, 141 were accurately measured. Univariate analysis did not reveal any significant modification of metabolite concentrations, and none of the multivariate models discriminated between the two groups of patients, even when the study was restricted to the severest forms of endometriosis.

Limitations, reasons for caution: One potential limitation of our study could be the fact that with this targeted metabolomics analysis we have explored only a limited portion of the FF metabolome.

Wider implications of the findings: The impact of endometriosis on the follicular microenvironment and the quality of the oocyte remains debated. In the absence of a metabolomic signature of endometriosis in the FF, our results indicate no micro-environmental impairment of the oocyte-cumulus complex in cases of isolated endometriosis among women undergoing IVF.

Trial registration number: na

P-286 5 years long term follow- up results of endometriosis combined with infertility patients after laparoscopic surgery

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Study question: Postoperative pregnancy rate of endometriosis women is the core part in gynecological field .How to improve the postoperative pregnancy rates of endometriosis patients with infertility?

Summary answer: EFI guidance, strict post-operation management , positive pregnancy scheme and GnRh-a use in severe patients can significantly improve pregnancy rate of endometriosis patients with infertility

What is known already: EFI score was a useful evaluation in predicting and guiding pregnancy in endometriosis patients combined with infertility.

Using GnRH-a 3-6 cycles in patients with r-AFS stages III/IV can decrease recurrence.

Study design, size, duration: From January 2010 to June 2011, after laparoscopic surgery, 146 endometriosis patients with infertility were divided into 3 major groups (EFI≥9 group, EFI5-8 group, EFI≤4 group). In each major group, patients with different situations were divided into more detailed small groups. Different group using different pregnancy guidance methods , these patients were also divided into 4 groups by r-AFS score/ stages to statistics pregnancy rates. All these patients had finished 5 years follow-up after operation.

Participants/materials, setting, methods: partiipant; Beijing obstetrics & gynecology hospital , gynecology department

146 patients were divided into 3 major groups by EFI score(EFI≥9 group, EFI5-8 group, EFI≤4 group). In each major group, patients with different situations were divided into more detailed small groups. Different group using different pregnancy guidance(methods including expectant pregnancy, ovulation detection/induction, artificial insemination and IVF-ET) ,

Main results and the role of chance: ∗The overall pregnancy rate was 89.04%. After 2 years of operation, the pregnancy rate was 95.7% (45 /47) in EFI≥9 group, 83.1% (69 /83) in EFI 5-8 group and 50% (8/16) in EFI≤4 group, the rate of the first two groups had no statistically significance(P=0.498), but had significant difference with the last group (P<0.001). These three groups were all reach satisfactory pregnancy rate.

∗Postoperative application of GnRH-a did not delay patient's relative pregnancy time. Patients who using GnRH-a with EFI ≥5 had highest pregnancy rate within 6 months after menstrual cycle recovery.

Patients with endometriosis stage III-IV were treated with GnRH-a 3-6 times after operation.If the gestation time was calculated from menstrual recovery after GnRH-a treatment, this study showed that the use of GnRH-a after endometriosis did not prolong the pregnancy time of patients

∗The r-AFS score had no statistics relativity with post-operation natural pregnancy rates(χ^2=4.069,P=0.254), term labor rates(χ^2=0.605,P=0.895)and spontaneous abortion rates(χ^2=0.394,P=0.942).

∗The EFI score could predict pregnancy rate of endometriosis patients combined with infertility effectively, EFI ≥9 group had the highest natural pregnancy rate, natural pregnancy rate was significant statistical different (P=0.001) in different EFI groups.

∗Postoperative 5 years recurrence rate was 9.6%(14/146).

Limitations, reasons for caution: Sample size is a little small, because it is not easy to get the proper patients. (We have strict criteria for study patients selection). Follow up for five years is also a difficult task.

Wider implications of the findings: There is a great controversy about whether to us GnRh-a after operations of endometriosis patients combined with infertility. Our research shows that postoperative application of GnRH-a did not delay patient's relative pregnancy time.

Trial registration number: 201015

P-287 High number of endometrial polyps co-exist at a high rate with chronic endometritis: a possible etiopathogenetic link

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Study question: Is there any association between high number of endometrial polyps (EPs' number ≥6) and chronic endometritis?

Summary answer: High number of EPs was closely related with elevated risk of CE, while the link between classical single EP and CE has not been found.

What is known already: CE and EP were both subclinical inflammatory diseases that may cause uterine bleeding, pregnancy loss and impaired reproduction. However, the association between CE and EP has not been studied. Previous studies found that women with high number of EPs(≥6)had a significantly higher risk of recurrence rate than those with classical single EP after polypectomy, indicating that it might be a distinct polyp subtype with different etiology.

Study design, size, duration: Retrospective study. From June 2017 to May 2018, endometrial samples were obtained from patients with high number of EPs or single EP under hysteroscopy during the proliferative phase. Non-polypoid endometrium specimens were obtained from infertile patients with normal uterine finding as control. All patients had no history of hormone or antibiotic treatment for at least three months. Repeated implantation failure, recurrent pregnancy loss, heavy uterine bleeding, potential neoplasms, submucosal uterine fibroids, intrauterine adhesion were excluded.

Participants/materials, setting, methods: Seventy-one patients with high number of EPs (≥6 polyps), 50 with single EP and 74 non-polypoid controls were enrolled. Clinical parameters were collected including age, BMI, infertile, parity and gynecological histories.CE was diagnosed based on conventional histology with immunohistochemistry to identify CD138 plasma cells. Density of plasma cell infiltrations and prevalence of CE were compared between the three groups and multivariable logistics regression was used to investigate the independent risk factors of CE.

Main results and the role of chance: Compared with the non-polypoid endometrium, single EP contained a similar density of plasma cells without statistically difference (2.5 [range 1.0-5.0]/HPF in single EP vs. 2.0[range 0.0-4.0]/HPF in non-polypoid endometrium, $p>0.05$), whereas the high number EPs had a significantly higher density of plasma cells (5.0 [range 3.0-13.0]/HPF). The prevalence of CE was significantly higher in women with high number of EPs than those with single EP(38 of 71, 53.5% vs. 13 of 50, 26.0% , $p<0.001$). However, there was no significant difference on the incidence of CE between single EP group and non-polypoid endometrium (13 of 50, 26.0% vs. 18 of 74, 24.3%, $p>0.05$). With univariable analysis, parity was associated with a lower risk of CE and high number of EPs was associated with increased risk of CE. Using multivariable analysis, high number of EPs was found to be associated independently with CE (Odds ratio [OR] 3.42, 95% confidence interval [CI] 1.86-7.06), while single EP did not increase the risk of CE as compared to non-polypoid endometrium (OR 1.065, 95%CI 0.46-2.50).

Limitations, reasons for caution: This is a retrospective study with small size. Results were relied on endometrial biopsy specimens, of which immunological conditions may not always represent the whole endometrium.

Wider implications of the findings: High number of EPs was closely associated with CE, suggesting that CE may play an important role in the development of this distinct subgroup. CE should be ruled out in women with polyps, especially those with high number EPs and appropriate treatment of CE may improve the pregnancy outcome.

Trial registration number: not applicable

P-288 Fulvestrant, an estrogen receptor antagonist, downregulates the expression of aromatase gene in eutopic endometrial stromal cells of women with endometriosis

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Study question: Does Fulvestrant change the expression of aromatase gene expression in eutopic endometrial stromal cells of women with endometriosis?

Summary answer: Fulvestrant could significantly suppress the aromatase expression in endometrial stromal cells of women with endometriosis.

What is known already: Endometriosis is an estrogen dependent disease caused by presence and growth of endometrial cells in extra uterine environment. Estrogens play their role in gene expression regulation through the estrogen receptors (ERs). Activation of ERs' transcriptional domains, leads to binding of these receptors to the regulatory region of many genes or recruitment of the other transcription factors. *CYP19A1* gene encodes Aromatase, the key enzyme in estrogens biosynthesis, and is stimulated by estrogens and upregulated via activation of aberrant promoters in endometrial stromal cells in endometriosis. This phenomenon is involved in formation of a positive feedback cycle in estrogen biosynthesis in endometriosis.

Study design, size, duration: We compared the *CYP19A1* expression between the control and treated endometrial stromal cells with different concentrations of a selective estrogen receptor down regulator, Fulvestrant, (10^{-6}-10^{-8} M) for 48 hours. In addition, a vehicle control group was considered to assess the effect of vehicle of Fulvestrant (DMSO). Endometrial tissues were obtained from patients with ovarian endometriosis (endometrioma) during the proliferative phase of the menstrual cycle. All the procedures were applied in three biological replicates.

Participants/materials, setting, methods: Human endometrial stromal cells were isolated from sample tissues and cultured in stromal cell medium (SCM) including phenol red free Dulbecco's Modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and sodium pyruvate 1%. The cells were treated with different concentrations of Fulvestrant, after evaluation of their purity by FACS in the third passage. The effects of Fulvestrant on cell viability and *CYP19A1* expression were examined by MTT assay and quantitative PCR respectively.

Main results and the role of chance: Fluorescence activated cell sorting (FACS) analysis showed more than 99% purity of stromal cells in the third passage of culture in which the treatment was applied. Our data demonstrated that Fulvestrant could significantly suppress the aromatase expression in endometrial stromal cells of women with endometriosis compared to non-treated control (P <0.0001) and vehicle control (P <0.05). In addition, no negative effect was observed on the cells' viability during the treatment with neither of Fulvestrant concentrations. It seems that Fulvestrant downregulates the *CYP19A1* expression and eliminates the positive feedback cycle in estrogen biosynthesis via suppression of estrogen receptors and their binding to the regulatory region of this gene in endometrial stromal cells of women with endometriosis.

Limitations, reasons for caution: This study only showed the Fulvestrant effects on human endometrial stromal cells in vitro and there should be more studies to investigate the potential role of Fulvestrant in endometriosis treatment, as selective estrogen receptors down regulators have been recently considered as a potential hormonal therapy targets for this disease.

Wider implications of the findings: Our findings are in agreement with other studies demonstrating that Fulvestrant antagonizes estradiol function in gene expression regulation via downregulation of estrogen-upregulated genes and upregulation of estrogen-downregulated genes.

Trial registration number: not applicable

P-289 In-vitro effect of Ulipristal on proliferative and apoptotic indices and steroid hormone receptor expression in endometriotic stromal cells

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Study question: Does ulipristal acetate (UPA) affect cell proliferation, apoptosis and expression of estrogen and progesterone receptors in human endometriotic stromal cells in-vitro?

Summary answer: In-vitro treatment of endometriotic stromal cells with UPA did not affect cell proliferation and apoptosis nor expression of steroid receptors.

What is known already: UPA is a selective progesterone-receptor modulator (SPRM), which binds to the progesterone receptor with high affinity and suppresses proliferation of endometriosis. In a rat model with surgically induced endometriosis, UPA treatment leads to regression and atrophy of endometriosis in terms of volume of endometriosis and expression of apoptotic and proliferative markers. Currently there are no data on the effect of UPA on human endometriotic tissue.

Study design, size, duration: Endometriotic cyst wall tissue was collected and dissociated with collagenase. Stromal cells were separated from epithelial cells using CD326 microbeads and cultured for 10 days until the cells reached 60-80% confluence. Equal portions of stromal cells were seeded into 6-well plates and treated with graded concentrations (0, 0.1, 1, 10 and 100 ng/ml) of UPA for 48 hours.

Participants/materials, setting, methods: Ovarian endometriotic cyst samples were obtained from four women undergoing ovarian cystectomy or salpingo-oophorectomy. Endometriotic stromal cells were cultured on 25-mm Thermanox coverslips and treated with UPA for 48 hours. Immunohistochemical staining for proliferative marker Ki-67, apoptotic marker Bcl-2 and receptors for oestrogen (ER-α) and progesterone (PR) was performed. H-score was used to evaluate the protein expression. Control cells were not treated with UPA.

Main results and the role of chance: Endometriotic stromal cells after treatment with UPA remained characteristically flat and spindle shaped demonstrating no morphological changes when compared with the control. UPA administration exhibited no significant change in the expression of proliferative (Ki-67) (1.55 +/- 0.77, 1.29 +/- 0.31, 1.36 +/- 0.56, 1.24 +/- 0.43, 1.35 +/- 0.22 for UPA concentration 0, 0.1, 1, 10 and 100 ng/ml respectively, p = 0.721) and apoptotic (Bcl-2) markers (2.59 +/- 0.66, 1.76 +/- 0.95, 2.58 +/- 1.42, 2.02 +/- 0.78, 1.86 +/- 0.55 for UPA concentration 0, 0.1, 1, 10 and 100 ng/ml respectively, p = 0.165). There were also no significant change in the expression of hormone receptors of ER-α (0.54 +/- 0.25, 0.63 +/- 0.18, 0.64 +/- 0.24, 0.69 +/- 0.26, 0.67 +/- 0.33 for UPA concentration 0, 0.1, 1, 10 and 100 ng/ml respectively, p = 0.76) and PR (2.21 +/- 1.16, 2.33 +/- 1.80, 2.54 +/- 1.17, 2.25 +/- 1.32, 2.56 +/- 1.34 for UPA concentration 0, 0.1, 1, 10 and 100 ng/ml respectively, p = 0.970).

Limitations, reasons for caution: The limited sample size contributed to high patient variation. Only one apoptotic and proliferative markers were assessed. There was no co-culture of oestrogen and progesterone with the endometriotic stromal cells which may underestimate the effect of UPA.

Wider implications of the findings: Whether there was any role of UPA on endometriosis remained uncertain. Evaluation of other apoptotic and proliferative markers should be performed for understanding the effect of UPA on endometriosis.

Trial registration number: Non-applicable

P-290 The effects of dienogest on ovarian endometriotic cyst are mediated by ER stress induction

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Study question: Whether dienogest induces endoplasmic reticulum (ER) stress in human endometriotic stromal cells and if this is associated with endometriotic cell apoptosis and invasiveness?

Summary answer: Dienogest upregulated ER stress in human endometriotic stromal cells, which led to increased apoptosis and decreased invasiveness.

What is known already: Dienogest, a specific progesterone receptor agonist, is used in the treatment of endometriosis. However, it is still unclear as to the mechanisms of therapeutic effects on endometriosis. Our recent study showed that endometriosis may be the result of aberrant ER stress induction due to progesterone resistance. This finding suggests that the regulation of ER stress induction may play a key role in treatment of endometriosis. Therefore, the anti-endometriotic effects of dienogest may be mediated by regulation of ER stress.

Study design, size, duration: To evaluate the effects of progesterone and dienogest on ER stress induction and its correlation with apoptosis and invasiveness in endometriotic cells, human endometriotic cyst stromal cells (ECSCs) were cultured with progesterone or dienogest. And, salubrinal (ER stress inhibitor) was added in culture medium to block ER stress. Specifically, unfolded protein response signaling pathways (PERK/elf2*a*/AKT4/CHOP and IRE1/TRAF2/ASK1/JNK) were evaluated to elucidate the effects of ER stress induction on apoptosis and invasiveness.

Participants/materials, setting, methods: The expression level of GRP78 and GRP94 was measured by Western blot to evaluate ER stress induction. The activity of ER stress-mediated PERK/elf2*a*/AKT4/CHOP and IRE1/TRAF2/ASK1/JNK pathways was elucidated by measuring PREK, CHOP, IRE1 and phosphorylated JNK expression. In addition, apoptosis and invasiveness of ECSCs were evaluated by apoptosis maker proteins (cleaved caspase-3 and PARP) and invasion associated proteins (MMP2 and MMP9) expression, respectively.

Main results and the role of chance: Progesterone treatment did not have any significant effect on GRP78, GRP74, cleaved caspase-3 and PARP, MMP2 and MMP9 expression in estrogen-treated ECSCs. However, dienogest treatment upregulated GRP78 and GRP94 expression, which was accompanied by increased expression of cleaved caspase-3 and PARP and decreased expression of MMP-2 and MMP-9. This dienogest-induced change in cleaved caspase-3, PARP, MMP-2 and MMP-9 expression was reversed by inhibition of ER stress using salubrinal (ER stress inhibitor). These results suggest that dienogest increases ER stress, which is involved in regulation of apoptosis and invasiveness in ECSCs. Furthermore, our results showed that dienogest-induced ER stress increases the expression of PREK, CHOP, IRE1 and phosphorylated JNK. Especially, the downregulation of PERK and IRE1 expression by siRNA, which blocked ER stress-mediated activation of PERK/elf2*a*/AKT4/CHOP and IRE1/TRAF2/ASK1/JNK pathways, decreased apoptosis and increased invasiveness of dienogest-treated ECSCs.

Limitations, reasons for caution: Only primary human cell cultures were used in this study, therefore the results may not fully represent the *in vivo* situation.

Wider implications of the findings: We found here that dienogest treatment regulates endometriotic cell apoptosis and invasiveness via ER stress, and we suggest that this pharmacological action of dienogest might contribute to an effective suppression of ovarian endometriotic lesion.

Trial registration number: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (grant # NRF-2016R1D1A1B03931692).

P-291 Autophagy role in the human endometrium: Effect of partial autophagy impairment on human endometrial stromal cells proliferation and differentiation

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Study question: Is autophagy important for the correct function of endometrial cells? Are the proliferation and differentiation of human endometrial stromal cells affected by autophagy impairment?

Summary answer: Autophagy flux is increased during human endometrial stromal cells (HESC) *in vitro* decidualization. Furthermore, the partial impairment of autophagy diminishes HESC proliferation and differentiation.

What is known already: Macroautophagy (autophagy) is a highly conserved intracellular degradation pathway that serves as an adaptive role against stressful situations, contributing to the maintenance of vital cellular functions. Besides, it plays an important role during cellular proliferation, differentiation and growth.

In the endometrium, the increase in autophagy markers correlates with the progression of the menstrual cycle, reaching its maximum towards the late secretory phase. Furthermore, autophagy has been studied in relation to endometrial pathologies, suggesting that defective autophagy can affect

endometrial function. However, to date, no functional assay has been performed to study in depth the role of autophagy in the endometrium.

Study design, size, duration: This study was performed using immortalized human endometrial stromal cells (HESC). HESC were decidualized (dec) *in vitro* with medroxyprogesterone_(MPA), estradiol_(E2) and 8-Br-cyclicAMP_(cAMP) or vehicle-treated (non-dec). To interfere with autophagy pathway, knockdown of the autophagy-related protein 7 (ATG7, component of the core machinery of autophagy pathway) with small interference RNA (siRNA) technology was performed. Decidualization experiments and proliferation assays were started 24hs after transfection of specific siRNA against ATG7 or a non-target (NT) siRNA into HESC.

Participants/materials, setting, methods: Changes in autophagy flux (AF) were compared between decidualized and non-decidualized cells by flow cytometry (FC) using CYTO-ID kit and chloroquine (CQ) to block autophagy. For siRNA experiments, knockdown of ATG7 was confirmed by western blot (WB) and RT-qPCR and the impairment of autophagy was evaluated by FC. Cell proliferation in siRNA-transfected cells was evaluated using MTS Assay and cell decidualization was evaluated by RT-qPCR of prolactin (PRL) and IGFBP1, both well-known decidualization markers.

Main results and the role of chance: Flow cytometry analysis showed that AF is increased during *in vitro* decidualization of HESC (CQ/no-CQ dec:1,35; non-dec:0,96; P<0,05) and that the overall median intensity (reflecting the quantity of autophagy vesicles -autophagosomes- per cell) is higher in dec compared to non-dec cells (dec: 2506±122; non-dec: 2093±150; P<0,05).

ATG7-siRNA transfection, performed to define the role of autophagy during decidualization, effectively down-regulated ATG7 expression both at the mRNA (fold change compared to NT-siRNA at day four of decidualization protocol: 0,11±0,01; P<0,001) and protein level (evaluated at day two of decidualization protocol). Furthermore, AF was partially diminished after ATG7-siRNA transfection.

In relation to HESC function, ATG7-siRNA transfection was effective to reduce cell proliferation (measured four days after transfection protocol) in non-dec cells (40%; P<0,005), however it had nearly no effect on proliferation of dec cells (9% reduction). Besides, cells transfected with ATG7-siRNA, followed by decidualization showed a decrease of PRL expression both at protein (ATG7-siRNA: 121,75±12,87mU/l; NT-siRNA: 196,75±18,54mU/l; P<0,001) and mRNA level (fold change over NT-siRNA: 0,59±0,03; P<0,001) and of IGFBP1 at mRNA level (fold change over NT-siRNA: 0,13±0,01; P<0,001).

These results indicate that autophagy contributes to the normal function of HESC, affecting the proliferation of non-decidualized cells and contributing with decidualization.

Limitations, reasons for caution: This study was performed using immortalized cells, further studies are necessary to define the role of autophagy during *in vivo* decidualization. Besides, the role of autophagy was evaluated in a system where AF was partially diminished, limiting the strength of our results. Non-autophagy functions of ATG7 should be also considered.

Wider implications of the findings: These results show that autophagy is an important cellular process needed for the correct function of HESC. Dysregulation of autophagy could be related to several endometrial-related pathologies. Further insight into the regulation and specificity of autophagy could facilitate the identification of new therapeutic targets for the improvement of women´s health.

Trial registration number: Not applicable.

P-292 Randomized controlled trial of intrauterine infusion of autologous platelet rich plasma (PRP) versus granulocyte colony stimulating factor (G-CSF) in thin endometrium in frozen embryo transfer.

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Study question: To compare the effect of intrauterine infusion of PRP and G-CSF on endometrial thickness and clinical pregnancy rate in resistant thin endometrium cases

Summary answer: PRP and G-CSF are both effective in improving endometrial thickness in resistant thin endometrium patients, which might improve clinical pregnancy rates in such patients

What is known already: Resistant thin endometrium still remains a big challenge for clinicians, creating burden in form of cycle cancellation, unplanned freezing of embryos and need for surrogacy in extreme cases. There is no proven recommended treatment for resistant cases of thin endometrium. Intrauterine infusion of PRP and G-CSF are newer modalities of treatment, found to be effective in unresponsive thin endometrium in various studies. The current study was undertaken to compare their effects on endometrial thickness and clinical pregnancy rates

Study design, size, duration: A prospective randomized controlled trial was carried out from 1st January 2018 to 31st Decemebr 2018 at a tertiary infertility centre in India. Forty patients undergoing frozen embryo transfer (FET) with history of thin endometrium (<7 mm) but with normal hysteroscopic examination were randomized by computer generated table into two groups of 20 each(Group A =PRP, Group B= G-CSF). Patients with platelet count < 1.50000/dl and with any history of systemic diseases were excluded.

Participants/materials, setting, methods: All patients received oral estradiol valerate (6 mg/day) from day 2 of cycle which was increased gradually up to 12 mg/ day. Patients with thin endometrium on day 11 were randomized to receive PRP (Group A) or G-CSF (Group B) and the dose was repeated after 48 hours if endometrial thickness was still < 7mm. Frozen embryo transfer was performed when the endometrium reached an optimal thickness (at least 7 mm) and appearance.

Main results and the role of chance: The mean pre- treatment endometrial thickness in Group A was 5.38 ±0.57 mm and in Group B was 5.24 ± 0.51 mm which significantly increased to 6.62 ± 0.98mm and 6.60 ±0.93 mm post treatment in Group A and Group B respectively, the difference was statistically significant (p< 0.001). In each group, 7 patients could not achieve an optimal endometrial thickness of 7 mm after treatment and embryo transfer was postponed. The positive beta Human chorionic gonadotropin rate in Group A and Group B was 53.84% vs. 38.46% (p=0.69), and clinical pregnancy rate in Group A and Group B was 38.46% vs. 23.07% (P=0.59), which was 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: Autologous PRP and G-CSF hold promise in the treatment of women with sub optimal endometrial thickness for embryo transfer. It would help to reduce the incidence of cycle cancellations and thus help reduce the financial and psychological burden of repeated cancelled cycles.

Trial registration number: MCDH/2018/65

P-293 Dienogest is effective for a progestin-primed ovarian stimulation protocol for in vitro fertilization while continuing the treatment of endometriosis

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Study question: Is progestin-primed ovarian stimulation (PPOS) protocol with dienogest effective for patients with endometriosis during controlled ovarian hyperstimulation (COH), compared to PPOS with dydrogesterone (DYG)?

Summary answer: Dienogest can be used as an appropriate progestin in PPOS protocol for women with endometriosis in terms of comparable clinical outcomes of PPOS with DYG.

What is known already: PPOS is a new COH regimen using a progestin as alternative to GnRH antagonist for blocking the LH surge, and several reports have shown that DYG is an appropriate progestin for PPOS protocol. 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. Dienogest also provides complete ovulation inhibition at a daily dose of 2mg, and a rapid return to normal ovarian function after cessation

of its administration. However, dienogest has not been used in PPOS protocol yet.

Study design, size, duration: This was a prospective controlled study of 84 women with endometriosis (aged <41) undergoing COH for IVF/ICSI and frozen embryo transfer (FET) at our infertility center from February to November in 2018. The patients were allocated alternately into two groups, one with dienogest (2mg/day) protocol (n=42) and the other with DYG (20mg/day) protocol (n= 42). Dienogest was administered orally from the previous cycle. DYG was started from day3 of COH cycle.

Participants/materials, setting, methods: Of the participants, 36 patients were histologically confirmed as endometriosis and 48 patients were diagnosed with published imaging criteria using transvaginal ultrasonography, respectively. Patients were administrated with 150-225 IU of human menopausal gonadotropin (hMG) daily for COH, both progestins were administered continuously until 5 days after the oocytes picking up.All viable embryos were cryopreserved for FET. The primary outcome was the clinical pregnancy rate. Statistical analysis was performed using unpaired t-test and chi-square contingency.

Main results and the role of chance: Patient characteristics: the age, body mass index, duration of infertility, AMH level, and endometriosis stage (r-AFS) were similar in the two groups. In the dienogest protocol, LH level on the trigger day was significantly lower than in the DYG protocol (1.44±1.60 vs. 2.98±2.34, P=0.002), suggesting that dienogest leads to stronger pituitary suppression. Still, no significant difference was found between the two groups for the hMG dose and the stimulation duration of hMG. Moreover, the number of oocytes retrieved, fertilization rate, and viable embryo rate were comparable between the two groups. During the follow-up period, the rate of mature oocytes in dienogest regimen was significantly higher than in DYG regimen [89.7% vs. 81.8%, P=0.005]. The clinical pregnancy rate did not differ between the two groups [Odds ratio (OR) 1.07, 95%CI: 0.490∼2.32, P=0.87]:40.0%for the dienogest group vs. 38.5% for the DYG group. Additionally, no significant difference was found in the ongoing pregnancy rate [OR 1.02, 95%CI: 0.45∼2.31, P=0.97]:31.1%for the dienogest group vs. 30.8% for the DYG group, and early miscarriage rate [OR 1.14, 95%CI:0.26∼5.03, P=0.84]:22.2%for the dienogest group vs. 20.0% for the DYG group.

Limitations, reasons for caution: Since the follow-up of these pregnancies is still ongoing, this study is lacking in the live-birth outcomes following PPOS. In addition, the sample size is small, further prospective studies including larger populations are needed to confirm the efficacy of PPOS protocol with dienogest.

Wider implications of the findings: This is the first study demonstrating that dienogest is an effective progestin for PPOS protocol in IVF, while maintaining the comparable outcomes of PPOS with DYG. Moreover, PPOS with dienogest has a number of potential advantages over DYG, including suppression of recurrence after surgical intervention and relieving pain with endometriosis.

Trial registration number: UMIN000031111

P-294 Should we care about polyps detected during the follicular phase of intrauterine insemination treatments?

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Study question: Does the suspicion of an endometrial polyp during follicle tracking for intrauterine insemination (IUI) change the cumulative reproductive outcome of these treatment cycles?

Summary answer: The detection of a polyp during follicle tracking for IUI does not seem to decrease cumulative live birth rates (CLBR).

What is known already: Endometrial polyps are a frequent uterine finding in infertility patients. Based on available evidence, there seems to be a benefit of removing a polyp when detected prior to intra-uterine insemination (IUI), regardless of its size, even though up to 27% of all small polyps (<10mm) regress spontaneously. However, the potential benefit of cancelling an IUI when a polyp is detected for the first time during follicle tracking is unknown.

Study design, size, duration: In this retrospective cohort study, all patients who underwent an IUI between May 2009 and March 2017 were included, all having a normal baseline uterine ultrasound and/or hysteroscopy. In 160 out of 6127 patients (2,6%) or in 415 out of 14498 cycles (2,8% of cycles) a polyp was diagnosed during the follicular phase. Each patient was included only once and performed a maximum of 3 consecutive IUI cycles.

Participants/materials, setting, methods: We compared the CLBR between women with and without newly-diagnosed polyps using multivariable Cox regression analysis in order to adjust for the following potential confounding factors: female age, body mass index (BMI), use of gonadotrophins for ovarian stimulation, peak estradiol level, number of follicles >14mm prior to the administration of human chorionic gonadotropin (hCG), as well as sperm concentration and motility.

Main results and the role of chance: Female age was significantly higher in the polyp group, compared with the control group (33.1±4.7 versus 34.9±4.9, p<0.001). Conversely, other relevant baseline characteristics did not vary significantly between both study groups, namely BMI (24.0±4.7 versus 24.3±4.2), sperm concentration after capacitation (32.7±40.4 versus 31.7±41.2), sperm motility after capacitation (99.9±11.4 versus 99.5±6.5), peak estradiol levels (380.0±367.2 versus 380.5±350.1), number of follicles >14mm (1.3±0.7 versus 1.3±0.8) and use of gonadotrophins (13.8% versus 10.8%). The unadjusted CLBR after up to 3 IUI cycles for the women with and without a polyp were 18.4% versus 26.0% (p=0.066), showing a deleterious effect of the presence of a recently-diagnosed polyp of borderline significance. However, after performing multivariate Cox regression analysis, the presence of a polyp detected during treatment no longer seemed to influence CLBR significantly (adjusted hazard-ratio 0.839, 95% confidence interval 0.568-1.239).

Limitations, reasons for caution: The presence of biases related to the retrospective design of this study cannot be excluded. Furthermore, as in-cycle hysteroscopies were not performed (to avoid a potential hindering effect on the IUI cycle outcome), one cannot exclude the possibility of misdiagnosis associated with the sole use of pelvic ultrasound.

Wider implications of the findings: This study, which included a large dataset, may be reassuring for physicians and patients, as the new detection of a polyp during the follicular tracking for an IUI cycle did not seem to be associated with a reduction in CLBR, if left untreated.

Trial registration number: B.U.N. 143201836012

P-295 Inhibition of STAT3 as novel immunotherapy target for treatment of endometriosis

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Study question: To identify specific immunomodulator targeting myeloid-derived suppressor cells (MDSCs) for potential therapy of endometriosis.

Summary answer: STAT3 inhibition by Sunitinib can eliminate MDSCs, inhibit G-MDSC immunsuppressive function and induce neutrophil production which suppresses the growth and development of endometriosis.

What is known already: The development of endometriosis involves alterations of immunological factors, disfunction of immune cells, secretion of immunsuppressive chemokine and cytokines, in order to facilitate ectopic endometrium escape from immune surveillance. Our team has previously reported that MDSCs are significantly increased in early development of endometriosis in mouse model which inhibit T cell proliferation and promote endometriosis development.

Study design, size, duration: Eight specific immunomodulators targeting various MDSCs pathways were administrated to mouse model of endometriosis. MDSCs subsets in peritoneal fluid, peripheral blood, bone marrow and endometriotic lesions during and after treatment were monitored and compared. $CD11b^{+}Ly6G^{+}$ cells from each compartment were isolated for further cellular and molecular characterisation. At least five mice were included in each pharmaceuticals in every experiment.

Participants/materials, setting, methods: C57BL/6 female mice age 7 to 8 weeks old were used. After treatment, peritoneal fluid, peripheral blood, bone marrow immune cells were collected, endometriotic lesions size and weight were compared. Mouse body weight was continuously monitored for side effect.

Main results and the role of chance: Sunitinib selectively targets JAK/STAT3 signallingfor exapanison of MDSCs significantly decreased endometriosis

lesion size and weight without affecting body weight. Sunitinib markedly increased the percentage of $CD11b^+Ly6G^+$ cells in peritoneal fluid. Morphology showed the $CD11b^+Ly6G^+$ cells with no MDSCs features but rather neutrophils. Sunitinib significantly decreased p-STAT3 expression in G-MDSC but not in M-MDSC; and significantly down-regulated TGF-β in peritoneal fluid. RNA sequencing of the isolated $CD11b^+LyG^+$ cells show Sunitinib with differential cytotoxic features which characterised its underlying anti-endometriosis mechanism.

Limitations, reasons for caution: Further experiments are need to be testified in human samples.

Wider implications of the findings: Inhibition of STAT3 can be developed as novel immunotherapy target for treatment of endometriosis.

Trial registration number: Not applicable.

P-296 Changes in expression of thioredoxin and thioredoxin binding protein-2 according to histone deacetylase inhibitor treatment in human endometrial cells from patients with endometriosis

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Study question: Does histone deacetylase inhibitor(HDACi) reverse cell proliferation and apoptosis associated with endometriosis by regulating thioredoxin(TRX) and thioredoxin binding protein(TBP)-2 expression?

Summary answer: Suberoylanilide hydroxamic acid(SAHA) may affect the cell proliferation and apoptosis induced by oxidative stress in development of endometriosis, via resuming the TRX and TBP-2 expression.

What is known already: Oxidative stress and chronic inflammation play important roles in the pathophysiology of endometriosis. TRX is a redox regulating antioxidant protein that prevents cell damage from oxidative stress and TBP-2 are regulatory protein of TRX. Altered TRX and TBP-2 expressions in endometrium are known to be associated with the development of endometriosis. SAHA, a HDACi, induces reactive oxygen species formation and activation of caspase pathway in transformed cells, leading to apoptosis. This mechanism appears to involve TRX system which makes normal cells resistant to SAHA-mediated cell death.

Study design, size, duration: In-vitro, experimental study using primary cell culture and molecular biologic methods. From July, 2015 to June, 2016, eutopic endometrium was obtained from the patients who had undergone hysterectomy, and ectopic endometrium from the patients who had undergone ovarian cyst enucleation due to endometriosis. Primary stromal cell culture was performed using eutopic and ectopic endometrial cells in vitro. Recombinant high mobility group box(rHMGB)-1 was used to induce oxidative stress on the cells.

Participants/materials, setting, methods: The cultured human endometrial stromal cells (HESCs) and ishikawa cells were treated with rHMGB-1 and SAHA for 48 hours. Cell proliferation was assessed by CCK-8 assay, cell apoptosis was measured by flow cytometry, and cell viability by MTT assay. To assess the effect via TRX, siTRX transfection was performed on stressed cell by rHMGB-1, and SAHA was treated. mRNA and protein expression of TBP-2, TRX and TRX/TBP-2 ratio were estimated.

Main results and the role of chance: rHMGB-1 treated HESCs and ishikawa cells showed increased cell proliferation with decreased cell viability, decreased apoptosis and increased cell necrosis on CCK, MTT and flow cytometry. After treating SAHA, cell proliferation was decreased significantly with increase of apoptosis, especially in ectopic HESCs and ishikawa cells. mRNA and protein expression of TBP-2 were decreased in HESCS and ishikawa cells, whereas it was reversed after SAHA treatment. TRX expression was significantly decreased after SAHA treatment comparing to pre-SAHA treatment in ectopic HESCs and ishikawa cells, however, increase of TRX expression was shown in eutopic HESCs after SAHA versus pre-SAHA treatment. After blocking TRX gene by siTRX transfection, SAHA treated cells showed increased TBP-2 expression comparing to the control.

SAHA has been suggested to act selectively in transformed cells(cancer cell), by targeting low TRX cells in transformed cells. Our study shows ectopic endometrial cells show reduced level of TRX than eutopic cells, increasing cell apoptosis by SAHA, so that may assume SAHA as a specific treatment for the endometriosis.

Limitations, reasons for caution: Our study is limited in that we only performed in vitro analysis using HESCs. Therefore, in vivo studies are required.

Wider implications of the findings: Our results support that cell proliferation and decreased apoptosis caused by oxidative stress in the progression of endometriosis may be reversed by SAHA only in specific transformed cells, leading a potential treatment option in endometriosis.

Trial registration number: This research was supported by a faculty research grant of Yonsei University College of Medicine for 6-2015-0466.

P-297 Innate immune function is altered in endometrial epithelial and stromal cells of infertile patients with endometriosis

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Study question: Is innate immune function altered in endometrial epithelial and stromal cells of patients with endometriosis?

Summary answer: Innate immune function is altered in endometrial epithelial and stromal cells of patients with endometriosis.

What is known already: Pathophysiology of endometriosis remains to be clarified. However, eutopic endometrium of patients with endometriosis may be responsible for the pathogenesis and related-infertility. There are growing evidence that the uterus is a non-sterile compartment. The human endometrium is an important site of innate immune defense. Autophagy balances inflammation in innate immunity and impaired autophagy may be involved in pathophysiology of endometriosis. Numerous studies showed that immunological dysfunction is involved in pathophysiology of endometriosis. However, it is not clear whether innate immune function is altered in endometrial cells of patients with endometriosis.

Study design, size, duration: For this laboratory study, endometrial samples of patients with endometriosis undergoing laparoscopy for infertility (n=46) were collected throughout the menstrual cycle. In addition, we included infertile patients with hydrosalpinx caused by chlamydia trachomatis infection (n=16), and healthy fertile women (n=18) with macroscopically normal pelvic cavities who undergoing laparoscopic tubal ligation or reversal of tubal sterilization as controls.

Participants/materials, setting, methods: We evaluated the effects of lipopolysaccharide (LPS; 0.1μg/ml or 1 μg/ml) or polyinosinic:polycytidylic acid (Poly I:C; 10 or 25 μg/ml) for 4h or 24h on cell proliferation, IL-1β and TNF α mRNA expression by real-time PCR, pro-IL-1β synthesis by western blot analysis and secretion of mature IL-1 β and TNF α by ELISA in endometrial epithelial and stromal cells, with and without autophagy inhibition by knockdown of the ATG13. Statistical significance was defined as $p<0.05$.

Main results and the role of chance: Endometrial epithelial cells (EECs) secreted significantly more mature IL-1 β and TNF α than endometrial stromal cells (ESCs).

Menstrual phase: Mature IL-1 β levels of EEC/ESC of fertile healthy controls (EEC/ ESC-C) were significantly decreased after 24h stimulation with LPS or Poly I:C compared to those at 4h, whereas autophagy inhibition further promoted mature IL-1 beta levels at 24h. Vehicle, LPS or Poly I:C treated EEC/ESC of patients with endometriosis (EEC/ESC-Endo) secreted no detectable or very low levels of mature IL-1 β. TNF α levels in vehicle, LPS or Poly I:C treated EEC/ESC-endo were significantly higher than those of EEC/ ESC-C.

Early- and mid-secretory phases: Vehicle, LPS or Poly I:C treated EEC-C&Endo and ESC-C&Endo secreted no detectable or very low levels of mature IL-1 β. Vehicle, LPS or Poly I:C treated EEC/ESC of patients with hydrosalpinx (EEC/ESC-H) secreted significantly more mature IL-1 β than those of the other two groups. TNF α levels were significantly lower in vehicle treated EEC/ESC-C compared to those of EEC/ESC-endo and EEC/ESC-H. Either LPS or Poly I:C did not significantly increase TNF α secretion in ESC-C, whereas both significantly increased TNF α secretion in EEC/ESC-endo. In EEC/ESC-H, only Poly I:C significantly increased TNF α secretion

Limitations, reasons for caution: This study was only performed in vitro and only proinflammatory cytokines, IL-1 β and TNF α were analyzed. Furthermore, no cellular interactions between endometrial and immune cells were investigated.

Wider implications of the findings: Altered endometrial innate immunity may facilitate the survival of shed endometrial tissue and have detrimental effect on endometrial receptivity in patients with endometriosis. Further studies that investigate the mechanisms underlying altered innate immune function of endometrial cells in patients with endometriosis will provide novel therapeutic strategies for endometriosis.
Trial registration number: N.A.

P-298 Biomarker discovery associated with endometrial-related conditions is biased by the menstrual cycle effect in the transcriptomic analysis

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Study question: Does menstrual cycle affect the results of case versus control gene expression studies searching for endometrial-related-condition biomarkers?
Summary answer: Menstrual cycle effect is a confounding variable that must be considered at case versus control gene expression studies to identify reliable biomarkers of endometrial-related conditions.
What is known already: The endometrium is a dynamic tissue involving many genes whose expression changes throughout the menstrual cycle. Consequently, it is important for differential expression analyses to accurately define at molecular level the cycle phase of the endometrial biopsies. Transcriptomic approaches are increasingly used in Reproductive Medicine to identify genes associated to endometrial-related conditions. However, the menstrual cycle has not been properly considered in these studies and it is an important issue to ensure that biomarker discovery is not biased by the menstrual cycle phase, masking the genes associated to the endometrial condition.
Study design, size, duration: This *in-silico* analysis involves case vs control studies evaluating endometrial-related conditions: 3 of endometriosis (n=37, n=19, n=111), 1 of recurrent implantation failure (n=115), 1 of recurrent pregnancy loss (n=20) and 1 of uterine pelvic pathology (n=71). The original raw data was re-analysed using the same procedure; and the menstrual cycle effect was evaluated comparing the differentially expressed genes with and without removing the registered menstrual cycle phase in the analysis.
Participants/materials, setting, methods: Keywords of endometrial transcriptomics and related conditions were searched at Gene Expression Omnibus (GEO). For each included study, raw data were pre-processed, normalized using quantile method and explored through Principal Component Analysis (PCA) to estimate the menstrual cycle effect on the data. Significant differentially expressed genes (False Discovery Rates (FDR) <0.05) were compared (Fisher's exact test, FDR) for each individual study before and after removing the menstrual cycle effect using linear models (Limma R-package).
Main results and the role of chance: A total of 42 gene expression individual studies evaluating endometrial-related conditions were retrieved from GEO. Of these, 14.3% had not registered the menstrual cycle phase at the time of endometrial biopsy collection. From the remaining 85.7%, the studies with cycle phase registered for all samples, raw data available, and n > 3 per group were included. In all of them, the three first PCA's components grouped the samples according to the cycle phase rather than to the condition. This menstrual cycle bias was demonstrated in all studies, as a significant increment of an average of 4.6 times more differentially expressed genes were detected when this effect was removed (Fisher's exact tests FDR < 0.0006, 4.40E-16 < FDR < 5.06E-04). For these studies, on average 46.78% new genes associated with the endometrial-related conditions were highlighted as new condition-specific biomarkers that would not have been detected without removing the menstrual cycle bias in the analysis. Consequently, the cycle phase of endometrial biopsies should not only be registered, but also controlled at a molecular level in the data analysis, to optimize the detection of reliable biomarkers of endometrial-related conditions.
Limitations, reasons for caution: The bias of the menstrual cycle effect in biomarker discovery has only been evaluated on the available GEO endometrial-related-condition studies that met the criteria. Since this effect has been demonstrated in all of them, it could be extrapolated to case vs control studies evaluating other endometrial-related conditions.
Wider implications of the findings: This research demonstrates the menstrual cycle bias in biomarker discovery of endometrial-related conditions, introducing a new biomarker taxonomy that could distinguish between genes affected by the menstrual cycle and/or the researched condition. These results establish new guidelines to accurately detect endometrial biomarkers, improving reproducibility and gaining statistical power.
Trial registration number: not applicable. Research supported by IVI Foundation, IVI-RMA Global. A.Devesa-Peiro is granted by the Ministry of Science, Innovation and Universities (FPU/15/01398).

P-299 Alteration in gene expression of CREB, TNF-α and BCL6 in endometriosis

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Study question: Is gene expression of CREB, TNF-α, and BCL6 different in women with endometriosis?
Summary answer: Overexpression of CREB, TNF-α, and BCL6 was detected in ectopic endometrium of women with endometriosis
What is known already: Endometriosis is a multi-factorial disease. Several genetic, immunological and hormonal factors are suggested in its pathogenesis. cAMP response element (CRE) binding protein (CREB) is a transcription factor that mediated many different processes such as cell proliferation, survival, and differentiation. CREB has a CRE site in the promoters of many different genes including interleukin 2 (IL-2), IL-6, cyclooxygenase-2 (COX-2), macrophage migration-inhibitory factor (MIF) and tumor necrosis factor alpha (TNF-α). TNF-α is one of inflammatory cytokines that participate in angiogenesis and immune responses. Another transcription factor is B cell lymphoma 6 (BCL6) which is associated with cellular proliferation as nuclear gene repressor.
Study design, size, duration: In this case-control study, 10 endometriotic lesions (ectopic), 10 endometrial samples (eutopic) of women with endometriosis and 10 endometrial control samples were studied. Control samples were obtained from women who had no evidence of endometriosis during laparoscopy. Ectopic samples were obtained during laparoscopy surgery. 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 tissue collection, RNA extraction and cDNA synthesis were done. Real-time PCR technique was used for quantitative gene expression of CREB, TNF-α, and BCL6. Gene expression data were analyzed based on $2^{-\Delta\Delta CT}$ to estimate the relative fold change value. One-Way ANOVA was used for data analysis.
Main results and the role of chance: Gene expression profile of CREB, TNF-α, and BCL6 was increased in ectopic lesions compared with control samples. These increases were statistically significant ($p<0.05$) except for TNF-α ($p>0.05$).

In addition, overexpression of BCL6 was detected in eutopic endometrial tissues compared to controls while this difference was not significant.
Limitations, reasons for caution: For getting more information, we can study these genes in a large number of women with and without endometriosis and also to investigate gene expression of other transcription factors influencing inflammatory cytokines expression. Epigenetic studies such as CREB and BCL6 binding to the promoter regions of target genes are recommended.
Wider implications of the findings: Dynamic expression of CREB, TNF-α, and BCL6 could play an essential role in the development of endometriosis. Higher expression of these genes in ectopic endometrial

lesions can be considered as a molecular scenario for the pathophysiology of endometriosis by induction of inflammatory cascades and cellular proliferation.
Trial registration number: N/A

P-300 Web-based survey of fertility issues in women with endometriosis

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Study question: What are the fertility issues and the attitude towards reproductive techniques and fertility preservation options in women with endometriosis?
Summary answer: The risk of infertility is a major issue for many women with endometriosis. Few women consider themselves well-informed about fertility preservation options.
What is known already: Fertility preservation in women with endometriosis in a debated topic with only scarce medical literature on the subject.
Study design, size, duration: We conducted a one-time web-based survey about fertility issues and attitudes towards reproductive techniques and fertility preservation in women of reproductive age with endometriosis over a four month period in 2018. Women were recruited via advertisement on social media or via the social media pages of local endometriosis-patients associations.
Participants/materials, setting, methods: There were 270 female participants, with a mean age of 31.9 years. Each participant completed the online survey, a questionnaire in French (37 items) exploring the following dimensions: socio-demographic (5 items) and medical data on endometriosis (6 items), family planning and fertility (7 items), knowledge and attitudes toward endometriosis and fertility (4 items), means used/wished to access information (5 items), attitudes in the context of infertility (10 items).
Main results and the role of chance: A majority of women are worried (96%) about the impact of endometriosis on their fertility. Half (52%) of the participants have received, from their doctor, enough information concerning the possible effect of endometriosis on their fertility, whereas 31% have discussed the issue but wish further information. In contrast, only a minority (27%) of women consider themselves well-informed about fertility preservation options. Information given by endometriosis and reproductive specialists is considered most useful. Information mediated through patient associations and group discussions is also highly rated, whereas information given by the general gynaecologist is considered less useful. A majority of women would consider assisted reproductive techniques (74%) or adoption (70%) in case of infertility. Interestingly 72% of women would undergo oocyte vitrification for fertility preservation, whereas only 37% would resort to oocyte donation.
Limitations, reasons for caution: Diffusion via social media allows the participation of women from the general population and avoids the bias of hospital recruitment. We can however suspect that women most concerned about fertility issues were more likely to participate. Another limitation is the self-declared nature endometriosis-diagnosis.
Wider implications of the findings: This is the first survey addressing the topic from the patient's perspective. Our results highlight the importance of addressing the issue of fertility in women with endometriosis. Special attention should be given to information and counseling about fertility preservation since most women consider their knowledge on the topic as insufficient.
Trial registration number: Not applicable

P-301 Anogenital distance and endometriosis: results of a case-control study

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Study question: Is anogenital distance (AGD) associated with the presence of endometriosis?
Summary answer: AGD is not associated with the presence of endometriosis.
What is known already: AGD is the distance measured from the anus to the genital tubercle. Recent evidence suggests that a shorter AGD, a sensitive biomarker of the prenatal hormonal environment, could be associated with higher endometriosis risk, in particular, with deep infiltrating forms. Prenatal exposure to androgens results in a longer AGD, whereas a prenatal estrogenic environment results in a shorter AGD. Hypothetically, a shorter AGD, reflecting the intrauterine hormonal milieu, could represent an indicator of the presence of endometriosis. However, studies investigating AGD in affected women are scanty.
Study design, size, duration: Case-control study recruiting nulliparous women (aged 18-40 years) with endometriosis between 2017 and 2018. Cases were women with a surgical diagnosis of endometriosis in the previous 24 months or with a current nonsurgical diagnosis of endometriosis. Controls were asymptomatic women referring for periodical gynecological care and without a previous diagnosis of endometriosis. They were matched to cases for age and BMI. Exclusion criteria included previous or current pregnancy and presence of genitourinary prolapse.
Participants/materials, setting, methods: 90 women with endometriosis (*n*= 45 with deep infiltrating endometriosis (DIE), and *n*=45 with ovarian endometrioma (OMA)) and 45 controls were enrolled. For each woman, two measures were obtained using a digital caliper: AGD_{AC}, from clitoral surface to the upper verge of the anus, and AGD_{AF}, from the posterior fourchette to the upper verge of the anus. Each distance derived from the mean of six measurements acquired from two different gynecologists.
Main results and the role of chance: Baseline characteristics were comparable in the three study groups (age, BMI, smoking status). The mean ± SD AGD_{AC}in women with DIE, OMA and without endometriosis was 76.0 ± 12.1, 76.1 ± 11.1, and 77.8 ± 11.4 mm, respectively (p = 0.55). The mean ± SD AGD_{AF}in women with DIE, OMA and without endometriosis was 22.8 ± 5.0, 21.7 ± 9.0, and 23.7 ± 7.8 mm, respectively (p = 0.38).
Limitations, reasons for caution: Selection and measurement bias need to be considered. The decision of performing six measurements for each AGD from two different gynecologists should have limited measurement variability.The choice of controls may be cause of concern; we cannot exclude to have inadvertently included some cases among controls.
Wider implications of the findings: Our study failed to find an association between AGD and presence of endometriosis. Therefore, our findings do not support a crucial role of intrauterine exposure to steroidal hormones in the pathogenesis of the disease. Moreover, AGD does not seem to represent a reliable indicator of the presence of endometriosis.
Trial registration number: Not applicable

P-302 Effect of metformin on endometrial receptivity of diet-induced obese mice

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Study question: Evaluate the effects of metformin on endometrial implantation markers expressed in endometrium of mice feed with standard or high fat diet.
Summary answer: Metformin therapy seems to exert a beneficial effect not only on body weight, but also on endometrial receptivity factors in diet-induced obese mice.
What is known already: Women withpolycystic ovary syndrome (PCOS) present with an increased risk of subfecundity and infertility, as partially associated with insulin resistance and obesity. Furthermore, significant changes in uterine receptivity and markers of decidualization and implantation have been

discovered in obese women, suggesting molecular mechanisms of endometrial dysfunction. Metformin is widely used as treatment for type 2 diabetes mellitus and it is considered a promising drug for treating anovulatory obese women with PCOS. However, the direct effects of metformin on endometrial receptivity of those patients remain unclear.

Study design, size, duration: This study is an *in vivo* experimental study using diet-induced obese female C57Bl/6J mice (~14 weeks) treated with or without metformin (~21 days) and their nonfat controls also treated with and without metformin (~21 days).

Participants/materials, setting, methods: Beginning at 3 weeks of age, female C57Bl/6J mice were fed with standard low or high fat (60% fat) diet for ~ 14 weeks, then each group either received or did not receive metformin (380mg/kg mice) via drinking water for 14 to 24 days. Animals were weighted before and after the intervention, endometrium was collected during secretory phase and the mRNA expression of *IL8*, *HbEGF*, *Maoa*, *IGF1* and *IR* was evaluated by qRT-PCR.

Main results and the role of chance: Animals who received standard diet (SD) did not change their body weight (BW) after metformin treatment, however metformin did reduce the BW of the animals who were fed a high fat diet (HFD). In the endometrium, metformin treatment did not interfere in the mRNA expression of *IL8* in SD animals, however in HFD animals the expression was upregulated. Metformin treatment increased the *HbEGF* expression of animals fed in both diets. Furthermore, the mRNA expression of *Maoa* and *IGF1* was downregulated with metformin treatment in SD and HFD animals. Additionally, the IR expression increased with metformin treatment in both SD and HFD, however more pronounced in SD animals.

Limitations, reasons for caution: These results are seen in an animal model, which does not perfectly reproduce the complex composition of human fertility.

Wider implications of the findings: Giving the pivotal role obesity plays in the etiopathogenesis and progression of PCOS, our data suggest that metformin might be considered an essential therapeutic option to ameliorate fertility of those patients.

Trial registration number: Not applicable.

P-303 Microenvironmental regulation of human endometrial mesenchymal stem-like cells

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Study question: Do myometrial cells function as niche cells regulating the biological activities of endometrial stem cells?

Summary answer: Soluble factors derived from myometrial cells increased self-renewal and proliferation activities of endometrial mesenchymal stem-like cells (eMSCs) by activating WNT/β-catenin signaling.

What is known already: Adult stem cells contribute to regeneration of endometrium. Data from human and mouse endometrium localized eMSCs preferentially to regions adjacent to the myometrium. In mice, active β-catenin was highly expressed in proliferating endometrial label-retaining cells after parturition. We hypothesized that the myometrial cells provided niche signals regulating the biological activities of eMSCs.

Study design, size, duration: Sequential beading with magnetic beads coated with anti-CD140b and anti-CD146 antibodies was used to isolate eMSCs from endometrial tissues from proliferative (n = 24) and secretory (n = 16) phase. The eMSCs were seeded at low density, and indirectly co-cultured with myometrial cells at ratio of 1:90 for 15 days. Functional stem cell assays were performed and the proportion of cell expressing the eMSCs markers was evaluated.

Participants/materials, setting, methods: Proliferative and secretory phase samples obtained from women aged 35- to 50-years undergoing total abdominal hysterectomy. Endometrial cells were isolated enzymatically and the percentage of eMSCs after co-culture with myometrial cells was analyzed by flow cytometry. Western blot analysis for active β-catenin validated the activation of Wnt/β-catenin signaling. The role of WNT/β-catenin signaling in eMSCs self-renewal was confirmed by gain-of (activator: Wnt3a conditioned medium and recombinant WNT3A) and loss-of (inhibitors: XAV939 and IWP-2) function approaches.

Main results and the role of chance: *In vitro* co-culture of myometrial cells enhanced the colony forming and self-renewal ability of eMSCs. The expanded eMSCs after coculture retained multipotent characteristic and exhibited a greater total cell output when compared to medium alone culture. The level of active β-catenin in eMSCs increased significantly after co-culture with myometrial cells suggesting activation of the WNT/β-catenin signaling. Secretory factors from myometrial cells produced the same stimulatory effect on eMSCs. The functional WNT/β-catenin signaling in eMSCs self-renewal was determined with WNT activator (Wnt3a conditioned medium) and WNT inhibitors (XAV939 and IWP-2). Addition of Wnt3a CM or recombinant WNT3A increased the clonogenic activity and number of eMSCs. While, the stimulatory effect of myometrial cells on eMSCs was suppressed by the Wnt inhibitors.

Limitations, reasons for caution: The behavior of the cells may be altered during culture. The functionality of myometrial derived factors on eMSCs needs to be determined with *in vivo* studies.

Wider implications of the findings: Myometrial cells provide paracrine factors to eMSCs via Wnt/β-catenin signaling. Secretory factors derived from the myometrial cells offer a specialized microenvironment modulating eMSC activities. By gaining a better understanding of the niche composition, it will be possible to recreate a milieu for stem cell expansion and control their biological activities.

Trial registration number: Not applicable

P-304 Laparoscopic investigation and correction of undiagnosed endometriosis may enable natural conception and avoidance of In Vitro Fertilization (IVF) overuse in unexplained infertility patients

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Study question: Could laparoscopic surgery on women with unexplained infertility and recurrent implantation failure (RIF) enable natural conception by identifying and correcting underlying endometriosis?

Summary answer: Employing laparoscopic surgery in women initially diagnosed with unexplained infertility and RIF, resulted to natural conception following correction of underlying endometriosis.

What is known already: Twenty-five percent of infertile couples are being diagnosed with unexplained infertility. RIF is a clinical categorization referring to patients subjected to over 3 IVF cycles associated with failure in achieving a clinical pregnancy following embryo transfers including good quality embryos. Endometriosis is a polymorphic disorder that could go undiagnosed, in influencing infertility, and ultimately hindering natural conception fueling the requirement for IVF treatment. If left undiagnosed this could lead to RIF and IVF overuse. The need to avoid IVF over-treatment and effectively manage patients that are categorized as unexplained infertility coupled by RIF has been identified and highlighted.

Study design, size, duration: Medical records between 2004 and 2017 from women initially diagnosed with unexplained infertility and RIF while still pursuing fertility treatment were retrospectively analyzed. One-hundred and twenty women underwent laparoscopy as a last resort leading to identification and correction of the hitherto undiagnosed endometriosis.

Participants/materials, setting, methods: The patient study group included women diagnosed with unexplained infertility and RIF. Laparoscopic surgery was performed under general anesthesia in Genesis Athens Clinic. Following identification and correction of endometriosis patients were invited to conceive naturally. Patients were divided into 2 groups depending on achieving clinical pregnancy status. Statistical comparison was attempted based on years of infertility, number of previous failed IVF attempts, grade of endometriosis, female age and CA-125 levels between the two groups.

Main results and the role of chance: Following laparoscopy 81 out of the 120 patients (67.5%) were diagnosed with endometriosis. Seven were diagnosed with stage I endometriosis, 31 with stage II, 35 with stage III and 8 with stage IV endometriosis. Eighty-one patients were diagnosed with endometriosis as the sole infertility finding. Endometriosis lesions were laparoscopically

corrected. The 81 patients were invited to conceive naturally. Forty-seven patients (58.02%) achieved a pregnancy within the 1-year time-frame. In fact, the time-frame required for pregnancy to be ensued was less than 6 months for the majority of our patients 36 out of 47 (76.6%) as presented in Table 1. It should be noted that women who achieved a pregnancy within the first year were statistically significant younger than women that failed to achieve a pregnancy (35.55±4.81 vs 37.50±4.42, p=0.02) and presented with statistically significant lower CA–125 levels (17.38±2.44 U/ml vs 23.79±2.40 U/ml, p<0.001). No statistically significant difference was presented regarding the years of infertility, previous failed IVF attempts or grade of endometriosis.

Limitations, reasons for caution: The retrospective nature along with the small sample of the study is an identified limitation. Moreover patients who achieved a natural conception were statistically significantly younger. The invasive nature of laparoscopy accounts as a reason for caution prior to concurring on optimal practice.

Wider implications of the findings: Laparoscopic investigation should be employed for women diagnosed with unexplained infertility coupled by RIF in order to exclude diagnosis of endometriosis or alternatively correct it. This approach appears to enable natural conception for couples that could otherwise run the risk of being subjected to futile IVF attempts resulting to overuse.

Trial registration number: Not applicable.

P-305 Efficacy of Dienogest versus oral contraceptive pills (OCPs) on pain associated with endometriosis: Randomized Controlled Trial

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Study question: Is Dienogest (Visanne) superior in efficacy to oral contraceptive pills (Yasmin) in controlling pain associated with endometriosis?

Summary answer: Dienogest at a dose of 2mg/day showed no significant benefit to OCP in relief of endometriosis related pelvic pain but had a better tolerability profile.

What is known already: Endometriosis is a chronic inflammatory disease, frequently associated with dysmenorrhea, dyspareunia and abdomino-pelvic pain limiting quality of life. Most medical therapies aim to alleviate the severity of symptoms. Recent guidelines recommend the use of either OCPs or progestins as a first-line treatment of pain associated with endometriosis. The effectiveness of both OCPs and dienogest, a fourth-generation progestin, for endometriosis treatment has been demonstrated. The literature is rich with data comparing the use of Visanne or OCPs to placebo or GnRH analogs. However, there are no head to head studies comparing their efficacy in the management of endometriosis associated pain.

Study design, size, duration: We conducted a blinded randomized controlled clinical pilot study that compared the efficacy of dienogest with that of OCP for the treatment of endometriosis patients. Sample size consisted of 50 patients, 25 in each arm. Endometriosis patients, 20-45 years of age, diagnosed clinically, surgically or via imaging analysis suggestive of endometrioma with no contraindications to suggested therapy were randomized to receive Visanne 2mg/day or Yasmin (0.03mg ethinyl estradiol and 3mg drospirenone) daily for 24 weeks.

Participants/materials, setting, methods: Fifty endometriosis patients were assessed at baseline, at 3 months and 6 months interval. The primary outcome was the change in endometriosis associated pelvic pain from baseline to end of treatment, assessed by the Visual Analogue Scale (VAS). The secondary outcome quality of life was assessed using Endometriosis Health Profile 30 (EHP30) and other secondary outcomes such as side effects on similar numerical scales from one to ten regarding the tolerability and satisfaction.

Main results and the role of chance: In total, 50 women were randomized to treatment with Dienogest (n=25) or OCPs (n=25). Overall, 20 women in the Dienogest group and 18 women in the OCP group completed the study. Both groups were comparable at baseline, with a mean BMI of less than 25 in 60% of patients and a mean age of less than 35 in 51 % of patients. The reduction in mean VAS score was not significantly different between the 2 groups at 3 and 6 months (p=0.51 and 0.27 respectively). The use of analgesics was comparable between the 2 groups at baseline. However, the intake was lower in the Dienogest group at 3 and 6 months when compared to the OCP, yet it did not reach any statistical significance (p=0.19, p=0.06 respectively). Dienogest was well tolerated with fewer adverse events; mainly significantly lower nausea and lower premenstrual symptoms (p=0.035, p=0.04 respectively). In terms of quality of life related to sleep or work, there is no significant difference between the use of dienogest and OCP (p=0.13 and 0.36 respectively).

Limitations, reasons for caution: The small sample size in a single center randomized clinical trial limits the generalizability of the results.

Wider implications of the findings: Our results show that dienogest is comparable to OCPs in controlling pain associated with endometriosis however with less side effects and hence better safety and tolerability profile. While larger studies are needed, such a difference between dienogest and OCPs is considered valuable from a public health perspective.

Trial registration number: No trial registration number

P-306 lncRNA MEG3-210 regulates endometrial stromal cells via PKA/SERCA2 and p38 MAPK signaling pathway through galectin-1 in endometriosis

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Study question: What is the role of lncRNA MEG3-210 in endometriosis?

Summary answer: lncRNA MEG3-210 regulates endometrial stromal cells migration, invasion and apoptosis via PKA/SERCA2 and p38 MAPK signaling through interaction with galectin-1.

What is known already: MEG3 is considered as tumor suppressor in many diseases. Expression patterns of MEG3 transcripts are specific to tissue and cell type. Galectin-1(Gal-1) is expressed in human endometrium and endometrial stromal cells selectively. Gal-1 is involved in cell proliferation, motility, apoptosis and angiogenesis. Gal-1 promotes angiogenesis and growth of lesions in endometriosis.

Study design, size, duration: Tissue specimens came from eutopic endometrium of women with endometriosis (EU, n=28) or not (NE, n=29). Endometrial stromal cells (ESCs) were isolated from eutopic endometrium of women with endometriosis (EuESCs, n=13) or not (NeESCs, n=14).

Participants/materials, setting, methods: We used RT-qPCR to determine the expression of MEG3-210 in tissues and endometrial stromal cells. Then we learned about the role of MEG3-210 in ESCs by loss-of and gain-of function assays. We performed RNA pull-down and mass spectrum analysis to explore the potential interaction between MEG3-210 and proteins in ESCs. We used RNA immunoprecipitation (RIP) to confirm the interaction. With the inhibitor of pathway, we identified the regulatory axis involved in biological functions.

Main results and the role of chance: MEG3-210 is expressed lower in eutopic endometrium and stromal cells from patients with endometriosis. Downregulation of MEG3-210 could promote NeESCs migration and invasion but inhibit apoptosis via PKA/SERCA2 and p38 MAPK signaling pathway. In contrast, overexpression of MEG3-210 could inhibit EuESCs migration and invasion but induce apoptosis. Gal-1 is confirmed to interact with MEG3-210 by RNA pull-down and RIP. Furthermore, Gal-1 could activate p38 MAPK signaling and inhibit PKA/SERCA2. Of note, p38 MAPK signaling was responsible for the effects of MEG-210 on apoptosis, and SERCA2 played a more important role in migration and invasion.

Limitations, reasons for caution: Our major limitation is lacking endometrium from women diagnosed with endometriosis in stage I or II according to revised AFS classification. Furthermore, the detailed relationship between Gal-1 and PKA/SERCA2 needs to be explored further.

Wider implications of the findings: Our study is the first to demonstrate the functions and potential mechanism of MEG3-210 in endometriosis. These findings suggest that MEG3-210 contributes to pathogenesis of endometriosis. These results indicate that MEG3-210 may be used as diagnostic marker and Gal-1/p38 MAPK axis could be the potential target of treatment.

Trial registration number: not available

P-307 Oxytocin receptor antagonism improves receptivity-related parameters of cultured human endometrium.

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Study question: What is the effect of oxytocin (OT), oxytocin receptor (OTR) antagonism and high concentrations of estradiol (E_2) on receptivity - related parameters of cultured human endometrium.

Summary answer: E_2 and OT limited endometrial viability and decidualisation, increased cyclooxygenase-2 (COX-2) activity and formation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). All were inhibited by OTR antagonist, atosiban.

What is known already: Endometrial receptivity is affected in patients undergoing controlled ovarian hyperstimulation (COH), mostly due to exposure to supraphysiological levels of E_2. It has been confirmed that E_2 stimulates formation of OTRs in uterus. OT is a known factor stimulating the local release of prostaglandins, which decrease endometrial perfusion and receptivity. It has been shown that OTR antagonists might improve embryo implantation, possibly by decreasing the uterine contractions. Such an effect was also observed in patients without elevated uterine contractions. It could point to an additional mechanism of support of embryo implantation in patients receiving OTR antagonists.

Study design, size, duration: In vitro study on cultured endometrial tissues samples collected by aspiration biopsy from 30 women undergoing In Vitro Fertilisation – Embryo Transfer (IVF-ET) treatment between 2016 and 2017.

Participants/materials, setting, methods: In vitro study performed on a model of endometrial tissues explants and endometrial tHESC cell line. Receptivity-related parameters were investigated using cells viability and COX-1/2 activity assays and $PGF_{2\alpha}$ and prolactin secretion measurements. Changes in expression of OT receptors and COX-2 genes (*OXTR* and *PTGS2*, respectively) were examined with qPCR.

Main results and the role of chance: High E_2 and OT limited the endometrial viability and decidualisation, stimulated COX-2 activity and formation of prostaglandin $PGF_{2\alpha}$. All the above were alleviated by OTR antagonist, atosiban.

Limitations, reasons for caution: It is a preliminary in vitro study. Its findings require further preclinical and clinical confirmation.

Wider implications of the findings: Once confirmed in further studies, the results might point that OTR antagonists could be helpful in improving endometrial receptivity in patients undergoing COH.

Trial registration number: Not applicable

P-308 Relationship between the percentage of senescent endometrial cells and pregnancy outcome in women with recurrent pregnancy failure

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Study question: Is there a relationship between the percentages of senescent cells in human endometrium during the mid-luteal phase of the menstrual cycle and pregnancy outcome ?

Summary answer: Women with miscarriage have significantly lower percentage of p16-positive senescent endometrial stromal cells during the mid-luteal phase of the menstrual cycle.

What is known already: Endometrial abnormalities that involve cell functionality changes could have an adverse impact on pregnancy outcome. Some of these transformations are probably associated with cellular senescence. Recently, it was found that p16-positive senescent cells in human endometrium are responsible for acute cellular remodelling at the time of embryo implantation. In addition, the number of senescent cells and their communication with uNK cells was suggested to be tightly bound with the endometrial receptivity. However, little is known about the influence of senescent cells on the pregnancy outcome.

Study design, size, duration: This is a cohort study of 90 women with recurrent implantation failure (RIF) who had an endometrial biopsy during the mid-lutheal phase and live birth or miscarriage after in-vitro fertilization (IVF) within 14 months of biopsy. We used immunohistochemical (IHC) biomarkers, p16 and p21, to identify senescent endometrial stromal and epithelial cells.

Participants/materials, setting, methods: Our study was performed at Nadezhda Women's Health Hospital, Bulgaria. Patient biopsies with known pregnancy outcome were retrieved from our tissue bank. IHC staining for senescence with antibodies do detect p16-positive and p21-positive cells was used to search for biomarkers that differentiated women who had a miscarriage (Group1, n=45) from those who had a successful pregnancy (Group2, n=45). The percentage of senescent cells was calculated after enumeration by two independent investigators in multiple endometrial sections.

Main results and the role of chance: The studied senescent markers p21 and p16 were expressed in the endometrial tissue samples of all patients with a relatively high variability in the percentage of p21-positive and p16-positive cells. The mean percentage of p16-positive cells was significantly higher in all tissue compartments compared to the percentage of p21-positive cells (3.91% vs. 0.01% stromal cells, 3.18% vs. 2.17% glandular cells, 32.46% vs. 11.23% luminal cells, respectively, $p<0.05$).

The performed t-test showed that there was no significant difference in female patient's age and blastocyst quality between the studied patient groups ($P<0.05$, Student t-test). The percentages of p21-positive cells in endometrial stroma, glands and luminal epithelium and those of p16-positive cells in glands and luminal epithelium were also not significantly different between women with miscarriage and live birth ($P>0.05$). However, the mean percentage of p16-positive stromal cells was significantly lower in women with miscarriage compared to patients with live birth (2.4% vs. 5.3%, respectively, $P=0.04$).

Limitations, reasons for caution: The study cohort was limited in sample size. Not all confounding factors such as potential immunological and genetic reasons for miscarriage have been taken into account. In addition, the endometrial biopsy could also have an effect on pregnancy outcome.

Wider implications of the findings: This work marked differences in the percentage of certain types of senescent endometrial cells (p16-positive stromal cells) in relation to the pregnancy outcome. These findings emphasize the essential impact of cell senescence in human endometrium on the normal course of pregnancy and potential complications.

Trial registration number: Not applicable.

P-309 Follicle-stimulating hormone (FSH) induces apoptosis in endometrial cells. Luteinizing hormone (LH) activity prevents this effect

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Study question: Are the exogenous gonadotrophins follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) able to alter the physiology of endometrial cells?

Summary answer: Different exogenous gonadotrophins specifically affect endometrial cell viability differently: FSH promotes autophagy and apoptosis while LH and hCG alone or combined with rhFSH does not.

What is known already: Within ovary, FSH induces autophagy and apoptosis of granulosa cells leading to atresia of non-growing follicles, whereas LH has documented anti-apoptotic functions. Endometrial cells express functioning gonadotrophin receptors (FSH receptor, FSHR; LH/hCG receptor, LHCGR). Physiological cross-talk between autophagy and apoptosis has been reported in decidualized endometrial cells in late secretory phase of the menstrual cycle.

Study design, size, duration: Endometrium sample biopsies were collected during the proliferative phase of the menstrual cycle from healthy patients undergoing diagnostic hysteroscopy, dissected in smaller pieces then cultured

for 24 hours to avoid side effects due to previous surgery. Primary cultured endometria were then treated for further 48 or 72 hours with supraphysiological doses (100 ng/ml) of FSH, LH or HCG alone or combined. Controls omitted gonadotrophins.

Participants/materials, setting, methods: Endometrium tissue cultures were adopted as experimental model. Controls and gonadotrophin-treated biopsies were paraformaldehyde-fixed or processed for total RNA extraction. Immunohistochemistry was performed using anti-microtubule-associated proteins 1A/1B light chain 3B (MAP1LC3B, autophagy marker) and anti-apoptotic protease activating factor 1 (APAF1, apoptosis marker) primary antibodies. Expression of *aromatase* (positive control of treatments), *hypoxia-inducible factor 1-alpha*, *MAP1LC3B*, *epiregulin* (anti-apoptotic), *FAS receptor* (pro-apoptotic) normalized by *β-actin* as reference gene, was evaluated by RT-qPCR. Reactions were repeated in triplicate.

Main results and the role of chance: Supraphysiological doses of FSH impairs the endometrial receptivity during the embryo window of implantation by promoting endometrial cells death. FSH induces primarily autophagy (48 hours) which triggers apoptosis with cross-talk mechanism in longer incubation (72 hours). FSH increases the relative expression of *MAP1LC3B* assumed as autophagy marker as well as the pro-apoptotic gene *FAS receptor* and the protein APAF1. LH/hCG alone or combined with FSH prevents autophagy and apoptosis by inducing the anti-apoptotic gene *epiregulin*. Culture conditions were investigated for effectiveness of treatments (positive controls) and hypoxia. Results were supported by both immunohistochemistry and RT-qPCR. Statistical analysis was performed with Kruskal-Wallis test followed by the Dunn-Bonferroni's test ($P<0.005$).

Limitations, reasons for caution: This study was conducted only *in vitro*. Results obtained should be confirmed *in vivo* in the context of the whole human endometrial tissue and hormonal milieu. Indirect effects of activated gonadotrophin receptors on genes transcription, that lead to the overlap of autophagy and apoptosis signalling pathways are actually under study.

Wider implications of the findings: This study demonstrates that supraphysiological doses of gonadotrophin FSH impairs the endometrial cell viability, promoting anticipated physiological cell death. These effects are prevented/inhibited by LH/hCG

Trial registration number: Not applicable

P-310 Obstetrical and neonatal outcomes for Fresh versus Frozen-thrawed embryo transfer in women with endometriosis

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Study question: Does the frozen embryo transfer (ET) affect obstetrical and neonatal outcomes compared to fresh ET in endometriosis pregnant women after In Vitro Fertilization (IVF) ?

Summary answer: Endometriosis-women who conceive after frozen-ET have increased risk of induced labor, caesarian and post-term delivery but lower risk of low birth weight compared to fresh-ET.

What is known already: In endometriosis, it is known that there are endometrial local modifications, apart from any ovarian stimulation. Furthermore, there is accumulating evidence suggesting that obstetrical complications may originate from local disturbances occurring at the time of implantation. Indeed, after IVF, obstetrical and neonatal outcomes seem modified by the type of embryo transfer –in frozen cycles, the endometrial environment during the implantation window is probably modulate thanks to the absence of ovarian stimulation contrary to the fresh ET.

In the specific population of endometriosis women, no data are available concerning neonatal outcomes according to the type of transfer.

Study design, size, duration: An observational cohort study in a tertiary care university hospital was conducted between march 2010 and June 2017. Endometriosis infertile women achieving IVF singleton pregnancies progressing beyond 12 weeks' gestation were included. The diagnosis of endometriosis was based on published imaging criteria (transvaginal sonography or magnetic resonance imaging) or on histology for patients with a previous history of endometriosis surgery. Pregnancies obtained after a frozen ET were compared to those obtained after a fresh ET.

Participants/materials, setting, methods: A total of 339 pregnant women were allocated to two groups according to the type of ET performed to obtain the pregnancy: The Fresh ET group (n=112) and the frozen ET group (n=227). Main outcomes were the birth weight and the rate of low birth weight (<2500g). Secondary analyses were performed for the most common obstetrical and neonatal complications. Statistical analyses were conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: Among included women, 109/112 (97.3%) and 222/227 (98.2%) women had a delivery of a live child after 24 weeks in fresh and frozen ET groups respectively (p=0.526). The mean birth weight was similar in the two study groups after univariate (3181,09 ± 572,28 and 3249,01 ± 519,59, respectively, p = 0.117) and multivariate analysis (p=0,578). Risk of low birth weight was significantly lower in the frozen ET compared to the Fresh ET group after multivariate analysis (OR 0,185 CI95% [0,040-0,856], p= 0,031). However, the rate of labor induction (93/222 (41,9%) and 20/109 (18,3%), p<0,001) and caesarian section (93/222 (41,9%) and 28/109 (25,7%), p=0.004) was increased in frozen ET group, compared to the fresh ET group after univariate and multivariate analysis (OR 6,9 CI95% [2,47-19,3], p<0,001 and OR1,9 CI95% [0,12-3,19], p=0,017 respectively). The rate of post term birth (>41 weeks) was also significantly higher after multivariate analysis in the Frozen ET group (OR 2,0 CI95% [1,0-4,1], p=0,042). The incidence of hypertensive disorders, gestational diabetes antepartum and post-partum hemorrhage, preterm rupture of membranes and neonatal problems did not differ between groups.

Limitations, reasons for caution: One limitation is linked to the observational design of this study: confounders cannot be totally excluded. In addition, women in whom the diagnosis of endometriosis was based on imaging are possibly not as accurately phenotyped as those who had surgery.

Wider implications of the findings: Our results suggest that in endometriosis infertile women, perinatal outcomes are negatively affected by ovarian stimulation associated with a fresh ET but also by the vitrification process in case of frozen ET.

Trial registration number: NA

P-311 IL-10-IL-10R pathway promotes angiogenesis in the development of endometriosis

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Study question: Elevated IL-10 level in endometriotic milieus has been considered as a critical factor in endometriosis; however, the detail mechanism and causal relationship remain unclear.

Summary answer: This study provides a scientific basis for potential therapeutic strategy targeting IL-10-IL-10R pathway in endometriotic milieu

What is known already: Elevated IL-10 level in endometriotic milieus has been considered as a factor in endometriosis

Study design, size, duration: A surgical endometriosis murine model and IL-10 knockout mice (IL-$10^{-/-}$) were used to elucidate the role of IL-10 in the early development of endometriosis.

Participants/materials, setting, methods: Standardized *in vitro* and *in vivo* assays were used to analyze the angiogenic activity of IL-10, including transwell migration, tube formation assay, *in vivo* matrigel plug assay and zebra fish model.

Main results and the role of chance: In a murine model, exogenous recombinant IL-10 (rIL-10) local treatment on the day of surgery significantly enhanced endometriotic lesion growth and angiogenesis, whereas blocking local IL-10 activity using mAb suppressed those effects. Adoptive transfer of IL-10-deficient plasmacytoid dendritic cells (pDCs) into mice significantly inhibited lesion development. *In vitro* analyses demonstrated that IL-10-IL-10R pathway stimulated the migratory and tube formation ability in human umbilical vein endothelial cells (HUVECs) as well as ectopic endometrial stem cells (ENMSCs), at least in part, in a vascular endothelial growth factor-dependent pathway. We also found that rIL-10 directly stimulated angiogenesis using matrigel plug assay as well as zebra fish model. Pathological results from human endometrioma tissues also showed the increased infiltration of $CD123^+$ pDCs and higher percentages of $IL\text{-}10R^+$ and $CD31^+$ cells compared to the corresponding normal counterparts. These results show that IL-10 secreted

from local pDCs promotes endometriosis development through pathological angiogenesis in the early stage.
Limitations, reasons for caution: Most studies are conducted in the animal model, the clinical interpretation needs further investigation
Wider implications of the findings: IL-10-IL-10R pathway can provide a novel therapeutic target for endometriosis.
Trial registration number: None

P-312 Identification of involvement of developmental, metabolic and nervous systems in endometriosis by network-based bioinformatics analysis of HOX genes and their related genes

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Study question: Which genes and gene networks contribute to endometriosis in eutopic and ectopic lesions and what role do they play in the pathogenesis of this disease?
Summary answer: Some networks were identified that involved in development processes especially skeletal system in ectopic and eutopic tissues and in nervous system in eutopic tissues.
What is known already: Endometriosis is a complex gynecologic disorder that affects as many as 10-15% of premenopausal women. The pathogenesis of endometriosis, as the presence of endometrium-like tissue outside the uterine cavity, is largely unknown. *HOX* genes, encoding homeodomain transcription factors, are dynamically expressed in endometrium, where they are necessary for endometrial growth, differentiation, and implantation. Some studies on *HOX* genes and other genes have shown that dysregulation of some of them such as *HOXA10* are important for the development of endometriosis. But few investigations have studied in this vast range of *HOX* family genes and with a network-based view.
Study design, size, duration: Fifteen samples of eutopic and ectopic endometrium collected from patients in proliferation phase. Five tissue samples in each ectopic, eutopic and normal group were pooled as three biological repeats, and all following analyses were performed on the nine sample groups. To minimize the genetic heterogeneity, the same patients provided both eutopic and ectopic endometrium. Fifteen control individuals who underwent diagnostic laparoscopy participated in this study as control group.
Participants/materials, setting, methods: PCR array was performed for 84 *HOX* genes and their related genes. The statistical significance was determined using t-test. PCA and Hierarchical clustering were carried out between different groups. The genes co-expression networks were constructed based on correlation between normalized gene expression data. Gene function was annotated based on Gene Ontology, the intervention in other diseases and expression in other tissues using the DAVID to clarify the function of important genes and mechanism of endometriosis.
Main results and the role of chance: Among the analyzed genes, 33.33% (28) genes had no significant alteration in none of the patient samples. The expression levels of 33 and 44 genes were significantly altered in eutopic and ectopic samples, respectively. PCA and hierarchical clustering analyses were performed using the normalized expression data of the DEGs (Differentially Expressed Genes) and non-DEGs. These analyzes of the DEGs revealed three distinct clusters of eutopic, ectopic and normal samples and the analyzes of the non-DEGs didn't separated precisely these three groups.

Five co-expression networks were constructed based on gene expression data. DEGs in diseased tissues were significantly abundant in the network B2, especially DEGs in the ectopic tissues(73.1%).

Many DEGs in ectopic tissues play a role in developmental diseases class, such as cleft lip and metabolic diseases class like bone mineral density. The most DEGs in the eutopic and ectopic tissues, and genes of networks A, D, C, B1 and B2, other than the PDHX gene in network B2, were involved in the development or morphogenesis of various systems, especially skeletal system. Also, most genes in network D play roles in the development of the nervous system. Most genes in each network were expressed in one or two tissues.
Limitations, reasons for caution: It would be interesting that more patient samples could be collected with more accurate phenotypic data in order to more precisely evaluate the relationship between phenotype and genotype. This helps in identifying the pathogenesis of the disease.
Wider implications of the findings: Non-DEGs that connected to many DEGs in networks are likely effective in development of endometriosis but their expression has not been significantly altered. More experimental researches such as epigenetic studies and investigations of the genes in many patients can determine the effect of the non-DEGs in the development of endometriosis.
Trial registration number: not applicable

P-313 Evidence synthesis on the effectiveness of endometrial scratching - where did we go wrong?

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Study question: Why meta-analyses on endometrial scratching prior to IVF or IUI suggested that the treatment is effective while we know now that it is not?
Summary answer: Meta-analyses suggesting endometrium scratching is effective are erroneous because of the overall low quality of included studies.
What is known already: The Cochrane Review on endometrial scratching in IVF published in 2015 reported that moderate-quality evidence suggested a beneficiary effect of endometrial injury on live birth or ongoing pregnancy (RR 1.42, 95% CI 1.08 to 1.85). The Cochrane review on endometrial scratching before IUI or sexual intercourse published in 2016 reported that very low quality evidence suggested a positive association between endometrial injury and live birth or ongoing pregnancy (RR 2.22, 95% CI 1.56 to 3.15). However, well designed and powered RCTs, including a recent study with 1300 women (Lensen et al., 2019), indicated that endometrial scratching is not effective.
Study design, size, duration: We analyzed the quality of RCTs included in two Cochrane Reviews investigating the effectiveness of endometrial scratching in IVF and IUI or sexual intercourse (Nastri et al., 2015; Lensen et al., 2016). We identified 18 RCTs with full text from these Cochrane Reviews (11 on endometrial scratching in IVF, 7 on endometrial scratching in IUI /sexual intercourse).
Participants/materials, setting, methods: We assessed the RCTs on the following criteria:

1. inadequate trial registration (no registration, registered during or after recruitment);
2. problematic randomization as assessed by the low probability of random sampling for baseline variables using Monte Carlo simulations;
3. possible inadequate data handling as indicated by the violation of Benford-Newcomb's law for the distribution of first leading digits;
4. low quality of statistical analysis (critical concerns about statistical methods description and not repeatable univariable statistical analysis using summary statistics).

Main results and the role of chance: There were only 3 (17%) out of 18 RCTs with adequate registration. The numbers of RCTs with no registration and late or retrospective registration were 7 (39%) and 8 (44%), respectively. There were 3 (17%) RCTs with possible problematic randomization as assessed from the baseline data, while the distribution of first leading digits of all numbers violated Benford-Newcomb's law in 7 (39%) RCTs. In total, 7 (39%) RCTs had critical issues in terms of the description of statistical methods. There were 12 (67%) and 9 (50%) RCTs that had not repeatable statistical tests for continuous and categorical baseline variables respectively. The statistical results

of outcome(s) were not repeatable in 12 (67%) RCTs. Overall, no RCTs were without any concerns.

Limitations, reasons for caution: Monte Carlo simulations tend to be stricter in RCTs using block randomization. Using Benford-Newcomb's law to examine the distribution of first leading digits has a high sensitivity but low specificity in detecting inadequate data handling. For statistical assessment, we could only use summary statistics but not individual participant data.

Wider implications of the findings: The initial promising reports on the effectiveness of endometrial scratching are misleading due to the low quality of the initial RCTs, which could not be identified by the conventional risk of bias assessments. Rigorous evidence synthesis incorporating RCTs of low quality cannot overcome bias from uncontrolled or poorly conducted studies.

Trial registration number: Not applicable.

P-314 Epigenetic Aberration of E-Cadherin Gene (CDH1) in Eutopic and Ectopic endometrium of Endometriosis Patients

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Study question: Is there any alterations in expression and epigenetic profiles of *E-Cadherin* in eutopic and ectopic tissues of women with endometriosis?

Summary answer: *CDH1* is down-regulated in ectopic endometrial tissues through hyper methylation and hypo acetylation of histone H3 in its promoter region.

What is known already: Endometriosis is defined as the presence of endometrial-like tissue outside the uterine cavity. There is evidence that endometriotic cells have behavior same as cancer cells. In addition, cell-culture studies of epithelial endometriotic and carcinoma cells indicated that both cell types have the ability to invade collagen gels, and this ability is associated with the absence of the intercellular adhesion molecules. One major member of the classical cadherin family is epithelial (E)-cadherin which plays an important role in cell-cell adhesion. On the other hand, accumulating evidence suggests that endometriosis may be an epigenetic disease.

Study design, size, duration: In this case-control study, patients with endometriosis (n = 36) and healthy fertile women (n = 21) were included in the study. Ectopic biopsies were obtained by laparoscopic procedure and eutopic and control biopsies were gained with Pipelle. Women with other uterine abnormalities were excluded. Informed written consent was obtained from all women according to local ethical approval.

Participants/materials, setting, methods: The expression level of *CDH1* gene was evaluated in eutopic, ectopic and normal endometrium during the menstrual cycle by the use of quantitative real-time (qRT)-PCR. Also, Chromatin Immunoprecipitation (ChIP) coupled with real-time PCR was used to determine the epigenetic modifications on the regulatory region of *CDH1* gene. All data were analyzed using One-Way ANOVA and the Tukey's test.

Main results and the role of chance: *CDH1* gene expression in endometriotic samples was significantly lower than normal endometrium. In addition, the expression level of *CDH1* in normal endometrium was significantly lower during proliferative phase *vs.* secretory phase. Otherwise, epigenetic analysis data showed hypoacetylation and hypermethylation of *CDH1* promoter in endometriotic samples *vs.* control.

Limitations, reasons for caution: For getting more evidence, we need a large number of patients and control groups to examine *CDH1* gene also to evaluate expression of other related genes. Moreover, other Epigenetic studies such as DNA methylation of *CDH1* promoter region are suggested.

Wider implications of the findings: Our study declared that epigenetic modifications in *CDH1* promoter cause to significant down-regulation of *CDH1* gene in ectopic tissues, which lead to invasion in endometriosis lesions.

Trial registration number: not applicable

P-315 Inhibition of KIF20A reduces endometriosis implants in a randomized xenograft mouse model

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Study question: To assess effects of *KIF20A* inhibition by BKS0349, a high-affinity new inhibitor derived from Paprotrain, in endometriotic lesions generated in an endometriosis xenograft mouse model.

Summary answer: *KIF20A* inhibition by BKS0349 decrease cell proliferation, induces cell cycle arrest and promotes apoptosis of endometriotic lesions, reducing their size in a xenograft mouse model.

What is known already: Several studies have reported that ovarian endometriotic may be the origin of ovarian carcinoma or ovarian endometrioid cancer types, suggesting a possible etiological association between endometriosis and ovarian cancer. In this regard, accumulating evidence has shown that ectopic *KIF20A* overexpression might confer malignant phenotype to ovarian tumors by promoting cell proliferation and inhibiting apoptosis. In addition, it has been reported that *KIF20A* downregulation inhibits cell proliferation, induces cell cycle arrest in G0/G1 phase, and promotes apoptosis, proposing *KIF20A* as a therapeutic target for numerous tumors. However, to date, no data about the role of *KIF20A* in endometriosis has been described.

Study design, size, duration: This is a prospective study in which human endometrial biopsies (n=4) were transfected by mCherry adenovirus and intraperitoneally implanted in mice, generating a xenograft mouse model of endometriosis. Female mice were divided in Vehicle group (n=8), BKS0349 group (n=8) and Cabergoline (positive control) group (n=8). Vehicle and BKS0349 were administrated once a week and Cabergoline was orally administrated every day for 21 days. Mice were sacrificed 72 hours after last administration.

Participants/materials, setting, methods: Human endometrial tissue was obtained from egg donor women at the time of oocyte retrieval procedure. mCherry adenovirus was used to label endometrial tissue. mCherry-labeled endometriotic lesions were introduced in 6-week-old athymic nude female mice and monitored over time using IVIS Spectrum Preclinical *in vivo* Imaging System. Cellular proliferation was assessed by immunohistochemistry for Ki67 and apoptosis was evaluated with TUNEL staining. *CCND1* gene expression was measured by qRT-PCR using StepOnePlus System.

Main results and the role of chance: A significant reduction in fluorescent signal was observed 72 hours after treatment end (D24) for BKS0349 group (*p*-value=0.0313) and from D14 for Cb2 group (*p*-value=0.0313 on D14-21; *p*-value=0.0156 on D24) compared to D0, while this reduction was not highly pronounced in control group. In addition, fluorescent signal on D24 showed a significant decrease in BKS0349 group compared to control group (40% ± 19.3 vs. 81% ± 30.7; *p*-value=0.0303), according with significant size reduction of endometriotic lesions observed in BKS0349 group (0.073mm^2 ± 0.022; *p*-value=0.0006) compared to control group (0.128 mm^2 ± 0.019) at the end of the experiment. Functional studies showed significant reduction of proliferating cells in BKS0349 group compared to control group (3.5% ± 3.4 vs 9.2% ± 3.8; *p*-value=0.0082), which was even more pronounced than our positive control group (Cb2) (5.7% ± 5.8). In addition, C*CND1* expression was decreased in BKS0349 group compared to control group (Fold Change=0.259; *p*-value=0.049). Finally, while endometriotic lesions in control group were practically absent of apoptotic cells (25.48% ± 13.3), an increase of apoptotic cells in endometriotic lesions from BKS0349 (39.73% ± 22.95) and Cb2 (37.7% ± 24.87) group was observed, being statistically significant in animals treated with the *KIF20A* inhibitor, BKS0349 (*p*-value=0.0317).

Limitations, reasons for caution: Results obtained in this mouse model of endometriosis could not be directly assumed in humans due to the phylogenetic distance with mouse.

Wider implications of the findings: KIF20A inhibition by BKS0349 induces apoptosis and inhibits cell proliferation by cell cycle arrest in G0/G1 phase and consequently reduces endometriotic lesions size. This suggest that KIF20A could

be used as a novel therapeutic treatment for endometriosis due to its important role in cell cycle regulation and apoptosis.
Trial registration number: NA

P-316 A 3D culture system to study early stages of endometriosis

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Study question: Is it possible to study early stages involved in the establishment of new endometriotic lesions in vitro?
Summary answer: The 3D culture based model system is suitable to study extracellular matrix remodeling and different forms of invasive growth, including mesenchymal and epithelial sheet-like migration.
What is known already: Early studies suggested endometrial cells are highly invasive. However, another recent study suggested that an ectopic-lesion derived lesion does only form small spheroids on top of Matrigel and does not invade the hydrogel. One approach to evaluate invasive behaviour in a more robust and reproducible manner is to use spheroids containing 5,000-20,000 cells. This approach additionally provides more insight into the mechanism by which the cells migrate and is routinely used in cancer research. Moreover, it has been recently demonstrated that such spheroids mimic several aspects of endometriosis better than single cells, partially also because these cells experience hypoxia.
Study design, size, duration: Experimental in vitro study using immortalized endometriotic epithelial (12Z), immortalized endometrial stroma (ST-T1b) and primary endometriotic stroma cells (N= 20,000 cells/culture) cultured in collagen type I or basement-membrane-like Matrigel matrices after formation of spheroids using the hanging drop method for 4 days. Experiments were replicated at least 3 times.
Participants/materials, setting, methods: Cell spheroids formed by the hanging drop method were placed on 3mg/ml collagen type I or Matrigel and their growth and invasive behaviour was monitored on days 1, 3, 5 and 7 using microscopy. qPCR for markers of epithelial-to-mesenchymal transition was used to evaluate matrix-dependent differences in cellular phenotype. A range of inhibitors (Rac, ROCK, MMP, miR-200b, miR-145) was assessed with regard to their migration/invasion modulatory effect.
Main results and the role of chance:
Our study revealed that all the studied cell types were able to form spheroids. Statistical analysis showed that the spheroids formed by 12z cells are bigger and thus likely less compact compared to the other cell types ($p<0.05$). Surprisingly, while both the primary cells and St-t1b cells have stromal cell morphology, they exhibited differing characteristics when placed on Matrigel. While the St-t1b cells did not migrate on Matrigel, 12z cells and primary ectopic lesion-derived cells were spreading on this hydrogel. On the other hand, when placed on collagen, both primary cells and St-t1b cells migrated on the material, frequently forming holes in the material. This process could be slowed down or abrogated using Rac and MMP inhibitor in St-t1b cells, but not by the ROCK inhibitor. The 12z cells on collagen exhibited sheet-like collective migration that was not affected by any of these inhibitors.
Limitations, reasons for caution: The use of immortalized cell linesis a limitation of the study, as is the study of singular cell types compared to the more complex in vivo environment. While collagen I and matrigel are representative of interstitial matrices and basement-membrane-like matrices, respectively, the extracellular matrix in vivo is more complex.
Wider implications of the findings: Diseased and healthy cells have a different phenotype with regards to their interaction with extracellular matrix components, which may contribute to their increased migratory potential. Our results suggest that all studied cell types thrive on collagen I, suggesting scar tissue might be especially prone to the attack by endometrial cells.
Trial registration number: not applicable

P-317 Presence of Focal adenomyosis based on MRI is associated with presentation for infertility

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Study question: Is there an association between the adenomyosis appearance (focal or diffuse) by magnetic resonance imaging (MRI) and presentation with infertility?
Summary answer: In a population of operated patients, presenting adenomyosis on MRI, focal adenomyosis was identified as a risk factor for infertility.
What is known already: Uterine adenomyosis is characterized by the presence of endometrial glands and stroma deep within the myometrium. Various forms have been described including Focal adenomyosis (Foc-ADE), which corresponds to well-circumscribed nodular lesions within the myometrium and Diffuse adenomyosis (Dif-ADE) characterized by endometrial implants scattered throughout the myometrium. Although adenomyosis pathophysiology is not clearly understood, several evidences suggest that those two forms could be considered as two distinct entities. This disease exhibits various clinical presentations, notably infertility but reasons for reproduction impairment are still unknown. The role that play one or other of the adenomyosis forms on infertility must be explore.
Study design, size, duration: This was an observational study using data prospectively collected in all non-pregnant patients aged between 18 and 42 years, surgically explored for benign gynaecological conditions at our institution between May 2005 and May 2018. For each patient, a standardized questionnaire was completed during face-to-face interview conducted by the surgeon during the month preceding surgery. Only women with a uterine MRIs performed by one senior radiologist during the preoperative work-up were retained for this study.
Participants/materials, setting, methods: MRI was performed in 496 women operated in our center for a benign gynaecological disease. Among them, 248 women have a radiological diagnosis of adenomyosis. According to MRI findings, women were diagnosed for Foc-ADE and/or Dif-ADE forms. Of them, two groups were compared: the infertile women (n=75) and the non-infertile women (n=173). Correlations were sought with univariate analysis and a multiple regression logistic model to determine characteristics of infertile patients compared to adenomyosis non-infertile patients
Main results and the role of chance: In infertile women suffering from adenomyosis, the presence of a focal lesion was significantly more frequent (59/75 (78,7%) versus 111/175 (64,2%); p=0,024) than in non-infertile women. The diffuse form was no more present in infertile than in non-infertile women. Distribution of others benign gynecologic disease, notably endometriosis or leiomyomas was not significantly different between both groups. After a multivariate regression model including women age and endometriosis phenotype, the presence of a Foc-ADE lesion was identified as risk factor for adenomyosis-related infertility: OR= 2.1; 95% CI: 1.1 - 3.9.
Limitations, reasons for caution: Inclusion of only surgical patients may constitute a possible selection bias. In addition, it cannot be excluded that other non–explored causes of infertility are present in infertile women.
Wider implications of the findings: In women presenting adenomyosis, the practitioner should perform an appropriate imaging work-up to plan the strategy of patient management in the setting of infertility.
Trial registration number: none

P-318 Impact of extra-ovarian deep endometriosis surgery on AMH, a prospective study

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Study question: Does the surgery of extra-ovarian deep endometriosis have an impact on AMH level at 6 and 12 months after surgery?
Summary answer: AMH level decrease 6 and 12 months after surgery of extra-ovarian endometriosis.

What is known already: Deep endometriosis is a usual gynaecologic disease, affecting women of reproductive age. The first-line surgical treatment of endometrioma is cystectomy but several studies show a decreasing AMH level after surgery, which is a hormone representing ovarian reserve. However, up to date, no study concerning AMH level after extra-ovarian deep endometriosis surgery has been published.

Study design, size, duration: Prospective single-center study comparing the variation in the AMH serum 6 and 12 months after surgery of deep endometriosis isolated (Group A) with deep endometriosis surgery associated with endometrioma's cystectomy (Group B) , in Gynaecological surgery departments of the University Hospital of Strasbourg (HUS), Francebetween June 2015 and March 2018. An AMH sample was collected at the HUS the day before or the day of the surgery, 6 months and 12 months after surgery

Participants/materials, setting, methods: 101 patients were included after exclusion of hysterectomy, adnexectomy or no deep surgery, 53 in the group A and 48 in the group B. Up this day, 29 AMH serum are available at 12 months in the group A and 20 in the group B. Primary outcome was the variation in the AMH level 12 months after surgery, secondary outcomes were pregnancy rate after surgery and variation in the AMH serum at 6 months

Main results and the role of chance: Patients in group A had a significantly decreasing AMH level of 1.07 ng/mL 12 months after surgery p: 0.00481 and patients in group B had a significantly decreasing AMH level of 1.37 ng/mL 12 months after surgery p: 0.00289, but no difference was founded between group A and B p :0.60.

Patients in group A had a significantly decreasing AMH serum of 0.85 ng/mL 6 months after surgery p: 0.00245 and patients in group B had a significantly decreasing AMH serum of 1.14 ng/mL 6 months after surgery p: 0.00022, but no difference was founded between group A and B p :0.47.

Type of surgery and AFS scale doesn't seem to have an impact on the AMH level's change.

However, in patients reporting preoperative infertility, we found pregnancy rates of 44 % in group A and 61 % in group B 1 year after surgery.

Limitations, reasons for caution: Final results of our study should be available in March 2019. Many patients were lost to follow-up during the study because all the samples were analysed in the same laboratory to limit AMH variation due to different laboratories, and some patients couldn't come 6 and 12 months after surgery.

Wider implications of the findings: the surgery of deep endometriosis seems to reduce AMH level 12 months after surgery even if endometrioma's cystectomy is not performed but pregnancy rate one year after surgery is encouraging.

Trial registration number: NCT02400684

P-319 Antibacterial and antifungal activity of the human endometrial fluid during the natural cycle

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Study question: What is the antibacterial protein content of human endometrial fluid aspirate (EFA) in IVF cycles?

Summary answer: Human endometrial fluid aspirate shows an antimicrobial activity that was always present against *S. aureus* and against *Candida spp.*, *S. agalactiae* and *E. faecalis*

What is known already: The role of uterine microbiota is not well understood. Some microbiota patterns have been associated with favorable IVF prognosis and others with some pathological conditions. On the other hand, the endometrial fluid aspirate (EFA) contains antibacterial proteins that are enriched in implantative IVF cycles. However, the antimicrobial effect of raw EFA, where interactions among different regulating factors are plausible, has not been addressed. Moreover, the possible modifications over the natural cycle have not been analyzed.

Study design, size, duration: During a 3 month period, 38 women were recruited for the analytical study. The study was approved by our Institutional Ethical and Investigation Board (CEIC code 11/45)

Participants/materials, setting, methods: EFA was obtained by means of an embryo transfer catheter, under ultrasound guidance in 38 healthy women, aged between 18- 40 years, with regular cycles. EFA was tested against 9 different microorganisms

Main results and the role of chance: All the tested samples exhibited antibacterial activity against *Staphylococcus aureus*. Apart from inhibiting *S. aureus*, 32.4% of EFA were active against only one of the remaining microorganisms assayed, 16.2% against two of them, and 5.4% against four microorganisms. Concerning other microorganisms (*Candida albicans*, *Candida glabrata*, *Candida krusei*, *Streptococcus agalactiae*, *Enterococcus faecalis*) the antibacterial activity was present in 10.8-24.3% of EFA. On the other hand, none exhibited antibacterial activity against *Escherichia coli* or *Klebsiella pneumoniae*. The intensity of antimicrobial activity differs considerably among EFA samples, and we failed to observe a cycle related pattern for antimicrobial activity.

Limitations, reasons for caution: This is the first report on the direct antimicrobial activity of EFA samples from healthy women. Further studies are warranted to characterize the endometrial fluid of women undergoing IVF procedures in order to assess if there is a relationship between raw EFA antimicrobial activity and implantation success.

Wider implications of the findings: Endometrial fluid aspirate exhibited antimicrobial activity, which can be described according to two patterns: a pattern common to all the samples and an individualized pattern. Our data suggest that the uterus plays a role controlling the uterine microbiota by means of the endometrial fluid components.

Trial registration number: not applicable.

P-320 Cumulus cells and follicular fluid show alterations in bone morphogenic protein 15 (BMP-15), growth differentiation factor 9 (GDF-9), and oxidative status in patients with endometriosis

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Study question Is there relationship between oocyte-secreted factors BMP-15 and GDF-9 of cumulus cells and oxidative stress in the follicular fluid in patients with endometriosis.

Summary answer: GDF-9 and BMP-15 levels were decreased in cumulus cells and oxidative stress levels were found to be increased in the follicular fluid of endometriosis patients.

What is known already: Both GDF-9 and BMP-15 are oocyte-secreted factors and belong to the transforming growth factor beta (TGF-β) superfamily. They have pivotal role ranging from follicle maturation, fertilisation, and early embryo development. Oxidative stress, on the other hand, is documented to be present in follicular fluid of endometriosis patients. However, although alterations in GDF-9, BMP-15, and oxidative stress levels are documented in endometriosis, it remains unclear to what extend the combined effects have on clinical outcomes.

Study design, size, duration: This was a prospective study investigating the relationship between GDF-9, BMP-15, and oxidative stress levels of patients with END. The study was approved by the ethical committee of Gazi University Faculty of Medicine and 10 patients with endometriosis(test) and 10 patients with male factor (control) without any documented female factor were recruited between March 2018 and December 2018. Two independent and blinded researchers run the experiments and analysed the data.

Participants/materials, setting, methods: Consented patients with endometriosis(test) and male factor (control) were recruited. Any further experiments were performed at Biochemistry and Histology Departments of Gazi University Faculty of Medicine. Cumulus cells were mechanically dissected, fixed on polylysine processing slides and pre-treated with paraformaldehyde for immunohistochemical assays to analyse GDF-9 and BMP-15. Follicular

fluids (without flushing) were collected during oocyte pick-up, centrifuged and subjected to total antioxidant status (TAS) and total oxidant status (TOS) assays using UV-visible spectrophotometry.

Main results and the role of chance: No significant difference was found in TAS levels of follicular fluid. However, TOS results of follicular fluid of endometriosis (22.527 ± 3,410 vs 10,631 ± 2,150, $p<0.05$) patients were significantly higher than control group. This finding showed increased oxidative stress level was not balanced with an anti-oxidant response in endometriosis group. Irregular cellular lines and nuclear fragmentations were detected in cumulus cells of endometriosis group. Both GDF-9 and BMP-15 markers were detected in cytoplasm of cumulus cells and found to bedecreased in the endometriosis group. Correspondingly, patients with low GDF-9 and BMP-15 findings in cumulus cells had high TOS levels and low TAS levels in follicular fluid. In response to these findings, reduced GDF-9 and BMP-15 expressions in cumulus cells and higher TOS values in follicular fluid of patients diagnosed with endometriosiswere interpreted with decreased long-term developmental potential of oocytes

Limitations, reasons for caution: Both the cumulus cells and follicular fluids were obtained from patients that received gonadotropins. The sample size of the study is small and further studies are required.

Wider implications of the findings: It is clear that GDF-9 and BMP-15 levels show differences in the cumulus cells as well as the TAS levels of the follicular fluid from patients with END. Further studies should be designed to confirm the therapeutic modalities targeting these findings.

Trial registration number: Not applicable

P-321 Transvaginal hydrolaparoscopy versus hysterosalpingography to assess tubal pathology in subfertile women: a randomised controlled trial.

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Study question: What is the cumulative live birth rate after a diagnostic strategy using transvaginal hydrolaparoscopy (THL) compared to hysterosalpingography (HSG) in subfertile women?

Summary answer: In subfertile women, a diagnostic strategy with THL and HSG results in comparable cumulative live birth rates.

What is known already: THL and HSG are safe and feasible diagnostic techniques for testing tubal pathology in subfertile women.

Study design, size, duration: We performed a multicenter randomised non-interiority trial (NTR3462) in 4 teaching hospitals in the Netherlands. After informed consent, women were randomised to a strategy starting with THL or a strategy starting with HSG. While we originally aimed to recruit 1,330 women, the study was halted after randomization of 300 women due to limited funding.

Participants/materials, setting, methods: We studied subfertile women scheduled for tubal patency testing. Women with a positive Chlamydia PCR, prior tubal testing or tubal surgery, retroverted uterus, masses or cysts in the pouch of Douglas, and allergies to iodine or methylene blue were not eligible. The primary outcome of the trial was conception leading to live birth within 24 months after randomisation.

Main results and the role of chance: Between May 2013 and October 2016, we randomly allocated 149 women to THL and 151 women to HSG. Bilateral tubal occlusion was detected in 1 versus 3 women (0.9% versus 2.2%) in the THL group and HSG group respectively, while unilateral tubal occlusion was detected in 7 (6.2%) versus 8 (5.9%) women.

In the THL group, 86 women (63.7%) had a liveborn child within 24 months after randomization compared to 85 women (59.9%) in the HSG group. Time to pregnancy and time to live birth were not statistically different in both groups. Miscarriage occurred in 16 women (11.9%) in the THL group, versus 20 women (14.1%) in the HSG group. Multiple pregnancies occurred in 12 women (8.9%) in the THL group compared to 19 women (13.4%) in the HSG group.

Limitations, reasons for caution: The calculated sample size of 1,330 was not reached, due to lack of funding. Although our data in 300 women suggest superiority of THL over HSG with respect to live birth rates, the non-inferiority limit delta of 6% was not reached and therefore, the results are still inconclusive.

Wider implications of the findings: Both THL and HSG are in terms of safety and pain acceptable methods to test tubal patency. THL gives more information about endometriosis and adhesions than HSG. In this study we found comparable live birth rates and no statistical difference in time to pregnancy.

Trial registration number: NTR3462

P-322 Changes in some transcription factors expression influencing on CD4+ T cells differentiation in endometriosis

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Study question: Is there any different in gene expression of transcription factors *c-Maf* , *FOXP3* and *GATA3* in endometrial tissues (ectopic and eutopic) of women with endometriosis?

Summary answer: The gene expression of c-Maf and GATA3 was up-regulated while FOXP3 was down- regulated in women with endometriosis compared to the control group.

What is known already: Endometriosis is a chronic inflammatory disease of women in reproductive age. This disease is one of the most common causes of infertility. Pathogenesis of endometriosis has not been completely understood, however several studies suggest that immunologic alterations contribute in initiation and progression of the endometriosis. One of these alterations is change in different subsets of T cells. Forkhead box P3 (FOXP3) is a master transcription factor of regulatory T cells (Treg), GATA binding protein 3 (GATA3) is a proprietary transcription factor of T helper 2 (Th2) while **c-**Musculoaponeurotic Fibrosarconna (c-Maf) is a transcription factor expressed in Th2, Th17 and Treg.

Study design, size, duration: In this case-control study, 13 women with endometriosis and 13 women without the disease were enrolled after diagnostic laparoscopy. Ectopic endometrial biopsies were obtained through laparoscopic procedure while eutopic endometrial tissues and endometrial samples of controls were obtained by pipelle. Women with any other uterine abnormalities were excluded. Informed consent was obtained from all women according to local ethical approval.

Participants/materials, setting, methods: After endometrial tissues collection, RNA extraction and cDNA synthesis was done. The relative mRNA expressions of *c*-Maf, FOXP3 and GATA3 were studied by quantitatively real-time polymerase chain reaction (real time-PCR). The data were analyzed using One-way ANOVA followed by post-hoc Tukey Test. The comparison between the gene expression of c-Maf –FOXP3 and c-Maf –GATA3 was analyzed using T-test.

Main results and the role of chance: The results showed significant increase in the gene expression of GATA3 in eutopic and ectopic tissues compared to controls ($p<0.05$). In addition, the gene expression of c-Maf was statistically higher in ectopic endometriotic tissues compared to eutopic tissues and control ones while a significant decrease in the gene expression of FOXP3 was detected in ectopic and eutopic tissues of women with endometriosis compared to the control ones ($p<0.05$). The results of T-test analysis indicated a significant increase of *c-Maf* gene expression versus *FOXP3* in eutopic and ectopic tissues.

In addition, a significant increase in *GATA3* was observed in comparison with *c-Maf* gene only in the eutopic tissue of women with endometriosis.
Limitations, reasons for caution: For getting more information, we need to evaluate these transcription factors in a large number of endometriosis women and also to investigate expression of other transcription factors influencing on T cells differentiation such as RORγt. Epigenetic modifications such as DNA methylation at regulatory regions of these genes are also recommended.
Wider implications of the findings: These data collectively identify *FOXP3* and *GATA3*, c-*Maf* as candidate transcription factors critically involved in development of endometriosis beyond their role in induction of $CD4^+$ T cells differentiation.
Trial registration number: -

P-323 Evaluation of tubal patency in 2018 : the place of ultrasound with the use of an hyperechogenic medium, a franco-belgian survey

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Study question: In the fertility workup, what is the current place of tubal exploration with ultrasound in France and Belgium, particularly in prescribing and performing Hycosy/Hyfosy ?
Summary answer: Despite its benefits, tubal exploration with ultrasound is largely under-prescribed, due to a lack of knowledge of prescribers and their poor availability in practice.
What is known already: The use of a contrast medium during vaginal ultrasound to visualize the flow through the fallopian tube to test its patency was first describe in 1991. The feasibility, diagnostic efficacy and patient tolerance were demonstrated with this new diagnostic tool named hysterosalpingo-contrast sonography (HyCoSy). In 2007, a new hyperechogenic fluid (Exem Foam Kit) was introduced, having a more steady state, used for hysterosalpingo-foam sonography (HyFoSy) with a better accuracy and a shorter learning curve. The tubal exploration with ultrasound is simple and well-tolerated by women. It allows advance selection of patients in whom more invasive diagnostic procedures may be required.
Study design, size, duration: A web survey was created using doc.google with 23 questions regarding fertility workup, especially uterine and tubal examination. The survey was created in french and flamish. The physicians, gynecologists and reproductive endocrinologists, were invitated to participate by french and belgian societies of reproductive medecine. The link to the survey was posted on their website. The survey was open from October, 2017 to November, 2018. The Exem Foam Kit is reimbursed by social security in Belgium.
Participants/materials, setting, methods: There were 369 participants in the survey of whom 270 French and 60 Belgian. 39 were from other countries and were excluded from the analysis. Both French and Belgian groups were comparable regarding age, part of reproductive medicine in their practice, as well as their use of ultrasound in routine practice. The answers to the survey between the 2 groups were compared with Chi2 or Fischer exact test.
Main results and the role of chance: French physicians prescribed hysterosalpingography (HSG) more frequently (82% versus 42%, $p < 0.001$), while Belgians prescribed more frequently HyFoSy/HyCoSy (48% vs. 7%, $p < 0.001$). Regarding tubal exploration with ultrasound, 57% of Belgians prescribe a HyFoSy vs 33% of French ($p = 0.004$). 44% of French physicians left the choice between HyFoSy and HyCoSy to the operator. 60% of physicians in both groups did not prescribe routine antibiotic therapy with HyFoSy/HyCoSy. 62% of French and 70% of Belgians ($p = 0.7$) performed a laparoscopy in a second step. Among the reasons of non-prescribing HyFoSy/HyCoSy by French and Belgian physicians, there was a lack of knowledge of the procedure in 37% and 58%, the lack of a competent correspondent for these techniques for 49% and 35%, and insufficient data validating the technique for 14% and 7%, respectively. These differences were not significant.
Limitations, reasons for caution: This is a study based on volunteering, we can not be certain that our ample is representative of all french and belgian physicians working in the field of reproductive medicine. In addition the number of Belgian physicians was significantly lower than the French.
Wider implications of the findings: There is often a delay between the theoretical validation of a technique and its wide dissemination. As HyFoSy has proven its effectiveness, physician need to be more wisely informed and trained to this technique. The reimbursement of the Exem Foam Kit by the French social security will probably help.
Trial registration number: Not applicable

P-324 Interleukin-33 modulates inflammation and may be treated as a biomarker in endometriosis

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Study question: Does IL-33 contribute an important role towards inflammation observed in endometriosis and can be considered as a biomarker?
Summary answer: IL-33 posts its importance as biomarker by perpetuating inflammation, angiogenesis and lesion proliferation; critical events in progression of endometriosis.
What is known already: Endometriosis is a chronic inflammatory, estrogen-dependent disease that affects 6–10% of reproductive-aged women. Interleukin (IL)-33 is a critical regulator of number of processes including chronic inflammation, vascularization, hyper-nociception, and fibrosis; known associates of the pathophysiology. Although increased plasma and peritoneal fluid levels of IL-33 and its receptor ST2 had been associated with deep infiltrating endometriosis, whether IL-33 is playing an active role in the disease progression or if it is a bystander, is unknown. In a syngeneic mouse model supported by *in-vitro* findings we tried to delineate the possible contribution of IL-33 to the disease pathology.
Study design, size, duration: Syngeneic model of endometriosis was established in ∼10-week-old Balb/C mice (n=20). Treatment and positive-control group/s (n=6) were administered intraperitoneally mouse recombinant (r)-IL-33 at 1mg/100mL biweekly for two weeks. Control group (n=8) received phosphate-buffered-saline (PBS) in parallel. Endometrial epithelial carcinoma cells (EECC) and Human Umbilical Venule Endothelial Cells (HUVEC) were treated *in-vitro* with concentrations (10, 50 and 100 ng/mL) of human rIL-33 for 24 hours. Study was approved by Animal Ethics Committee (IAEC-56/PC/2018/10) of the institute.
Participants/materials, setting, methods: Plasma cytokine profile and pro-inflammatory marker/s were assessed by mouse multiplex assay and immunohistochemistry (IHC) respectively. Cell proliferation was evaluated using IncuCyte cell confluence proliferation assay. Cell-supernatant cytokine/s and effect of human rIL-33 on proliferation of cell lines and/or angiogenesis was assessed using human multiplex assay and fluorescence-kit based method in EECC, and HUVEC respectively. Data were evaluated by two-way analysis of variance using Graphpad Prism 7.0. $P < 0.05$ was considered as statistically significant.
Main results and the role of chance: Syngeneic model of endometriosis demonstrated significantly higher ($p<0.01$) plasma cytokine (chemokine (C-X-C motif) ligand 1 (CXCL1), IL -5, 6, 7, 33, and Granulocyte-macrophage colony-stimulating factor (GM-CSF)) level/s on day 20, day 27 following mouse rIL-33 injection compared to control, cueing to systemic inflammation. Positive control cohort revealed significantly elevated levels ($p<0.01$) of plasma cytokine/s in parallel authenticating mouse rIL-33 can promote systemic inflammation. Treatment with mouse rIL-33 produced qualitatively larger lesions and enhanced vascularization compared to control. Supernatants from EEECs and HUVECs revealed significantly higher concentrations of angiogenic (VEGF and PDGF-AA; $p< 0.001$) and pro-inflammatory (IL-1α and Tumor necrosis factor-a (TNF-α); $p< 0.05$) cytokines compared to PBS-treated cells. Varying concentrations of human rIL-33 on EECCs, and HUVECs did not have any effect on proliferation, however, tubule branch length in HUVECs was significantly increased ($p<0.05$, $p<0.01$ $p<0.0001$ corresponding to (10, 50 and 100) ng/ml)). IHC findings of

endometriotic lesions showed significantly increased Proliferating cell nuclear antigen (PCNA) and cluster of differentiation (CD)-31 staining in rIL-33 treated compared to control. PCNA immunolocalization was widely dispersed whereas quantitative analysis documented with an increased trend of CD-31 in treated mice, however, not statistically significant ($p = 0.09$).

Limitations, reasons for caution: Syngeneic model of endometriosis may not be a true reflection of human condition. Additionally, influence of intrinsic estrogen along with evaluation of short soluble form of ST2 (sST2), mature IL-33, and complexes of sST2 and IL-33 haven't been done which may increase the understanding of IL-33-related pathophysiology in endometriosis.

Wider implications of the findings: Whether IL-33 is involved in endometriosis alone or in combination with other factors is unclear; however, this molecule is possibly a potential biomarker and novel therapy target for managing endometriosis. Furthermore, the inhibitor of IL-33 (CNTO-7160), currently examined in clinical trials, may possibly be developed as new therapy for endometriosis.

Trial registration number: Not applicable

P-325 INFLUENCE OF TUBAL PATENCY ON ENDOMETRIOSIS RECURRENCE

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Study question: Does bilateral non-patency or absence of fallopian tubes decrease the recurrence rate of endometriosis?

Summary answer: Lower endometriosis recurrence rates were observed in women with bilateral occlusion/absence of the fallopian tubes after endometriosis surgery, although differences were not statistically significant.

What is known already: Despite effective surgical treatment for endometriosis, disease recurrence remains an important issue with incidences ranging from 5% to 25% more than two years after treatment. The exact pathogenesis of neither endometriosis, nor endometriosis recurrence is known. However, retrograde menstruation may play an important role according to Sampson's transplantation theory. Our study hypothesis is that non-patency of both fallopian tubes (because of surgical removal or by disease) and therefore absence of retrograde menstruation will lead to lower rates of recurrence of endometriosis. If this hypothesis would be confirmed, this could be an indirect confirmation of the transplantation theory.

Study design, size, duration: Retrospective 2:1 matched case control study on data of 895 women who underwent laparoscopic treatment for endometriosis between 2010 and 2014. From these 895 women, 49 had bilateral non-patent or absent fallopian tubes. They were compared with 98 matched controls with at least one patent fallopian tube.

Participants/materials, setting, methods: All women underwent complete laparoscopic excision of any rASRM-stage endometriosis in a tertiary referral center. Matching was based on relevant determinants of recurrence (e.g. rASRM-stage, postoperative treatment. . .). Primary outcome was the recurrence rate over a mean duration of 20 months (range 0-83 months). Recurrence was analyzed on 3 levels: symptom recurrence, recurrence on imaging and need for reintervention. Linear mixed models were used for continuous variables, logistic regression was used for binary variables.

Main results and the role of chance: Analysis showed a general trend towards lower recurrence rates in patients with non-patent fallopian tubes, however these findings failed to be significant. The majority of patients in both groups were treated with hormonal therapy after surgery to prevent recurrence (58.2% of the control group vs 59.2% in the study group).

72% of the patients with child wish in the control group and 80% of the patients with child wish of the study group underwent an assisted reproductive treatment. Postoperative pregnancy rates for patients with child wish were similar in both groups i.e. 53.2% in the control group and 65.6% in the study group.

Symptoms reoccurred in 30.6% of the control group (n=30) and 20.8% of the study group (n=10). Recurrence was confirmed by imaging in 7.14% of the control group (n=7) and in none of the patients of the study group.

12 patients of the control group and 3 patients of the study group needed reintervention over a period of 83 months. In those undergoing reintervention, recurrence of endometriosis was confirmed by anatomopathological examination in 5 patients of the control group and in none of the patients of the study group.

Limitations, reasons for caution: A retrospective file study may over/underestimate recurrence rates, although this is applicable for both groups. Only a small number of women could be selected for matched analysis (even with 2:1 matching-ratio), due to the low prevalence of bilateral tubal problems (49/895 women or 5.5%), which may result in a type-II-error.

Wider implications of the findings: If the trend observed in our study could be confirmed more strongly in a future larger study, this indirectly confirms the transplantation theory of Sampson. In that case, bilateral tubal occlusion/resection may become a future option for prevention of endometriosis recurrence in women who have completed their family.

Trial registration number: Internal institutional study number: S59032

P-326 Biophysical and molecular characterization of endometrial exosomes as non-invasive biomarkers of physiological modifications during implantation window

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Study question: Does exosomal profile of different endometrial surrogate biofluids is a non-invasive biomarker source for guiding a successful embryo implantation?

Summary answer: The evaluation of the endometrial exosomes (EVs) isolated from the surrogate biofluids, indicates EVs as possible bio-vectors to depict a typical receptive endometrial profile.

What is known already: The implantation window is mainly detected by monitoring the hormonal profile and with invasive methods requiring an endometrial biopsy, followed by histological or mRNA expression profile testing. To regulate blastocyst development and modulate the embryo-endometrial cross talk, the endometrium releases several factors in uterine fluid. Some of these are sorted from cytoplasmic endosomal compartments into secretory EVs and are thus delivered to target tissues in a paracrine and autocrine manner. EVs contain and transport several molecules that include a wide range of proteins, lipids and nucleic acids, according to their biogenesis, biophysical properties, surface proteins, transported content and biological role.

Study design, size, duration: Different biofluids such as uterine flushes (UF), cervix brush (B), urine (U) and serum (S) were collected from women volunteers with proven fertility after signing an informed consent, from February 2017 to September 2018. All biofluids were collected at different times after the LH surge. EVs isolation and molecular analyses were performed by researchers blinded to the study.

Participants/materials, setting, methods: UF, B, U and S were scheduled in LH+3/LH+5/LH+7/LH+9, from women volunteers with proven fertility (n= 14) at the Couple Sterility Centre, Siena University Hospital and IMER, Clinica Fertilidad, Valencia. EVs were isolated by chemical precipitation and then the concentration and size distribution in the different EVs subpopulations were determined with Nanoparticle Tracking Analysis (NTA). Transmission Electron Microscopy (TEM) and immunoelectron microscopy as well as RTq PCR, TaqMan miRNA arrays and Elisa have been applied.

Main results and the role of chance: Exosomes largely contribute to a proteome of UF, as indicated by exosome associated CD9 levels. Relative EVs content maintained the same pattern. when compared across the cycle phases, while an increase of EVs was detected in B sample in advanced cycle phases.

TEM analysis via negative staining confirmed ELISA data, Immunogold further confirmed the identity of EV-like structure by detection of EV markers like tetraspanins, CD9, CD63, Flotilin1. The profile of EV RNA confirms a fair recovery of RNA from B and UF samples, with the typical profile attributed to EV RNA, enriched in small RNA portion. A consistent expression of endometrial markers, namely PAEP (glycodelin A) and receptors for estrogen and progesterone, (ESR1, PGR) has been detected in vesicles recovered from UF, confirming that EV encapsulated RNA holds the advantage of the stability and traceable tissue origin. We can overall conclude that both measurement of a vesicle number, of a specific EV protein and a mRNA content confirms the efficient recovery of EVs suitable for the analysis of either protein and RNA composition from all the samples, in particular from UF that is confirmed to be most relatively enriched with EVs

Limitations, reasons for caution: A larger cohort of patients/samples may be useful to validated the data. Moreover, the molecular crosstalk between the embryo and human endometrium involves a complex molecular network, therefore embryo derived exosomes must be taken into account and integrated with the present results concerning the maternal influence.

Wider implications of the findings: A totally non-invasive diagnostic method, limiting as much as possible the stress of the patients undergoing assisted reproductive techniques, could be based on the precise definition of the exosomal profile of the receptive endometrium, depicting the expression of validated biomarkers, for guiding a successful embryo implantation.

Trial registration number: None

P-327 Organoids of human endometrium: is this a powerful 3D culture model in replicating the endometrial glandular epithelium?

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Study question: Does a three-dimensional culture model of endometrial glandular organoids mimic the uterine microenvironment at the maternal-foetal interface?

Summary answer: Organoids replicate endometrial epithelium, including its glandular-type organization. They reproduce functional behaviour such as responsiveness to the core regulatory hormones, modulating Glycodelin A (GdA) expression

What is known already: Due to ethical concerns, most of knowledge on mechanisms underlining human implantation derives from animal models, but they cannot always be translated to humans. Therefore, the development of an *in vitro* model allowing the opportunity to study the endometrial microenvironment and to investigate molecular mechanisms underlying the implantation process, is desirable. Nowadays, three-dimensional culture gained increasing interest and considerable importance has been ascribed to uterine glands and their secretions. In this regard, few studies addressed the expression and the glycosylation pattern of GdA, one of the major glycoprotein synthesized by endometrium, strongly modulated during the menstrual cycle.

Study design, size, duration: Endometrial biopsies have been collected from healthy and endometriosis-affected women, who underwent laparoscopic surgery from january to october 2018. The epithelial cells were isolated and 3D culture was applied to obtain organoids. Morphology, ultrastructure as well as GdA expression profile have been evaluated, both in unstimulated condition and after hormonal treatments able to induce the morphological and molecular modifications typical of the proliferative and secretive phase of the menstrual cycle

Participants/materials, setting, methods: Endometrial specimens have been collected from women of proven fertility (n=3) and endometriosis-affected patients (n=3). Obtained epithelial cells organoids were exposed for 4 days to estrogen alone or in combination with progesterone and cAMP, mimicking the proliferative and the secretory phase, respectively. Then, organoids were fixed and processed for TEM analysis or solubilized and analysed by 2D-electrophoresis followed by immunoblotting with a GdA-antibody or fixed and treated for immunofluorescence analysis using the same antibody.

Main results and the role of chance: Our data highlight that organoids obtained from human endometrial epithelial cells represent a good model to study the physiological function of the endometrial epithelium. TEM analysis showed that organoids preserve glandular organization as well as ultrastructural characteristics. Moreover, organoids retain the responsiveness to hormone treatment mimicking the in vivo glandular aspect and functions specific of the corresponding phase of the menstrual cycle. This was demonstrated by the analysis of GdA, a cycle-dependent marker of endometrial receptivity, that resulted to be expressed in cultured organoids with the same pattern detected in the endometrium in vivo, thus confirming the cycle-dependent modifications typical of this glycoprotein in epithelial endometrial cells. Finally, the GdA glycosilation pattern in organoids isolated from eutopic endometrium of endometriotic women significantly differs from that detected in healthy organoids, confirming data from endometrial tissues and supporting the idea that GdA deregulation may be a typical feature of this pathology

Limitations, reasons for caution: Larger study needs to validate these findings. Organoids seem to mimic endometrial tissue modifications dependent on cycle phase, even in the absence of endometrial mesenchymal compartment. Nevertheless, co-culture with endometrial stromal cells is advisable, to investigate these complex molecular pathways in light of reciprocal epithelium-stroma interactions operating in the tissue

Wider implications of the findings: We describe an endometrial glands 3D culture closely recapitulating molecular and functional characteristics of their cells of origin. This model could represent a valuable research tool to study implantation in vitro, and to test therapies for endometrial pathologies or implantation problems, allowing the possibility to build up patient-specific biobank resurces

Trial registration number: Not applicable

P-328 Upregulation of ROS genes may contribute to poor oocyte quality in women with endometriosis.

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Study question: Are reactive oxidative species (ROS) genes upregulated in women with endometriosis contributing poor oocyte quality?

Summary answer: ROS genes and genes generating a response to ROS are upregulated in women with endometriosis

What is known already: Endometriosis is a chronic inflammatory disease which leads to infertility. Infertility in women with the endometriosis may be due to poor oocyte quality. A number of mechanisms are associated with oocyte maturation may be disrupted in women endometriosis thereby impacting oocyte quality. A balance between the ROS and antioxidants is essential for oocyte maturation and quality. This balance is dysregulated in women with endometriosis. Reactive oxidative species genes may be upregulated in women with endometriosis.

Study design, size, duration: A meta-analysis was conducted on data from 17 studies to compare women with and without endometriosis between 25 November 2018 and 30 December 2018.

Participants/materials, setting, methods: Suitable studies and data identified from electronic databases and gene expression repositories. Peer reviewed, publicaly available studies investigating genetic expression of endometrial tissue from premenopausal women with endometriosis were included. Studies had individual participant gene expression data and published gene lists available. Dysregulated gene expression was determined through meta-analyses of microarray data. Data analysis was conducted by GeneSpring version 4.0.4. T-test using P value <0.05 and fold-change expression >1.5 was applied for statistical significance.

Main results and the role of chance: This study has shown that in eutopic endometrium of women with endometriosis, genes involved in generation of reactive oxidative species and the genes involved in generating a response to oxidative stress were significantly upregulated.

Limitations, reasons for caution: The limitation of the study may be the sample size.

Wider implications of the findings: Upregulation ROS genes likely contributes to poor oocyte maturation and quality in women with endometriosis. Increased ROS may lead to decreased antioxidants levels. Increased oxidative stress leads to DNA damage, meiotic abnormalities and chromosomal

instabilities, impairing oocyte quality. IImpaired oocyte quality may contribute to infertility in women with endometriosis.
Trial registration number: NA

P-329 Uterine immune profiling and endometriosis: any particularities in patients with repeated implantation failures?

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Study question: Do patients with endometriosis and repeated implantation failures (RIF) exhibit any specificity regarding their uterine immune profile compared with RIF patients with male infertility ?
Summary answer: Patients with endometriosis and a RIF history show some immune particularities regarding their endometrial immune profile when compared with RIF patients with male infertility.
What is known already: Despite extensive studies, the pathogenesis of endometriosis is still perceived as complex and unknown and the pathogenesis of infertility associated with endometriosis remains elusive. The involvement of the endometrium itself as an actor of embryo implantation failures in patients with endometriosis is still a matter of debate.
Study design, size, duration: A Case – Control cohort analysis compared the endometrial immune profile among RIF patients with endometriosis (n=176 patients) versus male infertility (523 patients) between 2012 to 2017. The endometrial immune profiling details the Th-1/Th-2 immunoregulated equilibrium, as well as the maturation and activation state of uterine NK cells. A classification in four types of uterine immune profiles is established to detail the local equilibrium: normal, over-activated, low activated, mixed profiles.
Participants/materials, setting, methods: The immune profiling is a diagnostic method detailing from an endometrial biopsy collected in the mid luteal phase the uterine natural killer cells (uNK) mobilization/ activation/ maturation state as well as the local immunoregulated equilibrium between the Th-1 (cytotoxic)and the Th-2 (angiogenic/ immunotrophic) cytokines. The biomarkers IL-15/Fn-14 (maturation and hyper-activation state of uNK) and IL-18/TWEAK (Th-1/ Th-2 equilibrium) mRNA ratios were determined by quantitative RT-PCR and CD56 mobilization per Immunochemistry.
Main results and the role of chance: The global repartition of the distinct immune profiles was not significantly different among endometriosis or male RIF patients (p=0.07). However, patients with a low-activated immune profile were significantly over-represented in the endometriosis RIF group (33% versus 23% respectively, p=0.03). More local depletion of Th-2 cytokines (p=0.03) associated with an higher rate of immature uNK cells (p=0.03) were observed in the RIF endometriosis group when compared to the RIF male group.
Limitations, reasons for caution: The observed differences were based on the retrospective analysis of the two groups and could not exclude some bias.
Wider implications of the findings: As patients with endometriosis show some immune particularities, personalized measures based on the precise documentation of their uterine immune profile may be beneficial in order to promote embryo implantation.
Trial registration number: not applicable

P-330 Neuropathic endometriosis-associated pain: Can we subgroup women on the basis of their sensory descriptors?

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Study question: In neuropathic endometriosis-associated pain, do patients cluster on responses to PainDETECT questions? If so, are there clinically meaningful differences between these groups?
Summary answer: Two clusters are identified, with strength of abnormal sensations having importance in prediction. These clusters differ in psychological profile and pain intensity scores.
What is known already: Endometriosis-associated pain has been traditionally thought of as arising from peripheral mechanisms in the pelvis. However, it is only recently that the potential role of neuropathic pain has been considered. Our previous work suggests that 75% of women with endometriosis-associated pain have a neuropathic or mixed neuropathic/nociceptive component to their pain. We now investigate if there are clusters within this group which could be used to further stratify patients for optimum treatment.
Study design, size, duration: A novel questionnaire was created using the Bristol Online Survey Tool. It included painDETECT^{TM} (a measure of neuropathic pain) as well as other validated questionnaires. The survey was distributed to women with endometriosis to complete online through patient support groups (approved by the Central University Research Ethics Committee, University of Oxford). 1615 responses were received, with 1444 meeting the inclusion criteria of having had at least one laparoscopy and a diagnosis of endometriosis.
Participants/materials, setting, methods: painDETECT^{TM} was used to group participants into nociceptive, mixed and neuropathic pain groups (nociceptive $\leq$12, mixed 13-18, neuropathic $\geq$19). Only mixed and neuropathic groups were further analysed here (n=904).

Data were computed using SPSS. Two-step cluster analysis with Schwarz Bayesian Criterion (BIC) was used to determine the number of clusters. Responses to painDETECT^{TM} questions were used as classification variables. Subsequent group analysis was performed, using Mann-Whitney U tests.
Main results and the role of chance: Two major clusters were identified. For both clusters the variable which had the greatest predictive importance was 'Do you have a tingling or picking sensation in the area of your pain (like crawling ants or electrical tingling)?' (importance=1.0), followed by 'Do you suffer from a burning sensation (e.g. stinging nettles) in the area of your pain?' (importance=0.88), both of which indicate abnormal sensations in the area of pain. All other questions had predictive importance <0.2. Cluster 1 (n=388, 39.7%) had stronger responses to both these questions, with 'strongly' being the most common response for both questions. For cluster 2 (n=590, 60.3%) the most common response was 'slightly' for both questions.

Group comparisons demonstrated that those in cluster 1 have significantly higher scores for: depression (Beck's Depression Inventory); anxiety (Trait Anxiety Inventory); catastrophising (Pain Catastrophising); dysmenorrhea, dyspareunia and non-cyclical pain (Numerical Rating Scale) ($p<0.006$ corrected). Further analysis, limiting to only those with definite neuropathic pain (n=573), the same questions are identified as having predictive importance in clustering.
Limitations, reasons for caution: Participants self selected from patient support websites, potentially producing a biased sample.
Wider implications of the findings: These results suggest that the presence/absence of abnormal sensations in women with neuropathic endometriosis-associated pain may be of particular clinical relevance. A better understanding of the mechanisms generating these sensations and potential therapeutic options is therefore urgently needed to improve quality of life for these women.
Trial registration number: Not applicable.

P-331 Free 25(OH)-vitamin D and vitamin D binding protein are potential biomarkers for leiomyoma and are associated with the leiomyomas size

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Study question: Are there associations between serum levels of vitamin D binding protein (DBP) and free 25(OH)-vitamin D with leiomyoma and the lesions characteristics?
Summary answer: Free 25(OH)-vitamin D and DBP had respectively positive and negative correlations with the leiomyomas size and could be potential biomarkers for leiomyoma.

What is known already: Role of vitamin D particularly its free form and also DBP as the modulator has been demonstrated in tumor progression. Moreover, the association of the blood levels of total vitamin D with the prevalence of uterine leiomyoma has been reported. Furthermore, the inhibitory effect of vitamin D on leiomyoma formation and progression has been documented. Previous studies also showed significantly lower and higher levels of respectively vitamin D and DBP in the blood of women with leiomyoma compared to healthy subjects.

Study design, size, duration: Leiomyoma was diagnosed using transabdominal ultrasound screening and confirmed by histological analysis. In the patients, the number, size and location (Intramural, Subserosal, Transmural, Submucosal, Intramural- Subserosal, and Intramural- Submucosal) of the leiomyomas were determined.

Participants/materials, setting, methods: Forty-four leiomyoma patients aged 35.32±6.15 and forty-one healthy women with age of 32.69±6.44 were enrolled in this study. In all the participants, serum levels of total 25(OH)-vitamin D and DBP were measured using commercial ELISA kits. Furthermore, serum levels of 25(OH)-vitamin D/DPB ratio and free 25(OH)-vitamin D were calculated.

Main results and the role of chance: Levels of 25(OH)-vitamin D/DPB ratio and free 25(OH)-vitamin D were significantly lower in patients group compared to the controls ($p<0.001$). We also found statistically higher levels of DBP in the patient with leiomyoma in comparison with the healthy women ($p<0.001$). There was no significant association between the evaluated serum factors with the location and number of the leiomyomas ($p>0.05$). However, the size of the largest leiomyomas had negative correlations with 25(OH)-vitamin D/DPB ratio and free 25(OH)-vitamin D levels and a positive correlation with DBP. Based on receiver operating characteristic (ROC) analyses, cutoff values for free 25(OH)-vitamin D and DBP as markers for leiomyoma diagnosis were 4.36 (pg/ml) and 251.7 (µg/ml) with sensitivity 78% and 88% and specificity of 76.7% and 71%, respectively.

Limitations, reasons for caution: Our study had a relatively small sample size.

Wider implications of the findings: Free 25(OH)-vitamin D and DBP had a role in leiomyoma pathogenesis and were associated with the size of leiomyomas. Serum levels of these factors can be used as biomarkers for the leiomyoma diagnosis.

Trial registration number: not applicable

P-332 The effect of Thymoquinone on experimental endometriosis rat model

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Study question: To evaluate the effect of Thymoquinone (TQ) in a rat model of surgically induced endometriosis.

Summary answer: TQ significantly regressed endometriotic implants and decreased immunoreactivity for VEGF.

What is known already: Endometriosis incidence is a chronic disease. Although endometriosis is one the most investigated disorders of gynecology, it's pathogenesis is still unclear. Investigations have demonstrated a role for immune dysregulation, oxidative stress and angiogenesis in endometriosis. TQ is the principle active ingredient of Nigella Sativa seeds. TQ as a natural agent, has become a favourable compound in studies due to its potent anti-inflammatory, anti-oxidant, antiangiogenic and immune enhancement capacity. TQ, has attracted much scientific attention in recent years for its high biological activity with low toxicity. Therefore, this preliminary study was designed to evaluate the effect of TQ on surgically-induced endometriosis.

Study design, size, duration: This was an experimental animal model study. Fourty-four female rat with induced endometriotis were diveded in 4 groups. Group 1; 20 mg/kg TQ , group 2 ; 1mg/kg subcutaneus single dose leuprolide acetate, group 3; 1mg/kg LA and 20 mg/kg TQ , and group 4;control. After four weeks of medication rats were sacrificed and size, histopathology and immunreactivity to vascular endothelial growth factor (VEGF) of the endometriotic implants and ovarian follicular counts were evaluated.

Participants/materials, setting, methods: The formalin- fixed tissues (endometriotic foci and ovaries) were embedded in paraffin blocks and stained with H&E after being sectioned to 5 µm thickness. Tissue slides were evaluated by a blinded pathologist to determine endometriosis score by the persistence of epithelial cells in endometriotic implants. The immunohistochemical staining of endometriotic foci was evaluated by the sme pathologist blind to the groups using semiquantitative method.

Main results and the role of chance: The post-treatment volumes of implants in groups 1, 2, and 3 were significantly less than those of group 4 ($p<0.001$). Post- treatment implant volumes in group 1, 2, and 3 were comparable to eachother ($p>0.05$). The histopathological scores of the implants were compared between the groups. Immunohistochemical staining of implants to evaluate VEGF revealed significant reduction in scores in group 1, 2, and 3 when compared to group 4 ($p<0.001$). The lowest score was noted in group 3 which was comparable to that of groups 2 and 3 ($p>0.05$). Antral follicle count in TQ group was comparable that of control group in contrast to LA group.

Limitations, reasons for caution: The study was limited by its design as an experimental rat model, which may have limited applicability to human. Besides, we examined only VEGF expression on the endometriotic implants which may also be a limitation

Wider implications of the findings: Our study demonstrated for the first time the TQ significantly regressed endometriotic implants and decreased immunoreactivity for VEGF without affecting ovarian follicle numbers.

Trial registration number: not applicable

P-333 Analysis of mi20a_1, mi145_1 and 320a_1 levels in follicular fluid of endometriosis patients. Their role as putative biomarkers for outcome of assisted reproductive technology (ART).

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Study question: Could levels of mi20a_1, mi145_1 and 320a_1 in follicular fluid be used as biomarkers for fertility outcome in endometriosis patients?

Summary answer: The studied miRNAs increased in patients with endometriosis relatively to controls, suggesting their use as markers for assessment of outcome of assisted reproductive technology (ART).

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. Globally, endometriosis affects up to 40% of infertile women.

Endometriosis associates to an imbalance in microRNAs produced in eutopic and ectopic endometrial tissue that affects disease progression, angiogenesis and reproductive condition. MicroRNAs are found in Follicular Fluid (FF) and are easily collected from patients under fertility treatments. In this setting, the analysis of specific microRNAS in the FF from women with endometriosis could represent a promising approach to identify biomarkers with application in diagnosis and prognosis of fertility treatments.

Study design, size, duration: We used FF obtained from women aged 26-42 years (n=32) submitted to medically ART and divided in two groups according to the clinical infertility diagnosis: endometriosis and controls (male factor infertility). FF samples were assigned by CETI (Centro de Estudos e Tratamento de Infertilidade) – Porto, Portugal.

Participants/materials, setting, methods: Total MicroRNA were extracted from Follicular Fluid using miRNA easy mini kit. MicroRNAs 20a_1, 145_1, 320a_1 were quantified using RT-PCR. To compare the study variables, student t-test was used.

Main results and the role of chance: An increase of mi20a_1, mi145_1 and mi320a_1 was found in FF from women with endometriosis comparing with healthy women. The expression of mi20a_1 was found 4,5 times increased in the samples from endometriosis, comparing with controls (p=0,037); mi145_1 was 2,85 times higher in the samples from endometriosis, comparing with controls (p=0,003) and mi320a_1 was 4,5 times higher in the samples from endometriosis, comparing with controls (p=0,006). While mi20a is involved in hypoxia, angiogenesis, cell proliferation mechanisms, mi145_1 is involved in cell proliferation, and mi320a intervenes in the inhibition of cell proliferation, migration and invasion and may also be involved in embryo quality. The upregulation of mi320a may constitute a compensatory mechanism that counteracts the up regulation of mi20a and mi145_1, that favours the endometriosis pathology.

Limitations, reasons for caution: Despite the strong increase in mi20a_1, mi145_1 and mi320a_1 found in FF of patients with endometriosis, further investigation is needed to validate this data, including an increase of the number of studied women. As well, the correlation of levels of the studied microRNAs with ART success is ongoing.

Wider implications of the findings: The findings involving specific microRNAs help to refine diagnostic tools to be employed in FF analysis during ART. The increase of mi20a_1, mi145_1 and mi320a_1 in FF from women with endometriosis favors their ability to be accounted as biomarkers for pregnancy outcomes, after ART treatments.

Trial registration number: not applicable

P-334 INTRA -UTERINE INSTILLATION Versus SUBCUTANEOUS-INJECTION OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) BEFORE EMBRYO-TRANSFER IN RESISTANT THIN ENDOMETRIUM IN IVF-ICSI CYCLES: A comparative study

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Study question: Whether intrauterine administration of G-CSF (granulocyte colony-stimulating factor) prior to Embryo Transfer in patients with resistant thin endometrium yield better pregnancy rates than subcutaneous injection of G-CSF?

Summary answer: Intrauterine administration of G-CSF (granulocyte colony-stimulating factor) prior to ET in patients with resistant thin endometrium yields better pregnancy rates than subcutaneous injection of G-CSF

What is known already: Endometrial thickness is an important index evaluating endometrial receptivity .Endometrial thickness below a threshold is associated with implantation failure and reduced pregnancy rate . Many therapies have been attempt to enhance the endometrial thickness and improve the endometrial receptivity, such as extending estrogen administration , low-dose aspirin , combination pentoxifylline and tocopherol , vaginal sildenafil citrate , and stem cells treatment . These treatments have improved endometrial receptivity and increased implantation and pregnancy rate in ART cycles in some extent. However, many cases still remain unresponsive.Lately, granulocyte colony-stimulating factor (G-CSF) has been used in the treatment of thin endometrium.

Study design, size, duration: 112 Infertile patients with thin endometrium below 40 years age (2016 to 2018) were included in this study , in whom traditional treatment with estradiol, sildenafil etc had been unsuccessful.

Patients were randomly divided into two groups using computer generated list after proper consent .The study group received intrauterine & Subcutaneous G-CSF before Embryo Transfer in IVF-ICSI cycles.

Primary Outcome : Endometrial-thickness , Implantation & Pregnancy rates.

Secondary-outcome: Abortion-rate

Participants/materials, setting, methods: One group (n = 56) received intrauterine infusion of 300 microgramme / 1ml of G-CSF, and the other group (n = 56) underwent sub-cutaneous before Embryo-Transfer.

G-CSF was administered by an intrauterine catheter by slow instillation on the day of hCG administration.

If the endometrium had not reached at least a 7-mm within 48 h, a second dose was administered following oocyte retrieval.

Main results and the role of chance: G-CSF administrated via uterine infusion resulted in significantly increased Pregnancy Rates , whereas G-CSF administrated subcutaneously had less effect on the Pregnancy Rates.

The IR and PR were statistically significantly higher in the group receiving intrauterine G-CSF (18% and 32%, respectively) as compared to the subcutaneous group (10% and 16%, respectively).

Abortion Rates were comparable in both the groups and difference was not significant.

Intrauterine instillation of G-CSF is more effective in expanding chronically unresponsive thin endometrium, as compared to sub-cutaneous injection.

This treatment needs more multicentric trials to assess its potential in improving the implantation chances in IVF-ICSI cycles , thereby improving pregnancy rates.

Limitations, reasons for caution: Limitations to this study are the small sample size, and less number of cycles .

Wider implications of the findings: The ideal route of G-CSF administration has not been identified yet.

Studies have used G-CSF via either subcutaneous injection or intrauterine infusion.

In order to explore which route was better, we evaluated the effect on outcome with different routes of G-CSFWe found that G-CSF administrated intrauterine resulted in significantly increased results.

Trial registration number: not applicable

POSTER VIEWING
ETHICS AND LAW

P-335 Surrogacy in the Czech Republic from the point of view of the law office: experience for the years 2009-2018

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Study question: *What is the interest in surrogacy in the Czech Republic, who are the applicants and who are surrogate mothers?*

Summary answer: Interest in surrogacy in the CR is steeply growing, with growing number of non-standard applicants and surrogate mothers in a difficult financial situation.

What is known already: The Czech Republic has ratified the Convention on Biomedicine**,** the human body must not be a source of financial profit. Surrogacy is not legally regulated, it is carried out. The change of legal parenting is done through adoption. According to the recommendation of the Czech

Society for ART, surrogacy should be done solely for health reasons, donated gametes should not be used, the surrogate mother should be a citizen of the CR, she should have a recommendation of a gynecologist, practitioner and psychologist. Czech IVF centers generally accept only applicants who have evidence of consultation with a lawyer.

Study design, size, duration: Cross-sectional study, 330 serious candidates for surrogate motherhood who underwent a legal consultation between 2009 and 2018.

Participants/materials, setting, methods: Applicants for surrogacy who approached one of the very few Czech law offices dealing with this issue, with a request to consult the procedure, or the legal representation in the adoption process. In the documentation we looked back at data about the number of clients, some sociodemographic characteristics, or the continuation of the cooperation between the client and the law office. The data processing method is a basic retrospective descriptive statistics.

Main results and the role of chance: The interest in surrogacy is steeply increasing, from single candidates in 2009 to several candidates a week in 2018. Early on, only Czech heterosexual couples in reproductive age with medical fertility disorder appeared, at the moment there are also homosexual couples or individuals, as well as applicants who are not Czech citizens. Altogether 330 clients were interested in the procedure, mostly heterosexual couples. Increasingly, the applicants are less wealthy. Intrafamiliar surrogacy is rare, applicants with mothers are mostly contacted via the Internet. There is a growing number of candidates to surrogate mothers in a difficult financial situation. From 2009 to 2018, 103 children were handed over. There is one case in the CR where the disabled child was nothanded over.

Limitations, reasons for caution: We consider the study to be representative, other Czech law offices have probably not addressed the issue yet. However, it looks like the issue attracts interest of other Czech law officess.

Wider implications of the findings: *Lack of regulation can lead to trafficking in children and women. It is necessary to carry out an analysis of the motives of surrogate mothers.. This should be followed by the legislation of surrogacy, with taking into account that the CR is the target country for cross border care.*

Trial registration number: not applicable' for non-clinical trials.

P-336 Policy recommendations for queer and trans reproduction with Assisted Reproductive Technologies in Europe

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Study question: How can the access to Assisted Reproductive Technologies (ARTs) be improved for queer and trans people in six purposely

selected European countries (Austria, Estonia, Poland, Spain, Sweden, the UK)?**Summary answer:**

Improvements are needed in: providing information, change legal regulations for ARTs, for obtaining parenthood and citizenship, and for gender recognition, LGBT-training for medical staff.

What is known already: Queer, as in non-heterosexual relationships, and transgender kinship and family structures are increasingly formally recognized, and queer and trans research on ARTs is an established field (mostly in English-speaking countries, less in central and eastern Europe) and has demonstrated that a range of subjects use them.These studies show that one social group particularly affected by biotechnological and legislative changes around ARTs are queer and transgender people and that they are making use of cross-border reproductive care, which brings often challenges for obtaining legal parenthood and citizenship for their child.

Study design, size, duration: This is a participative, multi-method research project (2 years duration), drawing on a range of interconnecting qualitative sociological, ethnographical, feminist,queer, and philosophical (ethical) methods. It thus reflects the interdisciplinarity of the knowledge domains on which this project is based, where qualitative (interviews, focus groups, content analysis, discourse analysis, use of CAQDAS) but also quantitative (surveys) methods are used. So far 901 participants took part in the online-survey, interviews and focus groups.

Participants/materials, setting, methods:

Participants are self-defined queer and trans people (older than 21 years) who want to use or have used ARTs.

Methods/Materials: Literature review, document and content analysis of the ART guidelines in laws and recommendations show the differences between the 6 chosen EU-countries concerning ART and LGBT policies. Through purposive sampling data of the experiences of queer and trans people with ARTs is/was gathered with an online-survey, interviews and focus groups.

Main results and the role of chance: The main results of my Marie-Skłodowska Curie project (Horizon2020) "QTReproART -Towards an Inclusive Common European Framework for Assisted Reproductive Technologies (ART): Queer & Transgender Reproduction in the Age of ART" are policy recommendations and guidelines for the improvement of access to ART for queer and trans people in Europe, which cover the following areas: providing information, change legal regulations for ARTs, for obtaining parenthood and citizenship, and for gender recognition, LGBT-training for medical staff. A general recommendation for the EU is the main result, as well as individual results for each of the six selected EU-states. The details of the policy recommendations are presented at the conference, as the analysis of the collected data is still work in progress at the time of the submission of this abstract.

Limitations, reasons for caution: Not applicable.

Wider implications of the findings:

The findings of my project are mostly in agreement with studies on queer and trans reproduction so far, but my findings contain a more detailed analysis of different factors (e.g. where to find information about ART access for queer and trans people, legal challenges, treatment by medical and psychological staff).

Trial registration number: Not applicable.

P-337 Reproductive rights: status & future?

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Study question: What is the current human right status for the right to procreate, and which legal principles/developments are currently driving liberalization?

Summary answer: Human rights recognition of a right to procreate lacks, however increased marketization and due legal concern for children's rights has improved the legal status.

What is known already: International and regional human rights instruments protect the "right to found a family", the "right to respect for private and family life" and the right to have disease addressed through the "right to health" Yet, a right to procreate utilizing ART has not been recognized. However, when a child is born the best interests of the child shall be a primary consideration, enabling users of cross-border treatment to pursue legal recognition of their private and family life upon their return, by emphasizing the right of the child.

Study design, size, duration: Analysis of relevant Danish statutory law, Danish judgments and administrative decisions, human rights conventions and judgments.The project combines a dogmatic legal methodology with a sociolegal analysis of legal genealogy (Foucault 1980). Hence, the status for reproductive rights is expounded and it is demonstrated how subversive practices (cross-border reproductive care, and "activist"/reproductive justice practices meant to challenge prohibitive legislation) can drive legal development and effectively create an increased legal status for the right to procreate.

Participants/materials, setting, methods: Statutes, conventions, judicial and administrative decisions. The material is extensive – includes the legislative material regulating assisted reproduction as well as human rights, children's rights, international private law, EU law, and family and adoption law. Also, data retrieved from health authorities, patient organisations and professional societies describing the expansion in use of domestic and cross-border reproduction is included.

Main results and the role of chance: In Denmark, expansion and liberalization of reproductive rights is currently driven by the marketization of infertility care which allows for both cross-border circumvention of domestic regulation and allows for subversive practices at a scale that eventually drives regulatory response or that are designed to actively test the limits of the law by litigation. We demonstrate this using examples from Danish law and legal practice. Moreover, this development is not isolated to Denmark, but illuminate what is likely a European trend.

The status for reproductive rights is that to a high extend liberalization comes through the back door. For those who do not have the resources for cross-border care or for testing the law the improvements are smaller.

Limitations, reasons for caution: not applicable

Wider implications of the findings: Reproductive rights have been on the human rights agenda for 70 years yet the current human rights conceptualization of reproductive rights has seems to have reached the end of its evolutionary capacity. As the marketization of fertility care increases, so does the legal basis for bringing reproductive rights forward.
Trial registration number: not applicable

P-338 Egg providers' views on the use of surplus eggs in the UK, Spain and Belgium: implications for information giving and informed consent

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Study question: How do women who provide eggs for use in fertility treatment perceive practices related to the use, storage and distribution of surplus eggs?
Summary answer: Egg providers expressed concerns about the potential ways surplus eggs might be used following donation, suggesting that processes of information giving may need improvement.
What is known already: Within Europe, donor eggs are used in over 56, 000 cycles of fertility treatment per year. Vitrification allows clinics increased flexibility in the management and storage of eggs and permits separation of the traditional link between donor and recipient since technically they can be stored indefinitely. Vitrification and banking also increase possibilities for commercialisation and movement of 'surplus' eggs (i.e. eggs remaining after the initial recipient has completed their first cycle of IVF). Existing research tends to focus on egg providers' motivations and experiences, but little is known about their views of the use, storage and export of vitrified eggs.
Study design, size, duration: This paper is part of a larger, multi-phased comparative study (the 'EDNA' study, 2017-2020), which explores egg provision in the UK, Belgium and Spain, using multiple and largely qualitative methods of data collection. A maximum variation sample of egg providers was recruited from a range of sites in each country including fertility clinics, egg banks and via social media. This paper draws on interviews conducted between April 2018 and January 2019.
Participants/materials, setting, methods: In-depth, face-to-face interviews with 68 egg providers (n=27 in the UK, n= 20 in Spain, n= 21 in Belgium) were conducted. Interviews included questions about personal experiences as well as the use of elicitation techniques designed specifically to generate data on values and principles relating to the use, storage, and export of surplus eggs. Interviews were fully transcribed and entered into NVivo for analysis using a systematic, thematic method.
Main results and the role of chance: Egg providers were asked to consider a range of scenarios relating to possible uses for surplus eggs. Participants expressed uncertainty and that they did not feel well informed about the possible uses of surplus eggs. Women in all three countries supported eggs being divided amongst several recipients, but a majority (n= 51, 75%) expressed negative values relating to the trade of surplus eggs between clinics for profit. Shipping eggs to another country generated variable preferences: in Belgium there was a positive response (76% agreed), whilst in the UK and Spain half of women felt unsure or that this should not be possible. The majority of women in all three countries (n=55, 81%) felt positive about the option to store surplus eggs for their own personal later use. Our findings also demonstrate a desire for explicit informed consent with regards to the use of eggs and in some cases, for direct involvement in future decision-making about surplus eggs, especially after longer periods of storage.

Role of chance:

Our qualitative findings are not intended to provide statistical generalisation but instead aim to provide in-depth understanding and theoretical inferences about egg providers' motivations, experiences and moral reasoning about contemporary egg donation practice.

Limitations, reasons for caution: Despite the careful construction of the interview guide, participants may have given socially desirable answers, particularly given the sensitive nature of the statements. Given the sample size and character, views from specific groups may not be well-represented.
Wider implications of the findings: Egg providers may have concerns regarding the use of surplus eggs by clinics. Information giving and the taking and ensuring on-going consent need to ensure that egg providers have a more complete understanding of the possible ways in which eggs may be used or stored for future use.
Trial registration number: Not applicable

P-339 Should long-term follow-up post-mitochondrial replacement be left up to physicians, parents, or offspring?

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Study question: The UK requires only a long-term follow-up 'plan' for each mitochondrial replacement (MR) case. Then, should the follow-up be left up to physicians, parents, or offspring?
Summary answer: Physicians and parents should take coordinated efforts for the practical and offspring-centred follow-up, while entitling the resultant youth to refuse follow-up.
What is known already: Due to the experimental stage, MR can fail to prevent serious mitochondrial disease in offspring, or can unexpectedly affect the first and subsequent generations. Accordingly, the need for long-term and even intergenerational follow-up of resultant offspring born via MR has been underscored. Although some suggested making parental consent to long-term follow-up a condition for undergoing MR, parents can withdraw consent. Eventually, a UK regulator only requires a long-term follow-up plan in each MR protocol. Thus, follow-up post-MR is not mandatory. Those raise the question of whether follow-up post-MR should be left up to physicians, parents, or resultant children.
Study design, size, duration: First, case reports of germline mtDNA modification were surveyed. Then, identified cases were investigated as to whether resultant children were followed up. Next, major mitochondrial diseases were compared regarding those pathologies. After the ethical assessment of MR for prenatally preventing mitochondrial disease, practical and humane long-term follow-up post-MR was explored using knowledge obtained. Finally, its wider implications were considered regarding the use of MR for treating intractable female infertility.
Participants/materials, setting, methods: PubMed survey of germline mtDNA modification reports with English abstract, published until April 2018, identified 14 relevant cases. There was only one report that addresses the follow-up of resultant children, namely one survey of children born via ooplasmic transfer. For the pathological comparison, myoclonus epilepsy associated with ragged-red fibers (MERRF), mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and Leigh syndrome were selected.
Main results and the role of chance: Long-term follow-up post-MR to safeguard children from serious mitochondrial disease should not simply be left up to physicians, parents, or the resultant children.

At clinics, physicians must carefully inform couples of the risks of MR and the importance of long-term follow-up tailored to a specific mitochondrial disease. Namely, according to the presumed timing of onset of MELAS and Leigh syndrome, the clinically minimum required period for follow-up can be approximately one or two decades. Regarding MERRF, the minimum required period ranges from childhood to around 40 years of age. Importantly, an adequate follow-up plan might extend several decades even in cases of MR for preventing MELAS and Leigh syndrome.

Given that the use of MR is a responsible excise of one's reproductive freedom to have genetically-related children free from mitochondrial disease, parents who understand those points should ensure long-term follow-up be properly performed in the best interest of their offspring. On becoming legally competent, the resultant youth should be entitled to refuse follow-up if mitochondrial disease has been adequately prevented with medical proof. This offspring-centred follow-up approach is applicable to the use of MR for preventing early-onset type of MERRF as well as MELAS and Leigh syndrome.

Limitations, reasons for caution: This clinical and ethical consideration depends on the pathology of three major mitochondrial diseases and MR regulation in the UK. In other countries and/or other conditions, more practical and humane follow-up approaches may be conceivable.
Wider implications of the findings: This long-term follow-up approach could also be, to some extent, applicable to the use of MR for infertility treatment, although the primary endpoint is live births. At least, immediate follow-up of resultant infants should be performed through collaborative efforts by fertility physicians and pediatricians.
Trial registration number: not applicable

P-340 Strong fertility patients´ concerns about frozen embryos: a pilot descriptive study in an Argentinean fertility center

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Study question: What is the relation between an embryo representation and the disposition decisions among fertility patients in the countries with insufficient regulations like Argentina?
Summary answer: Several patients who undergo fertility treatments and don't have children represent the embryo as a child. When this happens, patients face difficulties in decision making.
What is known already: Reproductive decision making is a dynamic process. Many studies suggest that embryo representation is a crucial factor to identify those patients who experience difficulties in decision-making. This is shaped by multiple reasons, for instance, experience during past IVF treatments, life events, psychosocial factors, beliefs and personal values. However, despite this, the nature of the links between embryo representation and decision to donate or discard, remain unclear. It seems that couples undergoing fertility treatments find it difficult to donate embryos to other couples when they, themselves, are not guaranteed to have children of their own.
Study design, size, duration: A descriptive study using a survey method research was conducted to know what are the embryo perceptions and opinions of fertility patients at their first visit to a fertility center. The population under study was men and women who started fertility treatments in a specialized fertility facility of Buenos Aires City. This pilot study analyses a non-probabilistic sample and was performed between April and December 2018.
Participants/materials, setting, methods: 59 fertility patients were studied, 37.2% (22 participants) have cryopreserved embryos at the time the survey was administered. The participants are working Argentinean citizens with a good educational level (36.8% complete university studies) and are either married (46.7%) or in cohabitation (35%). The data was collected through an anonymous and self-administered questionnaire that was completed in the fertility center where the participants made their first time consultation.
Main results and the role of chance: Patients face uncertainties when they were asked to select between the conventional embryo disposition options: with 37% of respondents choosing *store for reproduction*; 8% *donate to another couple*; 2% would *thaw and discard*, but 25% have still not come to a decision and 28% do not know or do not answer. Among the patients who did not make any decision or do not answer, 47% have chosen "child" as the option that best represents the embryo. The absence of children in the majority of the sample (61.1% still have no children) makes '*store for reproduction*' the prevailing option. Although there are relatively few participants who have decided to donate their surplus embryos (5 participants), many of them reported having considering it (43%). When the patients were asked their opinion about the possibility that the Fertility Center could dispose of the embryos that are presumed "abandoned" (meaning 5 or more years without any contact with the progenitors despite the efforts made to contact them), the majority totally agree (41%), partially agree (29%), partially disagree (12%) and disagree totally (18%). The vast majority of respondents (86.5%) would not be willing to face the costs of maintaining long-term cryopreservation.
Limitations, reasons for caution: Sample size could be more robust. The conclusions are related to the context where the study was performed. The patient's indecisiveness regarding embryo disposition decisions are deepened in a context of legal uncertainty.
Wider implications of the findings: After more than three decades of assisted reproduction, the problem of the embryo moral status still persist. In this context, gaining in-depth knowledge of the decision-making process and factors that influenced it might be useful to improve the communication strategies.
Trial registration number: not applicable

P-341 Attitudes towards in vitro gametogenesis in a representative sample of the Belgian population

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Study question: What are the current attitudes towards the possibility of in vitro gametogenesis (IVG) in the Belgian population?
Summary answer: Attitudes towards the importance of genetic parenthood are ambivalent. There is limited acceptance of safety and efficacy testing of IVG (embryo and animal research).
What is known already: The great majority of people attending a fertility clinic prefer genetic over non-genetic parenthood. A previous Dutch study indicates that there is widespread acceptance of stem cell based fertility treatments, both for heterosexual and same-sex couples, but low acceptance for the treatment of single women and women who are infertile due to aging.
Study design, size, duration: A questionnaire was developed and incorporated into a web-based online survey and sent out to Belgians 18 years or older in September/October 2018 by a market research and polling agency (iVOX) until a representative sample (by age, gender and region) of 1000 participants was reached.
Participants/materials, setting, methods: The survey contained questions about the general attitude towards IVG, attitudes towards the different goals of IVG, the importance of several potential uses of IVG, risks and means of risk mitigation, different settings in which IVG might be used and other potential future reproductive technologies. Data were analyzed and correlated with socio-demographic variables.
Main results and the role of chance: There is generally an ambivalent attitude towards the importance of genetic parenthood. While 64,4% of respondents believe that families resulting from donor conception and families in which children are genetically linked to both parents are equally valuable, 26,5% of respondents indicate that a genetic relationship between parents and children is necessary for a good parent-child relationship and 67,6% consider the possibility of establishing genetic parenthood to be an important advantage of IVG. French-speaking participants adhere a greater importance to the genetic link than Dutch-speaking participants.

A quarter of our respondents (25.2%) objected to the use of IVG to accomplish full genetic reproduction in lesbian couples, while a majority (64.7%) objected to the use of IVG in post-menopausal women. The impact of religion and level of education was very limited.

A minority (16.7%) of participants was willing to accept greater risks for IVG than for other ARTs, but the use of spare IVF embryos to study those risks was acceptable for only 54.7% of participants, embryo creation for 39.7%, experiments on mice for 33.0% and on monkeys for 29.0% (with a significantly greater acceptance by men than women).

Finally, 85,9% agreed that the government should strictly regulate IVG.
Limitations, reasons for caution:

Between 8 and 15% indicated that they had "no opinion" about the statement. The characteristics of this group of respondents and the impact on the sample's representativeness need to be taken into consideration.
Wider implications of the findings: Balancing the benefits of IVG with the risks and the measures needed to mitigate those risks is compromised by a great diversity of attitudes towards the importance of genetic parenthood and the acceptability of embryo and animal research. There is a need for public education and dialogue on those topics.

Trial registration number: not applicable

P-342 The moral relevance of egg donors' drive to donate: a study of online forums in the UK

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Study question: How do (potential) egg donors frame egg donation on online forums and what is the moral relevance of this for the regulation of egg donation?
Summary answer: Apart from language of helping and giving, we found a discourse of egg donation as something one could experience as a deeply felt desire.
What is known already: Online information shapes egg donation practices in salient ways. Online spaces become ever more important in users' quests for information and support. Previous studies point to the gendered rhetoric of altruism that draws on constructions of ideal motherhood and to the use of emotional language (e.g. words as 'gift' or 'gift of life') to target egg donors. Studies have shown how agencies attempt to recruit potential donors through both monetary and non-monetary benefits that may focus on personal gain, rather than on other considerations.

This is the first study on egg donation and Internet forums in the UK.
Study design, size, duration: A google search using the words 'Egg donation' and 'UK' was conducted to replicate potential forum users' searches. Purposeful sampling was used to guarantee variation in user profiles and forum initiators. Four freely accessible forums (active between 2015 and 2017) were included led by patients (1), private fertility clinics (1), and parenting communities (2). Posts of eggs sharers and intra-family donors were excluded. We studied 193 posts from 33 threads involving 125 unique contributors.
Participants/materials, setting, methods: The length of posts varied between 16 and 295 words. The second author carried out the initial coding using in-depth inductive thematic analysis. Code samples were checked by the first author and discussed by all three authors until a consensus was reached about a coherent and internally consistent thematic map. Here, we describe three themes: 'presenting selves as available', 'helping by giving' and 'the drive to donate'. (Potential) egg donors are described here as 'donors'.
Main results and the role of chance: Forum posters represented themselves as the 'ideal donor' via messages varying between subtle offers of help to those in the style of personal ads, which were marked by positively articulated self-disclosure. Donors wanted to help others and strongly emphasised how special their donation was using phrases like 'the greatest gift'. Reimbursement was never directly mentioned as a motive and many women explicated that it was not their main motivation.

Donors often expressed the desire to establish some kind of relationship with the recipients and/or the donor-conceived child and several appeared to use forums as a way to side-step current UK regulations that guarantee anonymity for all involved parties until the age of 18.

In many posts, an eagerness to donate was phrased as 'wanted to for a long time' or 'nothing will stop me'. These posts described how the act of donating eggs required bodily investment or even a form of self-sacrifice and risk-taking for the sake of others. In these posts, concerns or even negative experiences were overruled by a clear drive to donate.

In addition, we will consider the moral acceptability of aspirations like physical self-sacrifice, attention seeking, and the desire for contact with and impact on recipients/children.
Limitations, reasons for caution: The research data were forum posts, rather than the donors posting them. Therefore, they may provide a partial and limited picture of lived experiences. This qualitative study does not aim to make empirical generalisations but rather to generate theoretical inferences. The ethical analysis uses the empirical data as cases.
Wider implications of the findings: In contrast to the dominant view of egg donors as passive and purely altruistically motivated responders to needs of others, we urgently need to construct a view that takes into account the interests as well as the ambitions of some donors and study the ethical implications thereof.
Trial registration number: not applicable

POSTER VIEWING
IMPLANTATION AND EARLY PREGNANCY

P-343 HIF2α in the uterine stroma permits embryo invasion through detachment of uterine luminal epithelium

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Study question: Hypoxia inducible factor 2α (Hif2α) is expressed in the mouse uterus during embryo implantation. This study aimed to clarify functions of uterine Hif2α using conditional knockout mouse models.
Summary answer: This study provided a new insight that stromal Hif2α allows trophoblast invasion through detachment of the luminal epithelium and activation of an embryonic survival signal.
What is known already: It has been reported that Hif2α, a major transcriptional factor inducible by low oxygen tension, is expressed in the mouse uterus during embryo implantation, its role in pregnancy outcomes remains unclear.
Study design, size, duration: In vivo study using mouse models
Participants/materials, setting, methods: Mice with deletion of Hif2α in the whole uterus (UKO) and in the stroma (SKO) were generated by crossing Hif2α-floxed mice with Pgr-Cre and Amhr2-Cre mice, respectively. Reproductive phenotypes of these mice were evaluated after mating with wild-type fertile male mice.
Main results and the role of chance: UKO mice showed infertility due to implantation failure. Supplementation with progesterone and leukemia inhibitory factor restored decidual growth arrest and aberrant position of implantation sites in UKO mice, respectively, but did not rescue pregnancy failure. Histological analyses in UKO mice revealed persistence of the intact luminal epithelium, which blocked direct contact between stroma and embryo, inactivation of embryonic PI3K-AKT pathway, and failed embryo invasion. UKO mice showed the reduced expression of membrane type 2 metalloproteinase, lysyl oxidase, VEGF, and adrenomedullin in the stroma at the attachment site. In addition, SKO mice showed infertility with impaired embryo invasion, suggesting the importance of stromal Hif2α in embryo invasion.
Limitations, reasons for caution: This study did not use human cells and tissues but mouse models.
Wider implications of the findings: There is a new therapeutic potential to prevent conception by blockade of HIF2α
Trial registration number: not applicable

P-344 Deciphering the trophoblast-epithelium dialogue: a time course study of in vitro early attachment

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Study question: What is the evolution of the transcriptional dynamics of both the trophoblast and the endometrial epithelium in early attachment during implantation?

Summary answer: Successful attachment produced an early and transient transcriptional response in the receptive epithelium, in contrast to a more dynamic transcriptional response in the trophoblast

What is known already: Embryo implantation is the most limiting step in success of infertility treatments. Many studies have focused on this process using *in vitro* models of embryo-endometrium co-culture as well as animal models. Although a number of molecules have been reported to be involved in the embryo-endometrium interaction, the bulk of molecular mechanisms regulating human implantation still remain elusive. In order to understand the requisites for successful implantation and the establishment of pregnancy, a comprehensive description of the compartment-specific gene expression responses that both the embryo and the endometrium undergo during implantation is urgently required.

Study design, size, duration: We developed an *in vitro* co-culture model of confluent monolayers of a cell line mimicking receptive epithelium (Ishikawa) combined with spheroids of an engineered, Green Fluorescent Protein (GFP) expressing, trophoblast line (JEG-3) in 96-well plates. After 0hrs, 8hrs and 24hrs of interaction, the co-cultures were pooled and sorted by FACS; GFP+ (trophoblast spheroids) and GFP- (epithelial substrates) as well as control fractions non-co-cultured were collected for RNA extraction (in triplicate) and analyzed by RNA-seq.

Participants/materials, setting, methods: The transcriptional changes of both compartments (GFP+ trophoblast and GFP- epithelium) at different time points were analyzed using Illumina Hiseq 2500 system. Differential expression was performed with DESeq2; statistical significance was set at Log2 fold change $\geq$1 and p-value $<$0.05. Gene set enrichment analysis (GSEA) was performed using GOBP and Broad Hallmarks databases, with false discovery rate cut-off $<$10%. Further, an *in-silico* strategy was used to predict protein interactions in the trophoblast-epithelium crosstalk.

Main results and the role of chance: After 8 hrs of co-culture, 200 genes were up-regulated and 95 genes were down-regulated in the epithelial compartment; from 8 to 24 hrs, 127 genes were up-regulated and 131 were down-regulated. Trophoblast challenge induced a wave of epithelial transcriptional changes that resulted in overrepresentation of epithelial to mesenchymal transition (EMT), cell movement, apoptosis, hypoxia, inflammation, allograft rejection, myogenesis and cell signalling (*e.g.,* TNFa/NFkb, KRAS, JAK-STAT cascades) at 8 hrs. Interestingly, most pathways subsided at 24 hrs (*i.e.,* EMT, cell movement, allograft rejection, myogenesis and cell signaling), while others did not change (hypoxia, inflammation and apoptosis). In the trophoblast compartment, the transcriptional changes upon co-culture were more dynamic. A total of 1201 and 46 genes were up- and down-regulated after 8 hrs, respectively; from 8 to 24 hrs, 458 genes were up-regulated and 23 were down-regulated. The GSEA revealed that angiogenesis and hypoxia were overrepresented at both 8 and 24 hrs, while EMT and cell signalling were only increased at 8 hrs; from 8 to 24 hrs, inflammation and estrogen response were increased, while proliferation was decreased. The *in silico* protein interaction analysis, based on our transcriptional profiling, predicted 87 early trophoblast-epithelium membrane interactions (e.g., LAMA3-CD44, CD44-EGFR and ITGA5- NRP1).

Limitations, reasons for caution: Our *in vitro* model is based on cell lines of carcinoma origin; therefore, caution is warranted when extrapolating our results to other systems. We describe a 2-D model that does not take into account the involvement of other cell types in the transcriptional changes during implantation.

Wider implications of the findings: We provide a comprehensive description of the molecular events regulating early implantation in a time and compartment specific manner; although the results cannot be directly extrapolated, our data can be a source of candidate molecules important for successful embryo attachment.

Trial registration number: not applicable

P-345 Endometrial preparation for frozen-thawed embryo transfer in patients with intrauterine adhesion

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Study question: Does induced cycles achieve the better pregnancy outcomes compared to hormone replacement therapy during endometrial preparation (EP) for frozen-thawed embryo transfer (FET) in women who suffered from intrauterine adhesion (IUA).

Summary answer: Induction cycles achieved significantly higher rates of live birth, ongoing pregnancy and implantation when compared to artificial cycles in patients with moderate to severe IUA.

What is known already: Artificial cycles (ACs) are most commonly used to prime the endometrium before FET. However, poor endometrial response to exogenous hormone treatment could be observed in many patients with IUA. Moreover, high estrogen exposure may accelerate endometrial fibrosis. Additionally, patients need to be administered the prolonged hormonal treatment in case of pregnancy, leading to an increased cost, inconvenience and risk of venous thrombosis. Alarmingly high pregnancy wastage in AC has been noted. There are many studies to research which is the best protocol to prepare the endometrium for FET, but there are no studies that aim at the IUA patients only.

Study design, size, duration: A retrospective, matched, cohort study including 202 women who underwent autologous FET after hysteroscopic adhesiolysis was conducted at a university-affiliated center between January 2017 and March 2018. The patients with induced cycle (IC) were matched to those with AC by using propensity score matching, in a 1:1 ratio. The matching variables were the age at FET, body mass index, American Fertility Society (AFS) score before operation and the number of embryos transferred.

Participants/materials, setting, methods: The inclusion criteria were: (1) age at FET $<$40 years, (2) BMI $<$30 kg/m^2, (3) moderate to severe IUA according to the AFS classification (1988 version) (a score of 5-12). The exclusion criteria were: (1) using embryos originating from donor oocytes or preimplantation genetic testing, (2) pathology conditions such as hydrosalpinx, uterine adenomyosis, uterine fibroids and uterine malformations, (3) IUA present at COOK balloon removal. The pregnancy outcomes were compared between ICs and ACs.

Main results and the role of chance: Of the initial 499 cycles, 150 ICs and 132 ACs met the inclusion and the exclusion criteria. A total of 101 ICs and 101 ACs were included in the final analysis after matching. There was no difference in patient baseline characteristics between the two groups. The rates of live birth (44.6% vs 30.7%), ongoing pregnancy (48.9% vs 36.2%) and implantation (45.3% vs 32.6%) were significantly higher in the IC group ($p < 0.05$). The clinical pregnancy rate (58.4% vs 45.5%) and early miscarriage rate (9.1% vs 18.6%) were similar between the IC group and AC group ($p > 0.05$). There were 9 (20.0%) pairs of twins in the IC group and 5 (16.1%) in the AC group, respectively. No differences were observed in adverse pregnancy outcomes including premature delivery (13.3% vs 9.7%), low birth weight (14.8% vs 13.9%), placenta accreta and placenta previa (6.7% and 4.4% in IC group vs 16.1% and 3.2% in AC group, $p > 0.05$).

Limitations, reasons for caution: The main limitation of the current study is its retrospective nature and the sample size was not large enough. Moreover, it should be cautious in interpreting the results in patients with severe IUA due to the small proportion.

Wider implications of the findings: This study supports the limited evidence that, for women with moderate to severe IUA, FET using IC results in significantly improved rates of live birth, ongoing pregnancy and implantation when compared with AC. A randomized controlled trial (clinical trial registration number: NCT03578172) with adequate sample size is being conducted.

Trial registration number: Ethical Committee of the hospital no. LL-SC-2018019.

P-346 Predictive factors for biochemical pregnancy in intracytoplasmic sperm injection cycles

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Study question: Which factors contribute to the incidence of biochemical pregnancy in intracytoplasmic sperm injection (ICSI) cycles?
Summary answer: The combination of suboptimal endometrial development, and poor semen and embryo quality contribute to an increased incidence of biochemical pregnancy in ICSI cycles.
What is known already: Human reproduction is marked by its inefficiency. It is estimated that 70% of all pregnancies are lost prior to live birth; among these, 25% to 50% end up as biochemical pregnancies (BP). Most BP losses go unrecognized in natural pregnancies, because the menstrual cycle is not significantly altered. Nevertheless, in patients undergoing assisted reproduction technology (ART) treatments, in which β-hCG levels after embryo transfer are actively monitored, BP is diagnosed in up to 20% of the cycles. Despite the high incidence of BP, its predictive factors and precise etiologies remain unknown.
Study design, size, duration: This historical cohort study included data from 3,134 ICSI cycles performed from June/2010 to September/2016. The sample size was determined by considering effect size of 10%, α of 5%, β of 80% and 11 covariates for 1,634 subjects. Cycles were split into four groups, depending on the pregnancy outcomes: Clinical Pregnancy (CP, n = 903), Biochemical Pregnancy (BP, n = 55), Miscarriage (Mis, n = 142) and Negative Pregnancy (NP, n = 2,034).
Participants/materials, setting, methods: The study was performed in a private university-affiliated IVF center. In the first analysis, the effects of controlled ovarian stimulation, laboratory data and seminal parameters on pregnancy outcomes from the four different groups were evaluated using general linear models adjusted for potential confounders. Then, a discriminant analysis was conducted by the stepwise method for ICSI cycle prediction of BP or CP outcomes, to establish cut-offs for BP.
Main results and the role of chance: The results from the first analysis showed that the total sperm count ($p = 0.035$), total and progressive sperm motility ($p = 0.001$ and $p = 0.023$, respectively), total motile sperm count (TMSC, $p = 0.029$) and the endometrial thickness ($p < 0.001$) were significantly lower in the BP group compared to the other groups. Lower rates of high-quality embryos on days two and three of development were observed in the BP group compared to the CP and Mis groups ($p < 0.001$). In the second analysis, the cut-offs for BP prediction established by the discriminant analysis were endometrial thickness < 11 mm, total sperm motility < 55.5% and total dose of FSH > 2,400 IU.
Limitations, reasons for caution: The main limitation of the present is the reduced number of cycles included in BP group. Moreover, cut-offs values for the prediction of BP must be used in combination, which prevents the use of a single parameter to predict BP chance.
Wider implications of the findings: Biochemical pregnancy can be predicted by utilizing combined cut-offs of endometrial thickness, sperm motility and total dose of FSH. This approach can both improve the understanding concerning mechanisms responsible for biochemical pregnancy and assist in the management of cases of previous pregnancy losses.
Trial registration number: None

P-347 Transcriptional analysis of the trophoblast-epithelium interaction: how do they respond to each other?

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Study question: Which are the transcriptional changes specific to the trophoblast *vs*. the receptive or non-receptive endometrial epithelium upon their interaction?
Summary answer: Endometrial receptivity requires a transcriptional signature that determines the trophoblast response. A series of coordinated compartment-specific transcriptional responses drive attachment and implantation.
What is known already: Human implantation is a highly orchestrated process essential for the establishment of pregnancy, and currently, implantation failure is a major cause of human infertility and the most limiting step in ART. Implantation failure can be caused by both maternal and embryonic factors, as well as by a defective crosstalk between them. However, the molecular mechanisms underlying human implantation are poorly understood and cannot be studied *in vivo*. Although several *in vitro* models have been developed to study human implantation, the specific transcriptional dynamics of the trophoblast and receptive *vs*. non-receptive endometrial epithelium during their interaction have not been described.
Study design, size, duration: We developed an *in vitro* model by co-culturing a) immortalized cell lines of non-receptive (HEC-1-A) or receptive (Ishikawa) epithelium and b) GFP expressing spheroids of a trophoblast line (JEG-3). 96-well plates containing a confluent layer of substrate were co-cultured with one spheroid per well, in triplicate. After 48h, all the wells of each plate were pooled and sorted by FACS for the isolation of epithelial and trophoblast compartments, including non-co-cultured controls of each cell line.
Participants/materials, setting, methods: The transcriptional profiles of the trophoblast (GFP+) and non-receptive *vs*. receptive epithelium (GFP-) were separately characterized by RNA-seq (Illumina Hiseq 2500). Statistical significance of gene expression differences was set at Log2 fold change ≥ 1 and p-value <0.05. Gene set enrichment analysis (GSEA) was performed to reveal the biological function of differentially expressed genes using GOBP and Broad Hallmarks databases, with false discovery rate cut-off <10%. An *in-silico* strategy predicted protein interactions in the trophoblast-epithelium crosstalk.
Main results and the role of chance: After co-culturing Ishikawa cells with trophoblast spheroids, 310 and 298 genes increased or decreased their expression compared to non-co-cultured Ishikawa control cells, respectively; only 9 genes were differentially expressed in HEC-1-A upon co-culture with trophoblast spheroids. Compared to HEC-1-A, the trophoblast challenge to Ishikawa cells differentially regulated the expression of 495 genes; these differences highlighted an important role for cell adhesion and extracellular matrix (ECM) molecules in the trophoblast-epithelium interaction. GSEA revealed enrichment of cell division, cell cycle regulation and metabolism in the Ishikawa substrate. Both differential gene expression and functional analysis suggested that the trophoblast spheroid response depended on the receptivity of the substrate. Comparing the gene expression profile of trophoblast spheroids revealed that 1877 and 323 genes were up-regulated or down-regulated when co-cultured on Ishikawa (compared to HEC-1-A) substrates, respectively. Pathways favourable to development, including tissue remodelling, organogenesis and angiogenesis were enhanced in the trophoblast compartment after co-culture of spheroids with the receptive epithelium. By contrast, the co-culture of trophoblast spheroids with the non-receptive epithelium enriched pathways mainly related to trophoblast cell proliferation and cell cycle regulation. Our *in silico* interactome network approach, based on our transcriptomic profile, predicted 21 direct protein partners presumably involved in trophoblast-epithelium crosstalk.
Limitations, reasons for caution: Our model, based on immortal carcinoma cell lines (widely used as models for receptive and non-receptive epithelium) may not exactly reflect the behaviour of primary endometrial epithelial cells. Also, the trophoblast spheroids may not fully represent the response of human embryos
Wider implications of the findings: Our system allows analysis of transcripts and proteins involved in embryo attachment in a compartment specific manner, superseding previous approaches which analyze both compartments together, and led to the identification of trophoblast or the epithelial molecules that might play a role in successful embryo attachment.
Trial registration number: not applicable

P-348 Frozen embryo transfer in hormone replacement cycle contributes to the development of subchorionic hematoma

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Study question: Does the difference in endometrium preparation [hormone replacement cycle (HRC) vs. natural cycle (NC)] in frozen embryo transfer affect the development of subchorionic hematoma (SCH)?
Summary answer: Based on our multivariate analysis, frozen embryo transfer in HRC increases the risk of developing SCH.
What is known already: Although this is debatable, SCH is known to be a cause of miscarriage and preterm birth. It was recently reported that frozen embryo transfer contributes to the development of SCH in assisted reproductive technology (ART). Moreover, it has been reported that taking aspirin during ART also contributes to increased SCH.
Study design, size, duration: This study was approved by the Institutional Review Board (IRB) of Tawara IVF clinic (Shizuoka, Japan). Between Mar. 2015 and Oct. 2018, 1947 pregnant women (HRC: n=1211, NC: n=736) involved in frozen embryo transfer in Tawara IVF clinic were included in this cross-sectional study. Pregnant women with echo-free space outlining the intact gestational sac who presented with vaginal bleeding at a gestational age of 9 weeks or less were judged to have SCH.
Participants/materials, setting, methods: In the HRC, a transdermal estradiol was started on day 2-4 of menstruation and was applied at 2.26 mg every 2 days until the 4th week of gestation, and continued at 1.44 mg every 2 days until gestation week 7. For luteal phase support, oral dydrogesterone (15 mg/day) was used for both HRC and NC. Furthermore, vaginal progesterone (300-800 mg/day) was used for HRC, but none or small amount of this was used for NC.
Main results and the role of chance: Patient backgrounds : Age, HRC 35.8±4.2, NC 35.8±4.1 (p=0.76); BMI, HRC 20.7±2.9, NC 20.8±2.9 (n.s.); Abortion history, HRC 0.38±0.74, NC 0.31±0.60 ($p<0.05$); Percentage taking aspirin during the cycle, HRC 23.0%, NC 10.9% ($p<0.05$). Pregnancy rate was not significantly different between HRC and NC (36.6% and 37.7%, respectively), and in the case of a single blastocyst transfer (SBT), not significant difference was also observed between both groups (42.6% and 44.4%, respectively). In total, 206 pregnant women had SCH (10.6%, only limited SBT, 10.0%). Univariate analysis showed that HRC increased the risk of developing SCH, compared to NC (14.3% vs 4.5%, respectively, OR: 3.55 [95% CI 2.45-5.30]; only limited SBT, OR: 3.93 [2.51-6.44]). In a multivariable analysis, controlling for age, BMI, abortion history, taking aspirin, the adjusted odds ratio (OR) for developing SCH was 3.08 [2.05-4.78] for HRC and 3.24 [2.00-5.49] for only limited SBT. Furthermore, we found that HRC also increased the risk of miscarriage after adjusting the confounding factors described above (OR: 1.71 [1.32-2.23], only limited SBT, OR: 1.66 [1.22-2.26]). However, in this multivariate analysis, the presence of SCH was not associated with increased risk of miscarriage (0.71 [0.46-1.06], only limited SBT, OR: 0.89 [0.53-1.45]).
Limitations, reasons for caution: This was a non-randomized study. The size and location of SCH were not considered in this study.
Wider implications of the findings: Interestingly, we found that frozen embryo transfer in HRC increases the risk of developing SCH. However, SCH does not contribute to increased miscarriage rates. It is necessary to further investigate long-term perinatal outcomes.
Trial registration number: not applicable

P-349 Serum progesterone level and ongoing pregnancy rate following frozen-thawed embryo transfer (FET) after hormone replacement therapy (HRT): a monocentric retrospective cohort study

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Study question: To compare, among patients with clinical pregnancy (CP) after FET with hormone replacement therapy (HRT), serum progesterone levels between those with early pregnancy loss or ongoing pregnancy (OP).
Summary answer: Serum progesterone level was lower in women experiencing early pregnancy loss after FET with HRT, potentially reflecting a lack of luteal phase support.
What is known already: Early pregnancy losses rate is higher with HRT for FET than with other protocols even though pregnancy rates are similar. A potential defect in the luteal phase (LP), maybe due to a suboptimal exogenous progesterone substitution, could explain these findings. Progesterone being exogenous in HRT, serum progesterone level reflects what was absorbed by the patient. Data is scarce and contradictory on whether this rate is related to ongoing pregnancy rate (OPR) and on the level required to optimize OPR after FET. The optimal route of progesterone for LP support in HRT is yet to be determined.
Study design, size, duration: A monocentric retrospective cohort study was conducted at a University affiliated fertility center in Paris, France. Between June 2016 and July 2017, 301 FET cycles were fulfilled entirely, thus included: 130 FET were performed after HRT; 171 after mild-ovarian stimulation protocol (OS). All women underwent FET in either cleavage or blastocyst stage. Embryos were mainly frozen by vitrification.
Participants/materials, setting, methods: For HRT, estradiol was administrated orally from the first day of the cycle (4-6mg/day). Vaginal micronized progesterone (600mg/day) was added once endometrial thickness reached 7 mm. Serum beta-hCG and progesterone rates were measured 10- or 12-days following FET, according to embryo stage, and every 48 hours until serum beta-hCG levels reached 1000 UI/ml. CP was defined as hCG>100 UI/ml and OP as fetal heartbeat at 12 weeks of gestation.
Main results and the role of chance: Among the 130 patients who had FET after HRT, 33 had clinical pregnancies (25.4%) and 18 ongoing pregnancies (13.5%). Among patients who had a CP after HRT, serum progesterone levels were higher in case of an OP than an early pregnancy loss at 3 times of measurements, but only significantly 48h after first pregnancy test (4 gestational weeks and 2 days) : PG0 12.4 ng/ml [7.5-14.6] vs 8.2 ng/ml [6.0-13.0], p = 0.320; PG1 15.0 ng/ml [14.0-15.9] vs 8.5 ng/ml [5.9-13.8], p = 0.048. No significative difference was found regarding demographic and cycles characteristics between women with early pregnancy losses and women with ongoing pregnancies. In the HRT group, PG0 (on the day of the first pregnancy test) was not different between pregnant and non-pregnant women (11.6 ng/ml [6.1-14.4] vs 11.2 ng/ml [8.2-14.1] p = 0.8). As expected, pregnancy loss rate was higher after HRT than after OS for endometrial preparation (OPR 13.5% vs 19.0%; p= 0.004) despite same clinical pregnancy rates (25.4% vs 23.4%; p = 0.7).
Limitations, reasons for caution: The small number of patients included and the retrospective nature of the study are the main limitation leading to heterogeneity of population. We calculated afterwards that 400 people per group were needed to show a statistically significant difference at PG0.
Wider implications of the findings: A cycle test before FET with HRT combining serum progesterone level measurement and if possible, an endometrial receptivity test, could help physicians to choose and to optimize the LP support dose and route of administration for each patient.
Trial registration number: The hospital ethics committee gave its unrestricted approval to use anonymously records from all patients who had previously given their informed consent (IRB 00006477).

P-350 Large refractile / lipofuscin bodies are present over a year prior to ovulation

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Study question: When do large refractile / lipofuscin bodies (LB) appear and is the presence of these large bodies determined by the patient's condition?
Summary answer: LBs are present over a year prior to ovulation and their presence seems to be patient- specific.

What is known already: LB is one of the main morphological abnormalities in the cytoplasm of human oocytes. It consists of a mixture of lipids and dense granular materials and exhibits the typical autofluorescence of lipofuscin. Lower fertilization rates and lower embryo development rates have been consistently reported when embryos with LBs have been transferred. LBs appear in both mature and immature oocytes and there is a strong tendency for recurrence in the same patient.
Study design, size, duration: This is a prospective study, conducted with a total of 585 primordial oocytes, which were obtained from four patients with gender identity disorder. Their oophorectomies were performed between November 2018 and January 2019. Informed consent for the use of their ovaries and institutional review board approval were both obtained for this research.
Participants/materials, setting, methods: Primordial oocytes were isolated from the ovaries after the oophorectomies of patients with gender identity disorder. The autofluorecence of the LBs was confirmed by fluorescent microscopy. The presence of lipids in the LBs was confirmed by lipid staining using Nile Blue and Sudan Black. The number of primordial oocytes with and without large LBs was recorded and the percentage of large LBs was compared among the four patients.
Main results and the role of chance: Small and large LBs were found in primordial oocytes from all four patients with gender identity disorder. Both large and small bodies exhibited autofluorescence and were positively stained by Sudan Black and Nile blue. The number of primordial oocytes with large LBs (>5μm) and with only small bodies was calculated for the four patients. The percentage of primordial oocytes with large LBs in patients #1 (28 years old), #2 (44 years old), #3 (30 years old), and #4 (36 years old) was 24.4% (30/123), 5.2% (8/154), 49.0% (70/143) and 28.5% (47/165) respectively.
Limitations, reasons for caution: These results are limited to patients with gender identity disorder who have been treated with testosterone injections until 6 weeks prior to oophorectomy. Therefore, the effects of testosterone on the appearance of LBs cannot be ruled out as effecting the results of this study.
Wider implications of the findings: As LBs were present in primordial oocytes prior to ovulation, and these oocytes require a full year to reach maturity, the cause of the bodies may not be related to stimulation protocols nor the timing of ovulation, but the environment in each patient's ovaries. Thus, recurrence is likely to occur.
Trial registration number: not applicable

P-351 Another cause of fertility-loss

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Study question: Is the loss of fertility due to changes in the extracellular environment?
Summary answer: Yes, it may be related to fibrosis beneath the tunica albuginea, which causes an increase in the total thickness of the dense connective tissue.
What is known already: Loss of fertility in women due to old age is a great concern, especially in developed countries. It is well known that the age-related loss of female fertility occurs as a result of the gradual decline in both the quality and quantity of eggs. A recent hypothesis suggests that the loss of fertility may be due to changes in the extracellular environment, which could negatively affect the quality of oocytes.
Study design, size, duration: This is a prospective study, conducted with a total of 18 ovaries, which were obtained from 18 patients with gender identity disorder. Their oophorectomies were performed between April 2014 and April 2016. Informed consent for the use of their ovaries and institutional review board approval were both obtained for this research.
Participants/materials, setting, methods: Slices of these ovaries, 5 μm thick, were prepared for histological analysis by picrosirius red staining (a specific dye for collagen I and III fibers). The mean thickness and density of the outermost part of the ovary, the tunica albuginea (TA) was calculated by computer-image analysis. To assess the effects of the patients' age and androgenic treatment on the ovaries; the data underwent statistical analysis by Pearson's correlation and Logistic regression.
Main results and the role of chance: The mean age of patients was 30.1 ± 7.5 (SD) years, ranging from 21 to 46 years old. There was no correlation between the dosage or length of the androgenic treatment and the thickness and density of the TA ($p = 0.76$ and $p = 0.14$, respectively). However, when the age of the donor was analyzed against the aforementioned parameters, a significant positive correlation ($r = 0.52$, $p < 0.05$) was detected between the thickness of the TA and the age of the patient. Furthermore, when the density of the TA was assessed, the results indicated the presence of another significant positive correlation ($r = 0.55$, $p < 0.05$) between age and TA density. More exhaustive histological analysis revealed that the increase in the thickness of the TA seems to be due to fibrosis beneath it, causing an increase in the total thickness of the dense connective tissue, whereas in normal ovaries, the thick connective tissue capsule of the TA is generally easily distinguished from the cortical tissue area. No interconnection with the pre-existence of polycystic ovarian syndrome was detected.
Limitations, reasons for caution: As human ovaries are a very valuable sample for the use of research, the number of samples available for this study was limited.
Wider implications of the findings: As an increased thickness in the dense connective tissue of the ovarian cortical area, which was found to be correlated with aging, makes it difficult for follicles to grow and ovulate, this could be a contributing factor as to why older women have a reduced oocyte development and ovulation.
Trial registration number: Not applicable

P-352 Concentrations of steroids in endometrium are not based on passive diffusion but altered by local steroid metabolism called intracrinology. A novel concept in endometrial receptivity

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Study question: Does the concentration of steroids in circulation reflect steroid concentrations in the endometrium of IVF patients and could IVF outcome be related to these differences?
Summary answer: This proof of principle study shows that steroid concentrations in circulation do not reflect the concentrations in the endometrium of IVF patients due to intracrinology.
What is known already: Too often, IVF cycles lead to disappointment for patient and doctor due to implantation failure *e causa ignota*. Omics' studies gave insight about expression of genes associated with endometrial receptivity and many are under influence of the ovarian steroid hormones estrogen and progesterone. Recent research suggests that the endometrium is not passively exposed to these steroids present in the circulation. Few reports showed that, to create the optimal steroid milieu, the endometrium can fine-tune intra-tissue steroid concentrations. This local steroid biosynthesis and metabolism process is referred to as 'intracrinology', a network of reactions that can synthesize, interconvert and metabolise steroids.
Study design, size, duration: Endometrial tissue and serum were retrieved from another study in which patients were randomized for endometrial biopsy or no intervention, to study the effectiveness of endometrial 'scratching' on pregnancy outcome (SCRaTCH-study). Patients were included after a first failed IVF treatment in which at least one embryo was transferred. Once randomized for the endometrial biopsy, ovulation was monitored and biopsies (Pipelle) and serum were obtained 5-7 days after a positive urinary LH-test in a natural cycle.
Participants/materials, setting, methods: After endometrial biopsy, patients continued with a second IVF treatment. Patients who became pregnant after fresh embryo transfer in the second IVF treatment (n=3) were compared to patients who did not (n=3). Cases and controls were matched for primary

versus secondary subfertility, embryo quality and age. Steroid concentration was determined in endometrial tissue homogenates and serum with the liquid chromatography mass spectrometry analysis. A Pearson Correlation and unpaired Student's *t*-test were used for statistical analyses.

Main results and the role of chance: The endometrial tissue concentrations of the steroids estradiol, estrone, 17-hydroxypregnenolone, 11-deoxycortisol, corticosterone, DHEA, testosterone, 17-hydroxyprogesterone, androstenedione, 21-hydroxyprogesterone and progesterone were not correlated to their corresponding concentrations in the circulating blood. Only the cortisone concentrations in blood were correlated to the concentrations observed in the endometrial tissue ($r=0.93$, $p=0.01$). The concentrations of estradiol, estrone, androstenedione and 17-hydroxyprogesterone were significantly higher ($P<0.05$) in the circulating blood than in the endometrial tissue. Testosterone, 17-hydroxypregnenolone, 11-deoxycortisol, corticosterone and 21-hydroxyprogesterone values were higher in the circulating blood as well, albeit not statistically significant. When the steroid concentrations in the endometrial tissue and circulating blood were compared between women who became pregnant after fresh embryo transfer in the second IVF cycle, and patients who did not become pregnant, we did not observe any statistically significant differences. Due to the pilot nature of this study, we restricted our analysis to only a low number of tissues.

Limitations, reasons for caution: We were unable to correct for confounders that determine endometrial receptivity due to a low number of included patients. Also, we were unaware of euploidy status of the embryo. Furthermore, the relative contribution of stromal versus epithelial cells in the endometrial tissue homogenate was unknown which might have caused bias.

Wider implications of the findings: This proof of principle study shows that concentrations of steroids in circulation do not reflect endometrial tissue concentrations in IVF patients. Although the concept of local steroid metabolism (intracrinology) is well established, its role in endometrium and especially in endometrial receptivity has not gained attention before and merits further evaluation.

Trial registration number: The SCRaTCH study is registered under NTR 5342

P-353 Is uterine natural killer (uNK) cells density related to the number of embryo transfer failure?

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Study question: Is uNK cells density around the time of implantation affected by the number of embryo transfer (ET) failure?

Summary answer: Uterine NK cells density significantly increased steadily with the number of ET failure

What is known already: Earlier studies have demonstrated an increase in number of uNK cells in women with recurrent implantation failure, compared with fertile controls. However, it is not known that if the number of ET failure has any impact on the uNK cells density.

Study design, size, duration: It was a retrospective cohort study. A total of 55 women were recruited in the study, including 12, 22, 14 and 7 women who failed to conceive after 2, 3, 4 and ≥5 embryo transfers, respectively. All endometrial biopsies were collected precisely on the putative day of blastocyst transfer of a mock non-conception hormonal replacement treatment (HRT) cycle, that is five days after initiation of progesterone (P+5).

Participants/materials, setting, methods: Endometrial sections were immunostained for CD56 to identify uNK cells. Image capture and cell counting were performed according to a standardized protocol. Results were expressed as percentage of positive uNK cells/ total stromal cells.

Main results and the role of chance: The median uNK cells density was 1.8% (range 0.9-3.2%), 2.1% (range 0.8-3.5%), 3.8% (range 1.7-5.8%) and 4.1% (1.9-5.8%) in women who failed to conceive after 2, 3, 4 and ≥5 embryo transfers, respectively. The median uNK cells density in women with 4 and 5 or more ET failures was significantly higher than those in women who had 2 or 3 ET failures.

Limitations, reasons for caution: The prognostic value of uNK cells measurement in women with recurrent implantation failure has yet to be confirmed.

Wider implications of the findings: The uNK cells density is related to the number of embryo transfer failure. The clinical value of uNK cells measurement in women with recurrent implantation failure is worthy of future study.

Trial registration number: None

P-354 Genome-wide DNA methylation analysis of chorionic villi and decidua in patients with recurrent miscarriage

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Study question: Is an altered DNA methylation program in chorionic villi (embryo factor) and/or decidua (maternal factor) associated with recurrent miscarriage (RM)?

Summary answer: Specific alterations of DNA methylation in gene regulatory regions in chorionic villi but not in decidua are associated with RM.

What is known already: While identifiable causes of RM include antiphospholipid syndrome, uterine anomalies and parental chromosomal abnormalities, we found that embryonic aneuploidy is the most common cause with a frequency of 41%, in a quarter of cases, the causes of this disease are unknown. Although disordered epigenetic transcriptional control owing to aberrant DNA methylation is a critical mechanism in abnormal fetal development, a simultaneous genome-wide DNA methylation analysis of chorionic villi and decidua in RM patients has not been carried out

Study design, size, duration: We examined genome-wide DNA methylation profiles in chorionic villi and decidua of 5 patients with unexplained RM (4.8±1.47) and compared them with 5 matched healthy controls who had undergone an artificial abortion (AA) in the first trimester. All chorionic villi were confirmed as karyotypically normal using comprehensive genome hybridization. Samples were collected in Nagoya City University Hospital from 2012 to 2017. In total, 19 patients and 19 control women were studied.

Participants/materials, setting, methods: DNA was extracted from each chorionic villi and decidua separately. Using the HumanMethylation450k BeadChip, we analyzed genome-wide DNA methylation in both chorionic villi and decidua, and the methylation profiles were compared using unsupervised hierarchical clustering analysis between patients and controls. Gene ontology (GO) analysis was performed to compare differently methylated genes in promoter and enhancer regions. Furthermore, the results were validated by bisulfite pyrosequencing using chorionic villi of 19 patients and 19 controls.

Main results and the role of chance: We extracted Infinium probes with a difference in the average b-value of >0.1 (mean level >10%), and found that 13,426 and 5,816 CpG sites were differently methylated in chorionic villi and decidua of patients in comparison with the controls, respectively. Interestingly, clear distinct DNA methylation signatures were observed between patients and controls by means of an unsupervised two-way hierarchical clustering analysis using the differently methylated probes, on analyzing chorionic villi, but not decidua. Notably, characteristic DNA methylation signatures were more obvious in gene enhancer and promoter regions, both of which are important for the regulation of gene expression. GO analysis revealed that the genes which were methylated differently in chorionic villi were associated with functionality related to multicellular organism processes and system development. Among these methylated genes, we identified gene X, whose enhancer region was highly methylated (mean methylation level, 23.8%) compared to the control ($P<0.001$). Taken together, our data indicate that an altered DNA methylation process during the early development of an embryo may affect the establishment and maintenance of a successful pregnancy.

Limitations, reasons for caution: Our sample size was relatively small. Therefore, a further study of a large cohort should enhance our findings. We are now investigating why the mechanism controlling the altered DNA methylation pattern was particularly established in embryos of RM patients.

Wider implications of the findings: This is the first analysis of genome-wide DNA methylation in chorionic villi and decidua of patients with RM. Our data show that an altered DNA methylation program active in early embryonic development may be one of the causes of RM.

Trial registration number:
not applicable

P-355 Effect of supplementation with oral dydrogesterone and vaginal progesterone for luteal-phase support in frozen embryo transfer at natural ovulatory cycle

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Study question: Does the difference in methods for luteal phase support (LPS) affect the rates of miscarriage in frozen embryo transfer (FET) at natural ovulatory cycle (NC)?

Summary answer: Compared to a combination of dydrogesterone (DG) and vaginal progesterone (P), LPS with oral DG had a lower rate of miscarriage in FET at NC.

What is known already: LPS with oral DG or vaginal P in FET during hormone replacement cycle (HRC) prevents miscarriage. In FET at NC with spontaneous or induced ovulation, LPS administered during the luteinized unruptured follicle (LUF) phase may possibly be effective in preventing miscarriage as well. However, it is unknown what type of LPS is effective for the prevention of miscarriage in FET at NC.

Study design, size, duration: In all, 3886 women undergoing single FET at the Tawara IVF clinic (Shizuoka, Japan) between March 2015 and October 2018 received oral DG and/or vaginal P for LPS. Of these, 1462 women who underwent good morphological blastocyst transfer with no history of miscarriage were included in this cross-sectional study (HRC: n=802, NC: n=660). In the FET at NC group, 230 women received oral DG, while 432 women received oral DG with vaginal P as LPS.

Participants/materials, setting, methods: For LPS, oral DG (15 mg/day) was administered for both, the HRC and NC groups. Additionally, vaginal P (90–800 mg/day) was used for HRC, while no or a small dose (100–400 mg/day) of vaginal P was used for NC. Pregnancy was defined as a gestational sac detected on ultrasound at a gestational age of ~8 weeks. Miscarriage was defined as loss of a pregnancy after the presence of gestational sac had been confirmed.

Main results and the role of chance: The rate of pregnancy was not significantly different between the NC and HRC groups (50.0% and 49.4%, respectively). However, the rate of miscarriage was significantly lower in the NC than in the HRC group (15.5% and 20.7% respectively, $p<0.05$). Furthermore, in FET at NC, the oral DG group had a lower rate of miscarriage than the oral DG with vaginal P group (10.4% and 17.8% respectively, $p<0.05$). The age-adjusted odds ratio (AOR) for the rate of miscarriage was 0.52 [95%CI: 0.25–1.02]. Next, we performed an age-related subgroup analysis. The rate of pregnancy was not different between the oral DG and oral DG with vaginal P groups in women aged less than 35 years and in those aged 35 years and above. Although there was no significant difference in the rate of miscarriage rate between the groups for women aged less than 35 years (7.7% in the oral DG group and 6.7% in the oral DG and vaginal P group: AOR 1.06 [0.27–3.79]), the rate of miscarriage was lower for women aged 35 years or above in the oral DG group than in the oral DG with vaginal P group (12.5% and 25.8%, $p<0.05$: AOR 0.41 [0.17–0.91]).

Limitations, reasons for caution: This was a non-randomized study. Moreover, women who underwent NC without any LPS were not included in this study. Therefore, the effectiveness of LPS for prevention of miscarriage in NC is not clear.

Wider implications of the findings: LPS during the LUF phase might be effective in preventing miscarriage. Our results suggest that LPS with oral DG may be more effective in the prevention of miscarriage, especially in women aged 35 and above compared to LPS using oral DG with vaginal P.

Trial registration number: not applicable

P-356 Single nucleotide polymorphisms in PAPP-A affects its proteolytic activity and the biological activity of IGFs: Potential effects on pregnancy outcome

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Study question: Does the common single nucleotide polymorphism (SNP), rs7020782, in pregnancy-associated plasma protein-A (PAPP-A) show functional effects and does it have prognostic potential?

Summary answer: The minor rs7020782 allele encodes a protein with reduced activity towards insulin-like growth factor (IGF) binding proteins, that may affect IGF bioactivity and pregnancy outcome.

What is known already: PAPP-A is an integral part of prenatal screening and adverse pregnancy outcomes have been associated with aberrant PAPP-A levels. The IGF signalling pathway is important for follicular development and pregnancy outcome. PAPP-A upregulates IGF signalling by liberating IGFs from their corresponding binding proteins. The rs7020782 SNP in PAPP-A has been associated with increased risk of recurrent pregnancy loss. Women homozygous for the minor SNP (7% of Caucasian women) displayed significant reduced levels of PAPP-A in ovarian follicles together with significantly altered hormone levels. Finally, this SNP is positioned in a potential N-glycosylation motif that may alter the properties of PAPP-A.

Study design, size, duration: Molecular characterization of the genetic rs7020782 (SNP) variant in PAPP-A was achieved by comparing recombinant produced PAPP-A proteins manufactured by mutagenesis and subsequently transfection into human embryonic kidney 293T (HEK293T) cells.

Participants/materials, setting, methods: HEK293T cells were transfected with cDNA constructs encoding the two rs7020782 PAPP-A variants ("serine variant": minor allele, "tyrosine variant": major allele). Levels of PAPP-A were measured with ELISA in the cell supernatants. Proteolytic activity of PAPP-A towards radiolabelled IGF binding protein (IGFBP)-2, -4, and -5 was quantified. PAPP-A complex formation with its inhibitors, proMBP and STC2, was evaluated by Western blotting. Cell surface binding of PAPP-A was examined using flow cytometry.

Main results and the role of chance: This study demonstrates for the first time a significant effect of the rs7020782 SNP in PAPP-A on the proteolytic specificity of PAPP-A. A significant reduced cleavage rate of radiolabelled IGFBP-4 was presented for the serine variant (minor allele) of the rs7020782 SNP compared to the tyrosine variant (major allele). In addition, a non-significant reduced cleavage rate of radiolabelled IGFBP-2 and IGFBP-5 was displayed for the serine variant. Unlike IGFBP-5, IGFBP-4 exhibits complex interaction with PAPP-A by interacting directly with substrate-binding exosite(s) in PAPP-A. The serine variant of the rs7020782 in PAPP-A may disturb this proteinase/substrate interaction and consequently result in reduced proteolytic cleavage. Thus, women carrying the serine variant of the rs7020782 SNP in PAPP-A may present with an altered IGF regulation with a limited amount of free IGF to initiate signalling, which in turn affect pregnancy outcome.

PAPP-A cell-surface adhesion to HEK293T cells was not observed to be affected by the rs7020782 SNP and adhesion does not seem to depend on this specific site of variation. Additionally, complex formation between PAPP-A and its inhibitors, STC2 or proMBP, was not influenced by the rs7020782 SNP.

Limitations, reasons for caution: Additional studies are needed to apply the genetic rs7020782 (SNP) variant as a biomarker in a clinical setting.

Wider implications of the findings: We suggest that the rs7020782 SNP in PAPP-A could be used to identify women with high risk of adverse pregnancy outcomes such as recurrent pregnancy loss. Identification of these patients may allow for risk-specific treatment and improve reproductive outcome.

Trial registration number: not applicable

P-357 Impact of MTHFR C677T polymorphism on the levels of vitamin D and homocysteine and NK cell cytotoxicity in women with recurrent pregnancy losses

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Study question: How does affect the MTHFR C677T polymorphism on the levels of vitamin D and homocysteine and NK cell cytotoxicity in women with recurrent pregnancy losses?
Summary answer: The levels of vitamin D and homocysteine and NK cell cytotoxicity in MTHFR C677T homozygous were significantly different from both heterozygous and wild-type genotypes.
What is known already: Vitamin D deficiency is a risk for recurrent pregnancy losses through modulating immunological pathways, such as peripheral natural killer (NK) cell activity. MTHFR C677T gene polymorphism, which plays a critical role in modulating plasma homocysteine concentrations, is also a risk for recurrent pregnancy losses.
Study design, size, duration: This study is a cross-sectional study of 837 women with recurrent pregnancy losses during 9 years.
Participants/materials, setting, methods: Total of 837 women with two or more unexplained consecutive recurrent miscarriage was registered, and routine biochemical data including plasma vitamin D and homocysteine and MTHFR C677T genotypes, CC (wild type), CT (heterozygous), TT (homozygous) were examined. We analyzed the immunophenotypes of peripheral blood mononuclear cells from the registered women, and NK cell cytotoxicity assay was performed.
Main results and the role of chance: The rate of MTHFR C677T genotypes with CC (wild type), CT (heterozygous), and TT (homozygous) were 47%, 43%, and 11%, respectively in women with recurrent pregnancy losses. Although the level of vitamin D in homozygous was significantly lower compared to that in heterozygous (p=0.001) and wild-type genotype (p=0.003), the level of homocysteine in homozygous was significantly higher than that in heterozygous (p=0.001) and wild-type genotype (p=0.001). There was a significant negative correlation between the levels of vitamin D and homocysteine in hetero- and homozygous but not wild-type genotypes (Pearson's correlation coefficient was 0.39, 0.41, and 0.23, respectively). In multivariate analysis, vitamin D insufficiency (<30 ng/ml) was an independent predictive factor for hyperhomocysteinemia (adjusted odds ratio 1.89, 95% CI 1.41-2.52). The value of NK cytotoxicity in homozygous was significantly higher than that in heterozygous (p=0.01) and wild-type genotype (p=0.04).
Limitations, reasons for caution: Since this study was a cross-sectional in design, it is insufficient to evaluate the effects of MTHFR C677T genotypes on the prognosis of subsequent pregnancy successes.
Wider implications of the findings: The MTHFR C677T gene polymorphism may involve the pathogenesis of unexplained recurrent pregnancy losses via elevated hyperhomocysteinemia, which is associated with an increased thrombotic risk. An improvement of vitamin D insufficiency might be effective for the success of subsequent pregnancy to those who have a certain condition.
Trial registration number: obgyne001

P-358 External validation of prediction model (M4) for pregnancy of unknown location (PUL) in IVF patients presenting at very erly gestation

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Study question: Is the M4 logistic regression model able to predict accurately ectopic pregnancy (EP) in women with IVF pregnancies less than 6 weeks gestation?
Summary answer: M4 prediction model has a high negative predictive value for ectopic pregnancies in women after IVF treatment, however the positive predictive value was relatively low.
What is known already: M4 is a multinomial logistic regression model based on initial serum b-HCG level and the HCG ratio (48h/0h). Entering these values into the M4 formula returns estimated risks of the PUL being failed PUL, intrauterine pregnancy (IUP) or EP. Previous study has demonstrated that this model can be reliably used to rationalize the management of PUL-s with a potential to reduce follow ups in 70 % of PUL-s by reducing the number of visits, scans and blood tests as well as intervention rates. The model demonstrated 80% sensitivity, 88% specificity and 97.5% negative predictive value (NPV) in symptomatic UK population.
Study design, size, duration: This was a retrospective observational study of all women who presented with symptoms of pain or bleeding at less than 6 weeks gestation diagnosed with PUL on transvaginal ultrasound between Jan 2015 and Dec 2017 in a single assisted conception unit. Women were classified as having a PUL if there was no evidence of intra- or extrauterine pregnancy on transvaginal ultrasound. In these women, the M4 model was used to predict the outcome of pregnancy.
Participants/materials, setting, methods: During the study period of three years, there were 3064 pregnancies, of which 408 women presented with symptoms before 6 weeks of gestation. Of these, 37.2% (152/408) were diagnosed with IUP; 26.2% (107/408) had PUL, 7.8% (32/408) had EPs and 28.5% (116/407) did not undergo transvaginal scan due to the rate of falling b-HCG-s. 14 of the PUL-s were excluded as hCG-s were inappropriately timed. The model was applied in 93 women diagnosed with PUL.
Main results and the role of chance: The M4 model predicted 24 failing PUL-s (25%), 18 IUPs (19%) and 51 EPs (54%). However, the final outcome in the studied cohort was as following: 48 failing PULs (51%), 20 IUPs (21%) and 25 EPs (26%).

The sensitivity for prediction of EP was 84%, specificity 72%, positive predictive value (PPV) 52.5% and NPV 92.4%. Comparison with studies in non-IVF population showed similar sensitivity and NPV, 80.8% and 98.6% respectively, with higher specificity (88.9%) and lower PPV (31.8%) in the non-IVF patients.

For prediction of low risk pregnancy (failing PUL and IUP) our results demonstrated sensitivity of 55.5%, specificity 84%, PPV 90.4% and NPV 41.1%. The results significantly differ from the data published for non-IVF population excluding the PPV.

One possible explanation for these differences is that IVF patients often present at much earlier gestation than their non-IVF counterparts. In addition, their characteristics may differ: we are certain about their gestational age and, frequently, the investigations are started because of patient's anxiety or having mild symptoms only.

In conclusion, M4 model has high ability to predict EP with some overestimation in an IVF population but lower ability to predict failing PUL and/or IUP compared with the one in non-IVF patients.
Limitations, reasons for caution: This is a retrospective observational study that includes relatively small number of patients. It included a selective population with IVF pregnancies from a single assisted conception unit. However this is the biggest population of IVF patients with PUL that has been studied.
Wider implications of the findings: According to our knowledge, this is the first study validating the M4 regression model in IVF pregnancies. The results confirm findings from other studies that M4 model has high sensitivity and NPV for EPs, however it has low PPV that can overestimate the risk of ectopic pregnancies in this population.
Trial registration number: n/a

P-359 Antibiotic treatment for chronic endometritis shows more efficacy in success rates of assisted reproductive technology than endometrial scratch in women with repeated implantation failure

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Study question: Is the efficacy of antibiotic treatment for chronic endometritis (CE) not due to antibacterial effect but the impact of endometrial scratch?
Summary answer: Antibiotic treatment for CE significantly improves the clinical outcomes in IVF over endometrial scratch alone.
What is known already: CE is defined as a local inflammatory disease and is diagnosed by the presence of plasma cells in the endometrial stroma. Some studies show that CE negatively impacts reproductive outcomes by endometrial receptivity and its prevalence is high in women with repeated implantation failure (RIF). Furthermore, it is also unknown that antibiotic treatment significantly improves the reproductive outcome in women with CE. Alternatively, some authors report that the endometrial scratch has a positive effect on reproductive

outcome. So, whether improvement of reproductive outcomes of women diagnosed as CE is due to antibiotic therapy or scratch remains controversial.
Study design, size, duration: Retrospective study in our clinic, between January 2017 to August 2018. Endometrial biopsy was performed in 156 women with RIF. CE was histopathologically diagnosed using immunohistochemistry. The histopathologic cure of CE was evaluated in the second/third-look biopsy obtained during the following menstrual cycle. Subsequent two cryopreserved-thawed ET cycles were follow-up.
Participants/materials, setting, methods: Biopsy was performed from 2-site in Uterus. CE was diagnosed with the presence of plural CD138 positive cells within the stromal in HPF. Women diagnosed as CE(Group1) had a 14-day oral antibiotic administration. After confirming the absence of CE, they had further embryo transfers (ET). Women without CE(Group2) had further ET promptly. Implantation rate (IR), pregnancy rate (PR), miscarriage rate (MR) and mean endometrial thickness on the day of ET (MET) were compared.
Main results and the role of chance: The prevalence of immunohistochemically confirmed CE was 38.5%(60/156). All of them underwent oral doxycycline(200mg/day) and metronidazole(1000mg/day) administration. In the second-look biopsy, the cure rate of CE was 71.7% (43/60). Seventeen women who did not confirm absence of CE were further treated with a combination of oral ciprofloxacin(800mg/day) and metronidazole(1000mg/day). The overall cure rate following two-step oral antibiotic treatment strategy was 98.3% (59/60). At IVF attempt after treatment, in both groups, reproductive outcome in cumulative two ET cycles was improved. Especially, a significantly higher IR and PR was reported in women from Group 1 compared with women from Group 2 (62.0 versus 35.8%, OR 2.60, 95%CI 1.44-4.20; 48.4 versus 29.3%, OR 2.22, 95%CI 1.52-3.80). Moreover, in MR, Group1 was significantly lower than Group2 (39.5 versus 54.5%, OR 0.51, 95%CI 0.30-0.85). MET was similar between the Group1 and Group2 (10.89±1.79mm versus 10.87±1.82mm p=0.97). No side effects due to antibiotics were observe.
Limitations, reasons for caution: Possible biases related to retrospective studies and to preferential referral of patients with CE, and limited number of cases. Moreover, embryo quality which potentially affect the reproductive outcome is not considered.
Wider implications of the findings: Antibiotic therapy for endometriosis has an positive effect for IVF success rate that exceeds endometrial scratch. This result is encouraging in women with RIF. Studies which consider embryo factor are required. And the association with endometrisis and uterine microbiota is very interesting.
Trial registration number: Not applicable

P-360 Study protocol: The association of paternal factors on recurrent pregnancy loss - REMI III study. (15/25 words)

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Study question: What is the effect of paternal lifestyle and somatic health factors on the development of unexplained recurrent pregnancy loss (RPL)?
Summary answer: We hypothesize that RPL is caused by both maternal and paternal factors; several paternal factors may be associated with the development of RPL.
What is known already: RPL, defined as 2 or more spontaneous pregnancy losses, affects 3% of all fertile couples. The current diagnostic work-up in these couples focusses on maternal conditions such as anti-phospholipid syndrome, structural uterine abnormalities, endocrine factors, thrombophilia and chromosomal translocations. Paternal investigations are currently limited to a male karyotype. Disappointingly, an underlying cause may be identified in only 30-50% of affected couples. Studies focusing on paternal influences are scarce, but some retrospective studies indicate an association of paternal factors such as advanced age, smoking and high sperm DNA damage with RPL or spontaneous miscarriage.
Study design, size, duration: This is a multicenter hospital-based research project consisting of two study designs: a case-control study to identify etiologic paternal factors for the development of RPL and a cohort study to investigate the prognostic effect of the paternal factors on the outcome of next pregnancies. Based on sample size calculations, at least 88 couples in each arm will be included prospectively and another 600 in each arm retrospectively. Follow-up of the RPL cohort is five years.
Participants/materials, setting, methods: Couples that visit the recurrent miscarriage outpatient clinics in the participating centers and fulfil the criteria of unexplained RPL will be included. Couples from the obstetrics outpatient clinic with ≤ 1 pregnancy loss will be included as controls. Obstetric and general medical history will be documented, data about lifestyle will be collected, as well as semen (for investigation of DNA fragmentation by TUNEL assay) and a peripheral blood sample (for immunomodulatory investigations) of the male.
Main results and the role of chance: The primary exposure of the case-control study is paternal age; secondary exposures are BMI, alcohol consumption, smoking, physical exercise, sperm DNA fragmentation and immunomodulatory factors in blood and seminal fluid. Primary outcome of the cohort study is the development of live birth within 5 years after initial visit of the recurrent miscarriage outpatient clinic. Secondary outcomes are development of ongoing pregnancy (>24 weeks), time interval until next pregnancy and development of pregnancy complications such as growth restriction, preterm delivery and preeclampsia. Since the design of this study is observational, there is need to control and adjust for confounding factors. Therefore, stratification and regression models will be used.
Limitations, reasons for caution: Besides the risk of bias by confounding, there also needs to be caution for the risk of recall and response bias with respect to data about lifestyle factors such as smoking and drinking behavior and physical exercise.
Wider implications of the findings: Findings of this study may ultimately lead to new diagnostic tools, new insight in therapeutic options and -by developing a couple-specific prediction model- to a tailored prognosis on future pregnancies for couples with RPL.
Trial registration number:
Not applicable.

P-361 Three different endometrial receptivity profiles can be defined in patients with previous failed embryo transfer

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Study question: What are the different profiles of the implantation window (IW) in patients with previous failed transfer under hormonal replacement therapy cycle?
Summary answer: Three profiles were observed: shorter IW (less than 48h), wide highly receptive IW (over 48h) and progressive sub-optimal IW (partially-receptive before becoming receptive).
What is known already: The implantation window is referred as a receptive period of the endometirum that is limited in time during which the embryo implantation is possible. This crucial step is a major limitation in the success of embryo transfer in IVF cycle. Recent studies have revealed that the timing and the length of the receptive stage is patient dependant. adhesio is a molecular diagnostic tool that determines the receptivity status by analyzing the expression level of 11 specific and predictive genes in the endometrial biopsies using quantitative RT-PCR.
Study design, size, duration: We analyzed the data from the patients that underwent an endometrial receptivity test (adhesio) at clinique ovo between June 2016 and December 2018 and had signed the research consent form. This retrospective study includes 118 endometrial biopsies form 51 patients with at least one previously failed transfer.
Participants/materials, setting, methods: In a mock hormonal replacement therapy cycle, biopsies were collected at progesterone (PG)+6 days and PG+8. Additional biopsies (PG+7, PG+9) were retrieved in a subsequent cycle if the initial samples were non-receptive. The expression level of 11 genes was quantified by qRT-PCR for the receptivity prediction. Using an algorithm, samples were classified receptive, non-receptive or partially-receptive and the optimal frozen embryo transfer day was recommended. The patient's mean count of previous failed transfer was 3.1±1.8.
Main results and the role of chance: The endometrial receptivity profiles measured with adhesio were grouped into 3 categories: shorter, wide highly receptive and progressive IW. A majority, 55% of the patients (n=28), had a

non-receptive endometrium in one sample and a receptive endometrium in the other sample collected at 48h interval. One patient had a very delayed and short IW: the PG+9 biopsy was the only receptive sample. 16% of the patient (n=8) had a wide highly receptive IW with both samples (PG+6, +8) being receptive, their IW lasted over 48h. 24% of the patients (n=12) had a partially-receptive endometrium and then a receptive endometrium 48 to 72 hours later. For 2 patients (4%), their endometrium was never found receptive after the analysis of 4 biopsies (PG+6, 7, 8 and 9). Twenty-two of the patients with a shorter IW had personalized frozen embryo transfer resulting in 32% cumulative pregnancy rate. Four patients with a wide IW had a personalized frozen embryo transfer resulting in a 50% pregnancy rate. Even through these patients had previous implantation failure, their infertility is clearly not due to the endometrial receptivity. Conversely no pregnancy was achieved in the progressive IW group (n=7), more investigation have to be complete for this profile.

Limitations, reasons for caution: The data was collected in a single IVF center. A multi-centric study with an increased sample size would give a better understanding of the endometrial receptivity profiles of patients with previous failed transfer under hormonal replacement therapy cycle.

Wider implications of the findings: This study demonstrates the variability in endometrial receptivity profiles and thus the importance of detecting the IW in patients with previous failed transfer in order to offer a personalized frozen embryo transfer.

Trial registration number: not applicable

P-362 Is the uterine immune profiling affected by maternal age?

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Study question: Is the endometrial immune profile influenced by maternal age as it is observed for the oocyte quality?

Summary answer: The endometrial immune profile does not seems to be influenced by maternal ageing in patients with recurrent implantation failures

What is known already: Advanced maternal age is associated with reduced fertility and adverse pregnancy outcomes. Maternal age mainly negatively affects the oocyte quality. Regarding the putative negative effects of ageing on the uterus, the association is largely undefined but potentially relates to impaired decidual and placental development and embryo interaction with the uterus. During "the implantation window", a transient immune switch of cells, together with an adequate uterine Natural Killer (uNK) cell activation occur. Such reaction appears fundamental in enabling the establishment of local maternal tolerance and survival of the fetus. We investigate the effect of age on this endometrial immune reaction.

Study design, size, duration: Between 2012 and 2018, 6151 patients with an history of implantation failures benefitted of an endometrial immune profiling because of their history of RIF. We compared the repartition of the uterine immune profiles among patients below 30 years (529 patients), between 30-35 y (1780 patients), 36-38 y (1552 patients),39-40 y (1030), 41-43 (944 patients), over 44 years old (382 patients).

Participants/materials, setting, methods: Patients included had an history of implantation failures with their oocytes (more than 6 embryos replaced) or after oocytes donation (more than 4 embryos replaced). An endometrial biopsy was performed under substituted cycle in the luteal phase. We quantified uNK by immunohistochemistry and mRNA of IL-15 (uNK cells activation/maturation state), IL-18 (Th-1/Th-2 cytokines balance) and TWEAK/Fn-14 (immuno-regulation) by Real-Time PCR. The uterine profile defined four categories of profile: not deregulated, over-regulated, under-regulated, mixt deregulation.

Main results and the role of chance: We recorded the same repartition of immune disequilibrium in the five categories of maternal age with no variation according to the category of age: 27.2% did not show deregulation, 30.6 % were over-activated, 31.6% were under-activated and 10.3% had a mixt profiles

Limitations, reasons for caution: The uterine immune profiling only explores immune local parameters related to the Th_1/ Th-2 equilibrium as well as recruitment, maturation state and activation of uNK cells. Immune profiling does not explore T regulatory cells, dendritics cells and macrophages recruitment and expression

Wider implications of the findings: As local disequilibrium does not seem not variate with maternal age, effective understanding of local uterine deregulations may be proposed in function of the clinical context but not iun function of the maternal age

Trial registration number: Not applicable

P-363 New biomarker predicts the odds of a live birth in women experiencing secondary recurrent pregnancy loss after a first-born boy

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Study question: Does soluble-leucocyte-associated immunoglobulin-like receptor-1 (sLAIR-1) in serum reflect immune activation and predict a poor pregnancy outcome in women experiencing secondary recurrent pregnancy loss (SRPL)?

Summary answer: High level of sLAIR-1 in gestational week (GW) 5 predicts low odds of live birth in women with SRPL after the birth of a boy.

What is known already: Immunology has been shown to be the cause of pregnancy loss (PL) in some women with SRPL. A first-born boy before the PLs is significantly more frequent than first-born girls and half of these pregnancies are obstetrically complicated, indicating that the first pregnancy initiated an abnormal immune reaction**.** LAIR-1 is an inhibitory immune receptor expressed by immature immune cells. The expression decreases in activated immune cells, partly by shedding the receptor, transforming LAIR-1 to soluble LAIR-1 (sLAIR-1). Thus, higher levels of sLAIR-1 in serum reflect an activated immune system and have been found in patients rejecting their transplanted organ.

Study design, size, duration: A single-center, double-blinded, placebo-controlled trial including 82 women with SRPL between August 2008 - August 2013. The patients were randomized to either infusions with "Immunoglobulin Human" 120 mg/ml or "Privigen® " 100 mg/ml (IVIg) or placebo infusions with "Human Albumin" 5 % infusion. Eight infusions were given between GW 5-16 or until PL. The patients were block-randomized 1:1 having either 4 or $\geq$ 5 previous PLs.

Participants/materials, setting, methods: The trial was conducted at the RPL Unit at Copenhagen University Hospital in Denmark. Eighty-two women with unexplained SRPL and at least four PLs (at least three consecutive PLs after birth) were included. Serum samples were taken before the first infusion in GW 5 in the trial and 77 women gave consent to participate in this study. Multiple logistic regression was performed to evaluate predictors of live birth.

Main results and the role of chance: Median sLAIR-1 serum level in GW 5 was 268 pg/ml (from not detectable – 5122 pg/ml) in 77 women. Thirty-nine of the women had a first-born boy. The trial pregnancy resulted in a live birth in 20/39 women with a first-born boy equally distributed between the intervention groups (IVIg and placebo, 1:1). Median age in the live birth group was 34.5 years which is comparable to 34 years in the PL group. Number of previous PLs, BMI and smoking were comparable between the two groups. sLAIR-1 was dichotomized as either positive or negative with a positive cut-off in sLAIR-1 based on a ROC-curve predicting outcome of pregnancy (sLAIR-1 >499 pg/ml). Among women with a first-born boy, positivity for sLAIR-1 was a significant negative predictor for live birth (aOR 0.19, 95% CI 0.04-0.83, *p-value*= 0.027) adjusted for number of previous PLs, female age and IVIg treatment, which were not predictors of live birth in a multiple logistic regression analysis. A follow-up in January 2019 showed no association between positive sLAIR-1 in the trial pregnancy and cumulative live birth rate. There was no association between positive sLAIR-1 and outcome of the trial pregnancy when including women with first-born girls in the analysis.

Limitations, reasons for caution: Even though the study includes highly selected patients with more than four previous PLs and no risk factors of RPL

after standard clinical evaluation the numbers are still relatively small. Despite the significant findings, they need to be evaluated in a larger study.

Wider implications of the findings: A high level of sLAIR-1 in GW 5 in women with SRPL after a first-born boy is a predictor of loss of that pregnancy, but not long-term outcome. Thus, sLAIR-1 has potential for optimal selection of women with suspected immunological disturbances eligible for participation in RCTs with immune modulatory treatments.

Trial registration number: Clinicaltrials.gov NCT00722475.

P-364 To screen or not to screen? Prevalence of thrombophilia in patients with recurrent miscarriage and healthy controls

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Study question: Does the prevalence of inherited and acquired thrombophilia differ between patients with recurrent miscarriage (RM) and healthy controls?

Summary answer: There is no difference in the prevalence, neither in inherited nor in acquired thrombophilia between RM patients and healthy controls.

What is known already: Screening for thrombophilia is discussed controversially in clinical guidelines for RM whereas screening for Anti-phospholipid Antibody Syndrome (APS) is highly recommended. Several studies investigated the impact of inherited and acquired thrombophilia in RM and found contradictory results. Possible pathophysiological mechanisms concerning thrombophilia and RM include development of microthrombosis in the early placentation. However, the association between thrombophilia and RM seems to vary according to the type of inherited or acquired thrombophilia.

Study design, size, duration: A retrospective observational study was performed. The study group consisted of n= 207 patients with ≥3 miscarriages, screened for inherited and acquired thrombophilia in the outpatient clinic of the Department of Gynecological Endocrinology and Reproductive Medicine, Medical University Innsbruck, between January 2010 and January 2018. The control group (n= 141) consisted of young healthy women who underwent a routine screening for inherited and acquired thrombophilia before prescription of contraceptives between 01/2011 and 04/2018.

Participants/materials, setting, methods: Medical history (gravidity, parity, number of previous miscarriages, history of thrombosis), anatomical malformations (myomas, adhesions, septate uterus), haemostaseological alterations (APC resistance, protein C/S deficiency, factor VIII activity), autoimmunological changes (anti-nuclear antibodies, anti-phospholipid antibodies, thyroid antibodies), endocrine disorders (thyroid function, PCOS) and genetic data was assessed in patients and controls. The prevalence of alterations in APC resistance, protein C/S deficiency, factor VIII activity and anti-phospholipid antibodies were defined as primary outcome measures and compared between groups.

Main results and the role of chance: The mean age was 33.4±5.2 years in the RM group and 15.6±1.9 in the control group (mean±SD, p< 0.001). The BMI was higher in the RM group (mean±SD: 24.1±4.4 vs. 21.5±4.4, p< 0.001). 1.6% of the RM patients had a history of venous thrombosis and 23.8% had a positive family history of thromboembolism, compared with 0.85% and 15.1% in the controls.

No significant differences in the prevalence of protein C/S deficiency, APC resistance and factor VIII elevation were observed in RM patients and controls (RM vs. control: protein C 0.51% vs. 0.0%, p> 0.05 ; protein S 2.5% vs. 3.55%, p= 0.09; APC resistance 4.85% vs. 11.35%, p= 0.29; factor VIII elevation 11% vs. 10.95%, p= 0.78). The occurrence rate of antiphospholipid antibodies was the same in both groups (RM vs. control: anti-phospholipid-screen IgG 0.99% vs. 0,72%; anti-phospholipid-screen IgM 1.48% vs. 0.72%; anti-prothrombin antibodies 2.96% vs. 0%; beta-2 glycoprotein antibodies 1.97% vs. 0.0%; anti-cardiolipin antibodies 0.99% vs. 0.0%). One patient in the RSA group fulfilled the Sydney criteria and was therefore diagnosed with an APS, whereas no patient in the control group was diagnosed with APS (prevalence for APS 0,5% vs 0%).

Limitations, reasons for caution: Due to the younger age of the control group it is not predictable whether they will experience RM in future. However, the control group is representative for the population.

Wider implications of the findings: Our data does not show a significant difference neither for inherited nor acquired thrombophilia in RM. The conflicting results of other studies may be due to small study sizes and the inconsistent definition of RM. Moreover, the prevalence of inherited or acquired thrombophilia may be overestimated in RM patients.

Trial registration number: not applicable

P-365 Is embryonic aneuploidy rate higher in abortuses of early spontaneous abortions of D6 blastocyst than D5?:a retrospective study

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Study question: The purpose of this study was to compare the aneuploidy rate in first trimester abortion after frozen-thawed Day 5 and Day 6 blastocyst transfer.

Summary answer: Embryonic aneuploidy does not account for inferior pregnancy outcome after D6 TBT. Women's age was independently associated with the embryonic aneuploidy rate.

What is known already: Although there is a better endometrial–embryonic synchronization in frozen cycles, many studies have reported lower clinical pregnancy rate and live birth rate while higher spontaneous abortion rate after D6 thawed blastocyst transfer (TBT). It remains uncertain whether the inferior outcome after D6 TBT is caused by embryonic chromosomal abnormalities. Some reports demonstrated higher aneuploidy rates in embryos that blastulate on D6 compared with those on D5. But some research did not find the same trend. However, all these studies were just on the basis of preimplantation embryos while we focus on arrested embryos during early pregnancy.

Study design, size, duration: This is a retrospective cohort study examining the rate of embryonic chromosomal anomalies in the abortuses of early spontaneous abortions between March 2012 and February 2018 in an university-based reproductive medicine center. All the recruited patients were conceived after frozen blastocyst transfer and only hormone replacement cycles were included.

Participants/materials, setting, methods: Chorionic villus samples were collected from a total of 324 patients defined as missed abortion. Conventional chromosome karyotyping and a multiplex ligation dependent probe amplification subtelomere assay combined with fluorescence in-situ hybridization were performed for genetic analysis. The aneuploidy rate was compared according to the blastocyst development stage: D5 (n =226) and D6 (n =98).Multivariate logistic regression model was conducted to demonstrate the risk factors affecting the embryonic aneuploidy.

Main results and the role of chance: Patients' characteristics, including age, BMI, basal FSH, type of infertility, IVF/ICSI procedures, gestational age did not differ between the two groups (all P values>0.05). Although mean number of embryo were significantly higher in D6 than in D5 group (P<0.001), the mean number of high-quality embryo were similar (P=0.334). In D5 group, 39.38% (89/226) of abortuses demonstrated aneuploidy, which was comparable with 40.82% in D6 group (40/98, P=0.808).

A multivariate logistic analysis was performed to adjust for potential confounders such as the women's age during OPU, BMI, and type of infertility, IVF/ICSI procedures, and the day of blastocyst (D5 or D6), number and quality of embryo. The results showed that women's age at the time of OPU was found to be independently associated with the embryonic aneuploidy rate (OR = 0.89; 95% CI: [0.85–0.96]; P =0.001). Other factors, including blastocyst expansion stage were not important predictors of aneuploidy (OR = 0.93; 95% CI: [0.54–1.59]; P =0.792). When we further analyzed them according to women's age (>35 years or ≤35 years),embryonic aneuploidy rates were both comparable in older group (62.0% versus 53.5%; P=0.468) and younger group (32.9% versus 35.71%; P = 0.68) after D5 or D6 TBT.

Limitations, reasons for caution: The study was limited by its inherent shortcomings of retrospective studies and relatively small sample size.

Wider implications of the findings: The inferior developmental potential of D6 blastocysts may not be due to chromosomal abnormalities. We need to focus on different aspects related to blastocyst development.

Trial registration number: not applicable

P-366 Early, previously unrecognized, implantation of a second embryo detected using single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT)

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Study question: Can single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) detect cell-free DNA (cfDNA) from two embryos in maternal circulation despite clinical records indicating a singleton pregnancy?
Summary answer: SNP-based NIPT can establish whether cfDNA is present from one or more fetal haplotypes in maternal blood from a double embryo transfer (DET), singleton pregnancy.
What is known already: The frequency of singleton pregnancies resulting from DET is ~50%. Early implantation, resulting in a non-viable embryo, is difficult to ascertain using late, first-trimester ultrasound and chromosomal abnormalities are common in this scenario. Quantitative NIPT results may be confounded if cfDNA from a chromosomally abnormal, non-viable embryo persists in the maternal bloodstream. These methodologies cannot account for additional haplotypes, indicative of a multiple gestation or triploid pregnancy. Studies have previously demonstrated the unique ability of SNP-based NIPT to detect the presence of cfDNA eight-fifteen weeks post fetal-demise, however this research did not specifically address the in-vitro fertilization (IVF) cohort
Study design, size, duration: This series includes nine pregnancies, noted as singletons on the test requisition, with SNP-based NIPT results between January 11, 2018 and November 28, 2018. All cases were identified by the testing algorithm as having results indicative of a pregnancy with a vanished twin, unrecognized multiple gestation or fetal triploidy, due to evidence of an additional haplotype. As part of standard protocol, the ordering clinician's office was contacted for further discussion.
Participants/materials, setting, methods: Confirmation of a viable singleton gestation achieved with in-vitro fertilization (IVF) and DET was confirmed by the ordering clinician's office. Records did not indicate evidence of vanished or ongoing twins and none of the cases had any reported clinical findings suggestive of triploidy in the first trimester. The gestational age of the cases at the time of the blood draw for NIPT ranged from 10w1d–13w1d.
Main results and the role of chance: In the setting of IVF pregnancies achieved with DET, obstetricians ordering NIPT at 9+ weeks gestation may lack clinical evidence regarding implantation of one or both embryos. This case series demonstrates the unique ability of SNP-based NIPT to identify the presence of multiple fetal haplotypes in this scenario. If one set of cfDNA is identified, during analysis, the provider can be confident that the results are representative of the viable singleton pregnancy. Conversely, if multiple haplotypes are seen, the patient is not a good candidate for any NIPT as cfDNA may persist in the maternal blood stream for an undetermined number of weeks. In addition, multiple haplotypes can be suggestive of a singleton triploid pregnancy, so further evaluation should be considered.
Limitations, reasons for caution: A limitation of this study is the incomplete follow-up with unobtainable clinical details such as early ultrasound and cytogenetic reports, and/or pregnancy outcome. Larger, blinded, prospective studies in collaboration with IVF centers utilizing SNP-based NIPT are needed to further investigate the frequency of unrecognized early implantation of a second embryo.
Wider implications of the findings: In singleton pregnancies achieved with IVF and two- embryo transfer, knowledge of additional haplotypes has implications for pregnancy management. The use of SNP-based NIPT should be considered in this setting in order to avoid false positive results leading to unnecessary invasive testing and parental concern.
Trial registration number: N/A

P-367 Increased miscarriage rates following follicular-phase endometrial scratching

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Study question: Does intentional endometrial injury (scratching) during the follicular phase of ovarian stimulation (OS) increase pregnancy rates in ART?
Summary answer: Pregnancy rates did not vary significantly between the endometrial injury and the control group. However, significantly higher clinical miscarriage rates were observed after endometrial injury.
What is known already: Intentional endometrial injury has been put forward as an inexpensive clinical tool capable of enhancing endometrial receptivity. However, despite its widespread use, the benefit of endometrial scratching remains controversial, with several recent randomized controlled trials (RCTs) not being able to confirm its added value. So far, most research has focused on endometrial scratching during the luteal phase of the cycle preceding the one with embryo transfer (ET), while only few studies investigated in-cycle injury during the follicular phase of OS. Also, the persistence of a scratch effect in subsequent treatment cycles remains unclear.
Study design, size, duration: This RCT included women performing IVF/ICSI in a gonadotrophin-releasing hormone antagonist supressed cycle between 2014-2018. Participants were randomized to either undergo an endometrial biopsy between day 6 to 8 of OS or to be in the control group. The primary outcome was clinical pregnancy at 7 weeks. Secondary outcomes included live birth, early pregnancy loss, procedure pain/bleeding and cumulative live birth following all ETs performed within 6 months of the study cycle.
Participants/materials, setting, methods: A total of 200 subjects (100 per study arm) were recruited, with 1 (in the control group) later withdrawing consent to participate further. In 4 patients allocated to the intervention group biopsy was impossible due to cervical stenosis or intolerable pain. The trial was stopped prematurely after an analysis in 200 of the required 360 patients. During this interim analysis, a statistically significant higher clinical miscarriage rate in the intervention group was found.
Main results and the role of chance: The study arms did not vary significantly in terms of relevant patient and IVF/ICSI cycle characteristics. The intention-to-treat clinical pregnancy rates did not vary significantly among the biopsy and the control arms (respectively, 44.0% versus 40.4%, p=0.61), nor did the live birth rates (respectively, 32.0% versus 36.4%, p=0.52). Biochemical pregnancy loss was comparable between both groups (10% in the intervention group versus 15% in the control, p=0.49), however, clinical miscarriage occurred significantly more frequent in the biopsy group (25% versus 8%, p=0.032). In the intervention group, 3% of the patients experienced procedure pain and 5% bleeding. Cumulative live birth rates taking into account all ETs performed within 6 months of the study cycle were not significantly different between the biopsy and the control group (respectively, 54.0% versus 59.6%, p=0.43).
Limitations, reasons for caution: These results are limited by the fact that the trial was stopped prior to the predetermined sample size. This, together with the pragmatic design of the study, may have limited the detection of specific subgroups of women who may benefit from endometrial scratching.
Wider implications of the findings: These findings add to the growing evidence that scratching in an unselected patient population, regardless of the period when it is done, seems to be useless. They strongly discourage intentional endometrial injury during the follicular phase of OS as it is potentially harmful. Moreover, no long-lasting benefit was found either.
Trial registration number: ClinicalTrials.gov identifier NCT02061228.

P-368 The use of granulocyte macrophage colony-stimulating factor (GM-CSF) containing media for embryo transfer increases the clinical pregnancy and ongoing pregnancy rate

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Study question: Is it possible to improve the clinical outcome of vitrified-warmed embryo transfer by using a medium containing GM-CSF as a medium for embryo transfer?
Summary answer: It was suggested that clinical pregnancy rate and ongoing pregnancy rate are increased by using GM-CSF containing medium as the embryo transfer medium.

What is known already: GM-CSF is one of various cytokines and growth factors present in the female reproductive tract that are known to play an important role in embryonic development, embryo implantation and subsequent development.The expression level of GM-CSF usually increases at the time of implantation. However, among those who have undergone implantation failure or repeated miscarriage, the expression level and the concentration of GM-CSF may be low. This condition is thought as one of the causes of recurrent implantation failure and miscarriage.

Study design, size, duration: A retrospective observational study was performed by 473 cycles with patient ages 30 - 39 who underwent vitrified-warmed single blastocyst transfer at our clinic from February to November 2018.

Participants/materials, setting, methods: We investigated 148 cycles using GM-CSF containing culture medium (SAGE 1-Step with GM-CSF; CooperSurgical, Denmark) (test group) and 325 cycles using GM-CSF free medium (NAKA ONE STEP MEDIUM; NakaMedical, Japan) (control group) at the time of post-thaw culture and embryo transfer.Clinical pregnancy rate, miscarriage rate and ongoing pregnancy rate were compared and examined.In both groups, embryo culture was carried out in the GM-CSF-free medium before the blastocysts were cryopreserved.

Main results and the role of chance: Clinical pregnancy, miscarriage and ongoing pregnancy rates after embryo transfer were compared between the two groups.There was no significant difference in the mean age between the test group and the control group (p = 0.24, 35.2±2.8years vs. 35.5±2.7years).The clinical pregnancy rate in the test group was higher than that in the control group (p = 0.045, 52.0% vs. 42.2%). The miscarriage rate in the test group was lower than that in the control group (p = 0.076, 10.4% vs. 19.7%). The ongoing pregnancy rate in the test group was higher than that in the control group (p = 0.007, 46.6% vs. 33.8%).

Limitations, reasons for caution: This study was limited by the data based on a small sample size and lack of data about live birth rates after embryo transfer.

Wider implications of the findings: It was suggested that medium containing GM-CSF improves implantation and ongoing pregnancy rate when used for embryo transfer.Using GM-CSF-containing medium for embryo transfer may be an effective treatment strategy for some patients by shortening the time to pregnancy.

Trial registration number: Not applicable.

P-369 The prognostic value of Endometrial Receptivity Array in women with Recurrent Implantation Failure

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Study question: To compare patients who had a receptive ERA to those who had a non-receptive ERA and to describe clinical outcomes of embryo transfer after a personalized ET (pET)

Summary answer: Women with advanced age and POR had higher probablilty of having nonreceptive ERA.

In RIF patients, a nonreceptive endometrium suggests a poorer outcome even after pET.

What is known already: Embryo implantation requires that the blastocyst will attach during the receptive stage of the endometrium, known as window of implantation (WOI).

Molecular analyses of endometrial receptivity demonstrates a personalized WOI (pWOI) that is displaced in one out of four patients suffering from recurrent implantation failure (RIF) of endometrial origin and illustrates the utility of a personalized endometrial diagnostic approach

Study design, size, duration: This is an observational study conducted between January 2013 and September 2018 at Milann Fertility Centre Bengaluru, India where 60 patients with recurrent implantation failure who underwent endometrial receptivity array were included.

Participants/materials, setting, methods: Out of 60 patients, 39 had an ET after ERA testing. Patient demographics and cycle characteristics were collected.

Baseline characteristics were compared between patients who tested receptive and non-receptive. Implantation rates were compared for the receptive and non-receptive groups after all subsequent ETs.

Pregnancy outcomes were compared for the receptive and non-receptive groups after the first ET after ERA.

Excel and SPSS were used to perform permutation analyses and t-tests.

Main results and the role of chance: Out of 60 patients,41(68.3%) had receptive ERA result while 19(31.6%) had non receptive ERA result.

Women with advanced maternal age(p =0.02) and poor ovarian reserve (p=0.08), had statistical significant probability of being non receptive on ERA.

30 patients out of 41 in receptive group and 9 patients out of 19 in the nonreceptive group underwent subsequent personalized embryo transfer.

When analyzing implantation rates and clinical pregnancy rates, clinical pregnancy was significantly higher (p=0.03) in the receptive group compared to the nonreceptive group even after pET.

Limitations, reasons for caution: Our studyis limited by its smaller sample size.

Wider implications of the findings: Furtherstudies should be done to confirm the benefit of pET in patients with a non-receptive endometrium.

Trial registration number: 043/I/19/02

P-370 Comparison of low molecular weight heparin (LMWH) plus Aspirin, versus Aspirin only treatment on pregnancy outcomes of unexplained recurrent early miscarriage: a randomized clinical trial

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Study question: Does LMWH medication affect pregnancy outcome of early recurrent pregnancy loss (RPL) concurrent with aspirin treatment?

Summary answer: Although a minimally improvement in pregnancy outcomes has been observed following LMWH medication in patients with RPL, but the difference was not statistically significant.

What is known already: Recurrent abortion is still an issue for both physicians and patients. Especially in early abortions when the cause is largely unexplained. Despite the fact that fetal aneuploidies is the most causes of early abortions, use of heparin and aspirin and other treatments continues in this group of patients. Previous studies were largely unproductive, since heterogeneity and intervention time were different.

Study design, size, duration: The study was performed as a single blind randomized clinical trial between years 2015-2018. The sample size was calculated 164 (two groups of 82 patients) and patient were included into the study timely. At the time of abstract submission, the outcomes of 132 patients were completely determined.

Participants/materials, setting, methods: Samples were selected from patients who was referred to Avicenna RPL clinic with a history of at least 2 previously happened early un-explained miscarriages (G.A <10 weeks) based on diagnostic laboratory, imaging and genetic tests. Patients who got pregnant recently were divided randomly in two groups: low dose Aspirin plus LMWH treatment (4000 IU) (group A) and low dose Aspirin treatment only (group B) and the patients were followed up till their pregnancy termination (delivery/abortion).

Main results and the role of chance: 132 Patients were divided in two groups (group A =65 and group B=67 patients). Considering the significant P-value level < 0.05 there were no difference between two groups in age (CI = -1.580 - 1.37) and BMI (CI = -1.26 -2.32). Both study groups were also homogeneous in terms of abortion time, primary and secondary miscarriage, interventions before and during pregnancy, doses and timing of prescribe heparin and aspirin and enough follow up till birth. The live birth rates in groups A and B were 80% VS 73.1 % respectively which did not show any statistically significant difference between Aspirin plus LMWH treatment comparing Aspirin treatment only (Pearson chi square P-value = 0.352). Based on multivariate analysis using logistic regression model there were significant adverse relationship between successful pregnancy outcome and both of age (β= -0.179) and the number of previous miscarriage(β= -0.827) but none of them did not affected by LMWH additive treatment.

Limitations, reasons for caution: Although evidences about positive effect of LMWH on un-explained RPL outcomes aren't convincing, the main limitation

was pseudo-ethical issue in depriving patients from conventionally prescribing of heparin. Patients with bad experiences of RPL (especially when abortion ≥ 5) hardly accept to be allocated in control group with minimal medical interventions.

Wider implications of the findings: In patients with early recurrent abortions, it seems aspirin prescription is good enough to reach a satisfying result and benefit of excessive anticoagulant was not proven statistically in the study. Similar trials are suggested for evaluation of anticoagulant treatments in 2nd trimester recurrent pregnancy loss.

Trial registration number: The"Clinical Trial of LMWH plus Aspirin versus Aspirin Alone in Unexplained Recurrent Miscarriage" has been registered in Iranian registry of clinical trial by number IRCT20160403218 9N1

P-371 Prospective comparative analysis of WNT4, WNT6 and b-catenin expression in trophoblast tissue from first trimester miscarriages versus terminations of pregnancy for social reasons

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Study question: Is there a difference in the expression of WNT4, WNT6 and b-catenin in trophoblast tissue from first trimester miscarriages versus normal controls (social abortions)?

Summary answer: WNT4 expression was statistically significantly altered in first trimester miscarriages versus controls. There was no significant difference for WNT6 or b-catenin.

What is known already: The role of wnt pathway in pre-implantation events remains to be elucidated. Animal studies have shown than wnt signaling is important for embryo development and is involved with blastocyst activation and implantation potential. Especially WNT4 expression has been associated with normal uterine decidualization in mice and seems critical for embryo implantation. Reduced WNT4 expression has been observed in women with severe preeclampsia. Furthermore, WNT6 seems critical for normal stromal cell proliferation in mice. B-catenin, key component of the canonical wnt pathway, also has a role to play in the regulation of implantation although the results so far have been conflicting.

Study design, size, duration: The gene expression of WNT4, WNT6, b-catenin and Alu repeat amplification (used for normalization) was studied using reverse transcription PCR and real time PCR in trophoblast tissue retrieved after evacuation of retained products of conception due to early embryonic demise, incomplete miscarriage or termination of pregnancy for social reasons. The protocol of the study was approved by the Institutional Review Board of Patras Medical School, in accordance with Declaration of Helsinki. Participants signed informed consent.

Participants/materials, setting, methods: 14 samples were included for each study group. Only euploid conceptions were included. The quality of the PCR reactions was confirmed by melting curve analysis and the $2-\Delta\Delta Ct$ algorithm was used to analyze the relative expression of the target genes in comparison to the Alu repeat amplification. Kolmogorov-Smirnov test and Shapiro-Wilk test showed that the ΔCp was normally distributed in all groups. Independent sample t-test was used to compare the data.

Main results and the role of chance: There was a significant difference in the ΔCp of WNT4 for terminations of pregnancy and miscarriages ($p=0.002$, power=0.98). Significant difference was also identified between terminations of pregnancy and complete miscarriages ($p=0.014$, power=0.73) and terminations of pregnancy and incomplete miscarriages ($p=0.004$, power=0.86). There was a 7.04 fold increase in the expression of WNT4 gene in miscarriages compared to terminations of pregnancy, 5.5 fold increase in early embryonic demise compared to terminations and 9.02 fold increase in incomplete miscarriages compared to terminations of pregnancy. No significant difference was documented for the expression of WNT6 and b-catenin. There were no significant differences in patients' demographic background and gestational age.

Limitations, reasons for caution: Gene expression was explored at the mRNA level. The next step will be to analyze protein expression since b-catenin is mostly regulated through proteasome degradation. Although in pregnancy terminations fetal heart activity was detected on the day of the procedure, we cannot be certain that these pregnancies would develop normally.

Wider implications of the findings: This is to our knowledge the first study in human trophoblast to demonstrate statistically significant alteration in the WNT4 expression in miscarriages versus normal pregnancies. This highlights a potential role of the WNT pathway in placentation and trophoblast function and offers a potential target for future strategies to prevent miscarriage.

Trial registration number: not applicable

P-372 New relation between dysbiosis of the vaginal and endometrial microbiota and RIF found

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Study question: Is dysbiosis of the vaginal and endometrial microbiota a causing factor of repeated implantation failure (RIF)?

Summary answer: The dysbiosis of vaginal and endometrial microbiota was identified to be a strong biomarker for RIF.

What is known already: It is now possible to comprehensively analyze the microbiota of reproductive organs by using the next generation sequencer (NGS), the results of microbiota analysis have been reported not only for the vagina but also for the uterus, which was conventionally thought to be aseptic. In the uterine microbiota analysis, it was reported that when the *Lactobacillus* ratio was 90% or more, the implantation rate, pregnancy rate, and birth rate were significantly higher.

Study design, size, duration:

This study was conducted from October 2017 to June 2018. It was performed retrospectively for 166 women (RIF patients : 145 , Controls : 21) who consented to participate. It was approved by the Saint Mother Clinic's ethical committee.

Participants/materials, setting, methods: 145 women with a blastocyst Gardner's classification of 3BB or higher who had had more than three unsuccessful embryo transfers were the subjects of this study..21 healthy women were also enrolled as controls.

We investigated their vaginal and endometrial microbiota using 16S rRNA gene pyrosequencing.

We measured the α diversity of the microbiota in the samples and then compared the bacterial percentages in the RIF and control groups.

Main results and the role of chance: The endometrial microbiota had a higher α diversity than the vaginal microbiota(Controls $p=2.41e-07$;RIF patients $p<2.2e-16$).

In the endometrial microbiota, 20 kinds of bacteria genera (*Delftia, Schlegelella, Burkholderia, Gardnerella, Sphingobacterium, Prevotella, Megasphaera, Cloacibacterium, Dietzia, Rothia, Enterococcus, Atopobium, Micrococcus, Staphylococcus, Ralstonia, Exiguobacterium, Hydrogenophaga, Sediminibacterium, Limnohabitans,* and *Vagococcus*) showed significantly different levels between controls and RIF patients (all, $p < 0.05$). In the vaginal microbiota, 7 kinds of bacteria genera (*Corynebacterium, Atopobium, Megasphaera, Varibaculum,*

Gardnerella, Peptoniphilus, and *Prevotella*) showed significantly higher levels in the RIF patients ($p < 0.05$).

Unlike previous reports, we discovered that there was no significant difference in the endometrial *Lactobacillus,* where average levels of *Lactobacillus* were 51.6 ± 38.33 % in controls and 51.15 ± 37.48 % in the RIF patients ($p = 0.961$). However, average levels of vaginal *Lactobacillus* were 91.8 ± 22.73 % in controls and 76.38 ± 38.85 % in the RIF group ($p = 0.015$) This is a significant difference.

Limitations, reasons for caution: Even thought we found that microbiota seems to play a role in RIF, it is not the only cause. We must conduct further studies to clarify this role.

Wider implications of the findings: Analysis of the vaginal and endometrial microbiota using 16S rRNA gene pyrosequencing may be a new biomarker of RIF, it may help treat it and consequentially help raise the implantation success rate of RIF patients.

Trial registration number: Medical Information Network-Clinical Trial Registration, Japan (UMIN-CTR000031731) on March 15, 2018.

P-373 Efficacy of intrauterine administration of autologous peripheral blood mononuclear cells prior to embryo transfer in patients with recurrent implantation failures in assisted reproductive technology programmes

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Study question: To evaluate the efficacy of intrauterine administration of autologous peripheral blood mononuclear cells (PBMC) prior to embryo transfer in patients with recurrent implantation failures in IVF programmes

Summary answer: Intrauterine administration of autologous PBMC prior to embryo transfer in an IVF/ICSI programme increases the efficacy of IVF programmes in patients with recurrent implantation failures

What is known already: The direct effect of PBMC on implantation was first investigated by Yoshioka who demonstrated that intrauterine administration of autologous PBMC prior to transfer of embryos cultured with hCG significantly increased the rates of implantation, clinical pregnancy and delivery in patients with recurrent implantation failures during an IVF programme.Later studies showed that intrauterine administration of PBMC increases the implantation rate in FET cycles in women with a history of up to 3 implantation failures.Further studies also demonstrated positive effects of autologous PBMC activated by hCG prior to embryo transfer in patients with a history of two or more implantation failures

Study design, size, duration: To obtain the PBMC fraction, blood samples were obtained from the ulnar vein, under fasting conditions: on the transvaginal ovum retrieval day during a stimulated cycle and on the first day of micronized progesterone administration during an FET cycle.

Participants/materials, setting, methods: Group 1-autologous PBMC activated with Pregnyl 500 IU prior to embryo transfer.Group 2-PBMC without hCG activation prior to embryo transfer.Group 3-intrauterine administration of saline prior to embryo transfer.Based on different ART protocols two subgroups were formed within each of the three groups:Subgroup 1a-PBMC+hCG group in stimulated cycle,Subgroup 1b-PBMC+hCG group in FET.Subgroup 2a-PBMC group in stimulated cycle; Subgroup 2b- patients of the PBMC group in FET;Subgroup 3a-placebo group in stimulated cycle;Subgroup 3b-placebo group in an FET

Main results and the role of chance: We performed a comparative assessment of ART outcomes obtained after intrauterine administration of autologous PBMC, with or without hCG activation prior to embryo transfer in a stimulated cycle or FET cycle in patients with recurrent implantation failures.During a stimulated cycle, the rates of positive blood beta-hCG tests, implantation, and clinical pregnancy were significantly higher in the group of patients receiving intrauterine administration of autologous PBMC, both with and without hCG activation, as compared with the respective rates in the placebo group ($p<0.05$).Patients receiving hCG-activated PBMC had higher rates than subjects who received PBMC without hCG activation; however, the differences were not statistically significant ($p<0.05$). During an FET cycle, the rates of positive blood beta-hCG tests, clinical pregnancy, and implantation were higher in the group of patients receiving hCG-activated PBMC, as compared with the respective rates in the non-activated PBMC group and in the placebo group ($p<0.05$). In the hCG-activated PBMC group, these rates were higher than in the non-activated PBMC group, although these differences did not reach statistical significance ($p<0.05$).

Limitations, reasons for caution: intrauterine administration of hCG-activated autologous PBMC prior to embryo transfer during stimulated cycles or FET cycles increases the implantation rate and the occurrence of clinical pregnancy in women with a history of recurrent implantation failures.The obtained data lead us to recommend this immune-targeted method for patients with recurrent ART failures.

Wider implications of the findings: no

Trial registration number: not applicable

P-374 Effect of autologous platelet-rich plasma treatment on refractory thin endometrium during the frozen embryo transfer cycle

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Study question: Does intrauterine administration of autologous platelet rich plasma (PRP) for treating refractory thin endometrium have a beneficial effect on the implantation and pregnancy rates?

Summary answer: The use of autologous PRP improved clinical pregnancy and live birth rates (LBR) of the patients with refractory thin endometrium.

What is known already: Thin or damaged endometrium remains to be an unsolved problem in the treatment of patients with infertility. The empirical preference for endometrial thickness (EMT) among clinicians is >7mm, and the refractory thin endometrium, which doesn't respond to standard medical therapies, can be the etiology of recurrent implantation failure (RIF). Autologous platelet-rich plasma (PRP) is known to help tissue regeneration and is widely used in various fields

Study design, size, duration: A prospective cohort study was conducted in a university hospital between January 2015 and December 2018. Women who had a history of two or more failed IVF cycles and refractory thin endometrium were enrolled in this study. The main inclusion criteria were EMT of < 7 mm after more than 2 cycles of previous medical therapy for increasing the EMT. 40 women were enrolled in this study

Participants/materials, setting, methods: The subjects were treated with intrauterine infusion of autologous PRP 2 or 3 times from menstrual cycle day 10 of their frozen-thawed embryo transfer (FET) cycle, and ET was performed 3 days after the final autologous PRP infusion. The primary outcomes were the ongoing pregnancy rate and LBR. The secondary outcomes were the implantation rate, clinical pregnancy rate, and EMT increment compared with those on the previous cycle

Main results and the role of chance: 40 women underwent FET, and 3 patients were lost to follow up. The clinical pregnancy rate was 29.7%. The ongoing pregnancy rate and LBR were 16.2% and 13.5%, respectively. All the differences were statistically significant. One patient is still on-going pregnancy state. The implantation and clinical pregnancy rates were 14.7%. The average increase in the EMT was 0.7 mm compared with the EMT of their previous cycle. However, this difference was not statistically significant. Further, EMT of 21 patients increased (mean difference: 1.6 mm), while that of 11 patients decreased (mean difference: 0.9 mm); the EMT of 5 patients did not change. There were no adverse effects reported by the patients who were treated with autologous PRP.

Limitations, reasons for caution: This is not a randomized controlled study and a relatively small number of patients were enrolled
Wider implications of the findings: We assume that the mechanism of endometrial receptivity restoration after autologous PRP treatment has some aspects other than increasing the EMT. The molecular basis of the treatment needs to be revealed in future studies
Trial registration number: KCT0003375 at https://cris.nih.go.kr/cris

P-375 Hysteroscopy, performed before IVF/ICSI in patients with a normal transvaginal ultrasound and/or hysterosalpingography, improves the clinical pregnancy rate. A meta-analysis of randomized controlled trials (RCTs).

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Study question: Does routine hysteroscopy improve the clinical pregnancy rate before the first IVF/ICSI cycle in patients with a normal transvaginal ultrasound and/or hysterosalpingography?
Summary answer: Routine hysteroscopy improves the clinical pregnancy rate in patients with a normal transvaginal ultrasound and/or hysterosalpingography scheduled for their first IVF/ICSI treatment.
What is known already: The embryo implantation failure still today represents a challenge for reproductive physicians. Hysteroscopy represents the standard procedure for evaluating uterine cavity and it is offered routinely in patients scheduled for IVF/ICSI, because it seems to be associated with higher success rates, as observed in previous meta-analyses (Pundir et al., 2014; Di Spiezio Sardo et al., 2016). Nevertheless, a well-designed multicenter RCT, involving 750 women (Smit el al., 2016) and not included in those meta-analyses, showed no effect of hysteroscopy before IVF/ICSI in improving the live birth rate. For better understanding the role of hysteroscopy, we performed a new updated meta-analysis.
Study design, size, duration: A meta-analysis, based on PubMed, ISI-Web, Cochrane CENTRAL, EMBASE, was conducted to verify the effectiveness of routine hysteroscopy in patients with normal uterine cavity detected at transvaginal ultrasound and/or hysterosalpingography, scheduled for their first IVF/ICSI cycle. According to *PICO* format, inclusion criteria were: *Population*, infertile patients; *Intervention*, hysteroscopy; *Control*, no hysteroscopy; *Outcome*, clinical pregnancy rate (CPR). Secondary outcomes were: live birth rate, implantation rate, miscarriage rate.
Participants/materials, setting, methods: A bibliographic search was undertaken from 1998 to 2018, yielding 327 studies. Two researches (A.M., S.G.) reviewed independently the studies. After the first screening (title, abstract), 312 articles were excluded and 11 after the second screening. The Mantel-Haenszel method was used to calculate odds ratios (OR) and heterogeneity among studies (I^2). The results were expressed as OR with 95% confidence intervals (CI). Standardized mean differences (SMD) between groups were used for continuous outcomes.
Main results and the role of chance: Four RCTs were included (El-nashar et al., 2011; Elsetohy et al., 2015; Smit et al., 2016; Alleyassin et al., 2017). No significant differences between the hysteroscopy group and the control group in female age (p=0.29), BMI (p=0.21), number of retrieved oocytes (t=1.33, p=0.18), fertilization rate (Z=0.82, p=0.41) and transferred embryos (t=0.75, p=0.45) were observed. The studies did not report sufficient data for evaluating total dose of gonadotropins, stimulation duration and number of mature oocytes.

CPR was reported in all the four trials with 1,279 participants and it was significantly different in women receiving hysteroscopy [357/640] compared with the controls [304/644] (OR 1.67, 95% CI 1.08 to 2.58, I^2=64%).

In the two studies reporting live birth rate and including 935 patients (Elsetohy et al., 2015; Smit et al., 2016), no difference was observed between the hysteroscopy group [265/466] and the control group [232/469] (OR 1.69, 95% CI 0.71 to 4.00, I^2=86%). The miscarriage rate, reported in two studies with 962 participants (Smit et al., 2016; Alleyassin et al., 2017), showed no significant difference between women receiving hysteroscopy [54/319] and controls [41/273] (OR 1.16, 95% CI 0.74 to 1.80, I^2=13%). The selected studies did not report sufficient data to analyze implantation rate.
Limitations, reasons for caution: The couples' basal characteristics (FSH, AMH, AFC, seminal parameters) were not indicated in all the RCTs, as the drugs used for the ovarian stimulation.
Wider implications of the findings: Hysteroscopy may be offered in women with a normal transvaginal ultrasound and/or hysterosalpingography undergoing their first IVF/ICSI cycle. Further multicenter, well-designed RCTs with the live birth rate as the primary outcome are necessary for obtaining stronger evidence to support the use of routine hysteroscopy before IVF/ICSI.
Trial registration number: Not applicable for non-clinical trials.

P-376 Prevalence of Thrombophilic and folate metabolism mutations in embryos with different clinical outcomes

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Study question: Is there an association of thrombophilic and folate metabolism mutations in preimplantation embryos with pregnancy outcomes?
Summary answer: Homozygous FXIII (V34L) mutation is associated with poor implantation. MTHFR1298 AC/CC and MTHFR1298 AC (CC)/MTRR 66 AG(GG) genotype are associated with early miscarriage.
What is known already: Embryo implantation is a complex interplay of embryonic and maternal factors that requires controlled placental vascular invasion. Maternal mutations affecting coagulation cascade and one carbon metabolism are explored in cases of repetitive miscarriages or implantation failures, and for some of them, there is an established association with subfertility. Mutations in folate metabolism may compromise methylation and metabolism of DNA and proteins that can potentially affect embryo development in the early stages of implantation and placentation. The frequency of mutations in thrombophilia and folate metabolism genes in preimplantation embryos and their association with pregnancy outcomes is lacking.
Study design, size, duration: This is a retrospective study on 96 blastocysts with known clinical outcome after single euploid embryo transfer (41.6% - not-implanted, 33.3% - ongoing pregnancy and 25.0% - early miscarriage <12 gestational weeks). Clinical and demographic data of the patients were obtained (n=77). 47.9% of the embryos were derived from oocytes of young anonymous donors (25.3±3.4 years); 17.7% of women <35; 27.1% of women 35-40 and 7.3% of women >40. 19.8% were transferred to gestational carriers.
Participants/materials, setting, methods: 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-1 -675 4G/5G, FGB -455G/A, MTHFR-C677T and -A1298C, MTR-A2756G, and MTRR-A66G) using single base sequencing method. Stored whole genome amplified (WGA) DNA from trophectoderm biopsies (4-6 cells) of euploid blastocysts with known pregnancy outcomes was used. SPSS software was used for statistical analysis, and p<0.05 with CI 95% was considered as significant.
Main results and the role of chance: Embryos that implanted, did not implant or resulted in a miscarriage had similar allele and genotype frequencies for MTHFR C677T, MTR A2756G, Factor V-Leiden G1691A, Factor V-H1299R and Factor II-G20210A mutations. PAI-1 -675 4G/4G genotype was more frequent in embryos that did not implant (12.5%) vs implanted (1.9%) (p=0.08, non significant-NS). Interestingly FGB -455G/A mutation was only found in the embryos that implanted (n=56, 8%, NS). Homozygous XIII-V34L mutation was associated with lower embryo implantation potential (OR 2.2, 95% CI[1.06-4.6]). Minor allele and genotype frequency of MTHFR A1298C mutation were significantly higher (p=0.03 and p=0.009 respectively) in embryos that miscarried (q=0.35, [AC] genotype=0.66, [CC] genotype=0.042) compared to embryos with ongoing pregnancy (q=0.20, [AC] genotype=0.34, [CC] genotype=0.03) and non-implanted ones (q=0.175, [AC] genotype=0.3, [CC] genotype=0.025). Furthermore this effect of the MTHFR A1298C mutation was potentiated when a mutation in another folate metabolism gene MTRR A66G was present (OR 4.2, 95%CI[1.32-13.47]).
Limitations, reasons for caution: The small sample size and the data was obtained from a single IVF center. Although powered to detect difference in

allele and genotype frequency of individual mutations, larger sample size will allow for examination of the compound effect of more than one mutation on embryo implantation and development potential

Wider implications of the findings: Our results present for the first time the frequency of 10 mutations associated with thrombophilia and folate metabolism, and association of embryonic MTHFR A1298C genotype with miscarriages. Embryo selection for transfer can be improved by testing for these mutations at the same time of ploidy screening.

Trial registration number: not applicable

P-377 Diagnosing the Endometrium after Recurrent Implantation Failure (RIF)

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Study question: What endometrial dysfunctions can be identified in a cohort of women with RIF, and do they point to independent causalities?

Summary answer: Multiple endometrial aetiologies are observed in RIF, no single endometrial receptivity test can fully guide management. uNK-cell-counts correlate to histological and gene-markers of endometrial maturation.

What is known already: The endometrial factor is recognized as a cause of RIF. However management remains largely empirical and of questionable efficacy. There is a need to guide therapy by making an endometrial diagnosis.

Study design, size, duration: Prospective cohort study. Between November 2017 – January 2019 80 women with a history of RIF were referred to a new University based 'Implantation Clinic'. Patients underwent timed endometrial diagnostic profiling in a hormone substituted cycle. Endometrial biopsies were analysed by histology, immune cell profiling, and ERA testing (Igenomix). The vaginal microbiome was analysed (ART-PRED) and blood profiling for putative causes of implantation failure was performed.

Participants/materials, setting, methods: RIF was defined as failure to conceive after at least three transfers of high quality, fresh and/or frozen embryos. All participants were treated with a standard hormone substituted cycle using oral estradiol and vaginal progesterone. All samples were taken after 5 full days of progesterone. Blood tests included estrodiol, progesterone, prolactin, TSH, thrombophilia screening, and vitamin D. CD56 and CD16 cell counts were divided into three categories: low, medium and high based on pathology description.

Main results and the role of chance: In 67% of the women one or more possible explanatory reasons for implantation failure was identified. A low serum progesterone $\leq$ 35 nmol/L suggesting poor vaginal absorption was observed in 41%. The ERA revealed displacement of the receptive window in 51%. Endometrial CD56 and CD16 counts were low in 30% and 23% of the women, respectively, and high in 6% and 19% women, respectively. Only one patient was identified to have antiphospholipid antibodies, and none showed evidence of chronic endometritis. uNK cell counts in the endometrium showed a positive correlation with the maturity of the endometrium assessed independently by both the ERA (p-value 0.0003) and by Noyes histological criteria (p-value 0.0064). The proportion with an abnormal vaginal microbiome is under analysis and will be presented.

Limitations, reasons for caution: The tests performed in the study were taken in a standard hormone substituted cycle and results should be interpreted in that clinical content. The findings require comparison with a control group and this work is ongoing.

Wider implications of the findings: In the majority of women with RIF a potential endometrial factor can be identified. Diagnosing the endometrium could reduce the empirical use of unproven treatments, by providing a rationale for individualised treatment. Future trials of therapies for RIF should target the underlying diagnosis rather than test their empirical use.

Trial registration number: REG-117-2017 Registered with the local data protection authority.

P-378 Endometrial scratching for women with one or more embryo transfer failures: a systematic review and meta-analysis of randomized controlled trials

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Study question: To examine endometrial scratch injury (ESI) as an intervention to improve in vitro fertilization (IVF) outcomes in women with history of previous embryo-transfer (ET) failure

Summary answer: ESI improves IVF success in patients with $\geq$2 previous ET failures undergoing fresh ET; its timing and technique may provide variable effects on embryo implantation

What is known already: It is assumed that up to two-thirds of implantation failures could be secondary to defects in endometrial receptivity. Endometrial scratch injury (ESI) is an intervention widely offered to tnhance endometrial receptivity in women with a history of IVF failure. The rationale of ESI is to trigger a local acute inflammation, with release of cytokines and growth factors, that could help the implantation process. Howeverthere is still no agreement on the most appropriate timing (day of the menstrual cycle) and tecnique (number of performed endometrial injuries and optimal device) to be used

Study design, size, duration: This is a systematic review of all randomized controlled trials (RCTs) investigating the effects of ESI on IVF outcomes in women with at least one previous ET failure. Electronic databases were searched from their inception until November 2017. Ten studies were included (1,468 partecipants), of which 733 were assigned to the intervention group and 735 to the control group. All studies were open-label except for one trial (double-blinded) . three studies were multicentre trials

Participants/materials, setting, methods: Meta-analysis was conducted using the random-effect model with an intention-to-treat approach. Ovarian stimulation protocols included FSH or FSH/hMG mainly adapting dose of gonadotropins (150–375 IU daily) on patients' characteristics. The mean number of previous failed ET was between two and three in all studies, except for two. All patients received ESI during the menstrual period preceding the ET. The majority of women underwent a single homologous IVF-ICSI cycle with fresh ET

Main results and the role of chance: The meta-analysis included 1.468 partecipants from 10 RCTs. Intervention group showed higher live birth rate (LBR) (RR 1.38, 95% CI 1.05–1.80, P = 0,02) and clinical pregnancy rate (PR) (RR 1.34, 95% CI 1.07–1.67, P =0.01) compared with controls, without difference in terms of multiple PR, miscarriage rate (MR) and ectopic pregnancy (EP) PR. Subgroup analysis showed that double-luteal ESI with pipelle had maximum effect on LBR (RR 1.54, 95% CI 1.10–2.16, P = 0.01) and clinical-PR (RR 1.30, 95% CI 1.03–1.65, P = 0.03). Single follicular ESI with Novak curette showed too benefit on LBR (RR 2.36, 95% CI 1.16–4.81, P = 0.03) and clinical-PR (RR 1.78, 95% CI 1.07–1.96, P =0.03). No effects of single luteal ESI by pipelle and double follicular-luteal ESI were observed. ESI was beneficial only for patients with two or more previous ET failure (LBR: RR 1.64, 95% CI 1.21–2.21, P =0.001; clinical-PR: RR 1.57, 95% CI 1.22–2.03, P =0.0005). ESI improved IVF success only in women undergoing fresh ET cycles (RR 1.44, 95% CI 1.05–1.97, P =0.002 - RR 1.49, 95% CI 1.12–1.99, P =0.006). No complications associated with ESI occurred

Limitations, reasons for caution: The main point of weakness of this study is the heterogeneity between original studies in patients' characteristics, tools for identification of uterine diseases, IVF-ET cycles tecniques, types of embryos transferred and cointerventions. In addition, ebryo aneuplidy and endometrial inflammation as caouses for implantation failure were not ruled out

Wider implications of the findings: We recommend further well-designed RCTs on ESI, focusing on patients with homogeneous characteristics and avoiding bias due to cointerventions and due to the lack of preimplantation genetic testing. Future RCTs comparing types of intervention are needed to establish the most effective numnber, timing and device for performing ESI

Trial registration number: PROSPERO CRD42017082777

P-379 Placental histopathology in IVF pregnancies resulting from the transfer of frozen-thawed embryos compared with fresh embryos

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Study question: Do placentas of pregnancies resulting from fresh embryo transfer (ET) contain more histopathologic lesions compared with placentas of pregnancies resulting from frozen embryo transfer (FET)?
Summary answer: Placental histopathology analysis was comparable between pregnancies resulting from fresh ET and FET.
What is known already: An increasing amount of evidence has shown that pregnancies resulting from FET are less likely to be complicated with preterm deliveries, intrauterine growth restriction, and even antepartum death. It has been suggested that the supraphysiological estradiol levels in fresh cycles interfere with placentation, resulting in obstetric complications later on during pregnancy. It is yet unknown whether the differences in obstetrical outcome between fresh and FET cycles are also associated with certain patterns of placental histopathology.
Study design, size, duration: A prospective cohort study of pregnancy outcome and placental histopathology, in 89 women who conceived by IVF and delivered in a single tertiary medical center, between December 2017 and December 2018. Exclusion criteria were multiple pregnancies and egg donation. The prevalence of different placental histopathology lesions was compared between women who conceived after either fresh ET or FET. Thirty-eight placentas in each study group were sufficient to detect a 60% increase in vascular malperfussion lesions.
Participants/materials, setting, methods: During the study period, 89 women gave birth in our center and met the inclusion criteria, out of whom 49 had a fresh ET and 40 had a FET. Placental histopathology lesions were classified according to 'Amsterdam' criteria into maternal and fetal vascular malperfusion lesions, acute inflammatory response lesions, and chronic inflammatory lesions. All placentas were examined by a single pathologist who was blinded to the type of embryo transfer.
Main results and the role of chance: Women conceived after fresh ET did not differ from women conceived after FET with regard to maternal age (34.3 vs. 33.3, respectively, p=0.38), gestational week at delivery (38.3 vs. 38.9, respectively, p=0.33), preterm birth (14.3% vs, 5.0%, respectively, p=0.14), mean birthweight (2995 vs. 3219 gr, respectively, p=0.08), birthweight $<10^{th}$ percentile (14.3% vs. 12.5%, respectively, p=0.80), or the incidence of hypertensive disorders (14.3% vs. 5.0%, respectively, p=0.17), gestational diabetes (10.2% vs. 7.5%, respectively, p=0.72) and cesarean delivery (36.7% vs. 25.0%, respectively, p=0.23). Placental histopathology analysis from pregnancies conceived by fresh ET was comparable to pregnancies conceived by FET, with regard to the prevalence of maternal vascular malperfusion lesions (49.0% vs. 45.0%, respectively, p=0.83), fetal vascular malperfusion lesions (14.3% vs. 22.5, p=0.31), acute inflammatory response lesions (26.5% vs. 30.0%, respectively, p=0.71), and chronic inflammatory lesions (6.1% vs. 12.5%, respectively, p=0.45). Mean placental weight was comparable as well (453 vs. 476 gr, respectively, p=0.33).
Limitations, reasons for caution: The main limitations of the study are its small sample size and the limited information regarding the characteristics of IVF cycles.
Wider implications of the findings: In this preliminary study we did not find significant differences in pregnancy outcome and placental histopathology between IVF pregnancies conceived by either FET or fresh ET. These results are reassuring for clinicians and patients who wish to pursue with transferring fresh embryos.
Trial registration number: 0181-17-WOMC

P-380 Endometrial three-dimensional ultrasonographic evaluation is not associated to higher clinical pregnancy rate in infertile women submitted to frozen embryo transfer

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Study question: Is endometrial three-dimensional (3D) ultrasonographic (US) evaluation associated with higher clinical pregnancy rate in frozen embryo transfer (FET) cycles?
Summary answer: The clinical pregnancy rate is not higher in women submitted to endometrial evaluation performed by 3D US in FET cycles when compared to two-dimensional(2D) evaluation.
What is known already: Although some studies have evaluated multiple markers of endometrial receptivity through Doppler 3D US, endometrial thickness has been the most widely used variable in clinical practice. Despite this, there are no studies that have compared the measurement of endometrial thickness through 2D and 3D ultrasonography in frozen embryo cycles.
Study design, size, duration:
Retrospective study with infertile women submitted to FET and recruited between 01.08.2017 and 30.10.2018. Considering a clinical pregnancy rate of 40% and that a 20% difference between the groups would be significant, 97 patients per group (194 patients total) would be required for a power of 80% and alpha 5%.
Participants/materials, setting, methods: Three hundred and thirteen women aged 39 years or less were recruited and were divided into two groups: 1)2D:endometrial evaluation by 2D US(n = 135) and 2)3D: endometrial evaluation by 3D US(n = 109). All women underwent 2D or 3D evaluation until the day of embryo transfer. The examinations were performed by two observers through the Voluson E8 Expert (GE Healthcare). Seventy-four women were excluded [uterine alterations low ovarian reserve, poor embryo quality, thin endometrium.
Main results and the role of chance: Clinical pregnancy rate was similar between the 2D and 3D groups (p = 0.32). Logistic regression showed that the type of US (2D vs. 3D) [Relative Risk (RR) 0.84 Confidence Interval (CI95%) (0.58-1.2), p = 0.34], age [RR 0.97 (0.94-1.0), p = 0.06] and the cause of infertility [(Endometriosis - 0.95 (0.53-1.71), p = 0.86), (Polycystic ovary syndrome - 0.95 (0.53-1.71), p = 0.86), (Unexplained infertility - 0.98 (0.44-2.18), p = 0.95), (Tubal factor - 0.53 (0.25-1.14), p = 0.1), (Male factor - 0.96 (0.62-1.47), p = 0.84)] were not independent predictors of clinical pregnancy rate.
Limitations, reasons for caution: As the realization of 3D US depends on 2D image capture of reasonable quality, 3D evaluation may present limitations in some cases. However, to minimize such effect, changes in the uterine anatomy were considered as exclusion criteria and all exams were performed by two observers.
Wider implications of the findings: The assessment of 3D endometrial thickness is not associated with a higher clinical pregnancy rate in relation to 2D analysis in frozen embryo transfer cycles.
Trial registration number:
NOT APPLICABLE

P-381 MicroRNA let-7 in endometrial extracellular vesicles induces embryonic diapause

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Study question: How does let-7 suppresses implantation? Does it affect human blastocyst implantation?
Summary answer: Let-7 in endometrial extracellular vesicles induces embryo diapause to suppress implantation in both human and mouse models.
What is known already: Let-7 force-expression suppresses mouse embryo implantation. Mouse embryos can be induced to undergo diapause (delayed implantation), when the blastocysts become dormant and do not implant in the uterus but can be activated to implant and produce pups. The expression of let-7 family members is high in the dormant mouse blastocysts. Endometrial epithelial

cells including those from humans secrete extracellular vesicles containing microRNAs including let-7.

Study design, size, duration: Laboratory experimental study

Participants/materials, setting, methods: The actions of let-7 on survival of mouse blastocysts in vitro and in delayed implanting mice, and on differentiation and implantation potential of human blastocyst surrogates were studied.

Main results and the role of chance: Electroporation of let-7 prolonged the survival of mouse blastocysts in vitro. The treated blastocysts had reduced proliferation, nutrient metabolism and apoptosis when compared to the control blastocysts and the normal Day 4 blastocysts prior to implantation. They produced pups after transfer to foster mothers.

Two observations demonstrated that the let-7 in delayed implanting mouse blastocysts were derived from endometrium: (1) the expression profile of let-7 in endometrial epithelial cells, extracellular vesicles of uterine luminal fluid and blastocysts before, during diapause, and after estradiol-induced activation were identical; (2) Wildtype mouse blastocysts acquired a unique let-7 transgene after transfer into delayed implanting transgenic mice carrying the sequence. Let-7-enriched extracellular vesicles from endometrial epithelial cells prolonged mouse blastocysts survival in vitro. The treatment also down-regulated the target genes of let-7 in the treated embryos.

Human embryonic stem cells were induced to form spheroids and differentiate into human blastocyst surrogates; they possessed blastocoel-like structure, expressed trophoblast genes and attached specifically onto receptive human endometrial epithelial cells. Treatment with let-7-enriched extracellular vesicles reduced mRNA expression of syncytiotrophoblast and extravillous cytotrophoblast marker genes, suggesting blockage of trophoblast differentiation. The attachment potential of the treated spheroids on receptive human endometrial epithelial cells was also significantly reduced.

Limitations, reasons for caution: The observations on human blastocyst surrogates may not truly reflect those that would happen in human blastocysts.

Wider implications of the findings: Embryonic diapause is an evolutionary conserved phenomenon. The results suggested that endometrial let-7-enriched extracellular vesicles retarded embryo development. Thus dysregulation of microRNAs production may alter developmental rate of embryos causing embryo-endometrium asynchrony and infertility. (The study is supported by GRF 17119117, GRF 17107915 and NSFC 31471398)

Trial registration number: Not applicable for non-clinical trials.

P-382 Recurrent Implantation failures – Is there a role for PGT-A?

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Study question: Is Pre-Implantation Genetic Testing for Aneuploidy (PGT-A) a beneficial intervention for patients with Recurrent Implantation Failures (RIF)?

Summary answer: PGT-A for RIF doesn't seem to improve Live Births. Considering lesser multiple pregnancies and lesser time to pregnancy we could still consider PGT-A for RIF.

What is known already: Role of PGT-A for couples with RIF is still un-certain.

Study design, size, duration: This is a retrospective study of couples that underwent fertility treatments at our center from 2014-2017 with history of RIF.

140 couples with RIF underwent PGT-A screening, of which 68 underwent a successful transfer with one Euploid Blastocyst (n=68).

34 couples with history of RIF underwent blastocyst transfers without PGT-A screening (n=34).

Participants/materials, setting, methods: Patients who had minimum two transfers (Fresh or Frozen) and not conceived were classified as RIF and recruited. Retrospectively we grouped the couples into PGT-A (n=68) and Non PGT-A group (n=34)

All women underwent Blastocyst transfers in a frozen cycle (FET)

For PGT-A, embryos were biopsied at Blastocyst stage and subjected to Next Generation Sequencing (NGS)

Multiple pregnancy rates (MPR), Live Births (LBR), number of FET attempts to pregnancy and time to pregnancy were compared between the groups

Main results and the role of chance: LBR in PGT group was 61.7% (n=42/68), all pregnancies were singletons with no incidence of multiple gestations.

LBR in Non-PGT-A group was 55.8% (n=19/34) with 11.7% multiple pregnancies.

Most of the women in PGT-A group conceived in the first embryo transfer (40 women conceived in the first FET) and the average time take to conceive was there months.

In Non PGT-A group women conceived either in the 3rd or 4th FET attempt and took an average of 9months to attain pregnancy.

Though the live births were comparable between both the groups, time to pregnancy was shorter in PGT-A screened group with lesser attempts.

Considering the shorter time to conceive and lower incidence of multiple pregnancies, PGT-A could still be offered to couples with RIF.

Limitations, reasons for caution: Retrospective data, Small sample size

Wider implications of the findings: Though live births do not seem to be better after PGT-A intervention, considering the psychological aspects associated with every failed attempt; PGT-A still could be a beneficial intervention to shorten time to pregnancy. PGT-A could be an active intervention for clinics with a strict policy for elective single embryo transfer

Trial registration number: NA

Retrospective Data

P-383 E4 allele of Apolipoprotein E (APOE) gene is associated with recurrent implantation failure

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Study question: Present study was aimed to evaluate whether there is an association between *APOE* genotypes with recurrent implantation failure cases.

Summary answer: Endometrial gene expression pattern is an important determinant of its receptivity during implantation, and allelic polymorphisms may alter the level of expression in particular genes.

What is known already: Recurrent implantation failure (RIF) which involves 10% of assisted reproductive technology (ART) treatments may happen due to maternal or embryonic etiologies. Previous studies have shown an up-regulation in *APOE* gene expression inside the endometrial tissue during the window of implantation. Furthermore, several reports have emphasized on the association of *APOE* allelic polymorphisms with female infertility and recurrent pregnancy loss. *APOE*, an important effector in cholesterol and lipid metabolism and transport, is encoded by three major polymorphic alleles (E2, E3 and E4) which may produce six different genotypic combinations.

Study design, size, duration: 100 infertile women (age<40 years) with at least three episodes of ART failure with minimum of four good quality embryo transfers and any known risk factors were compared to 100 normal fertile controls with at least one live birth and no history of infertility.

Participants/materials, setting, methods: Genomic DNA was extracted by standard salting out method from 5ml peripheral blood samples of all participants and amplified through polymerase chain reaction. finally genotyping and allele determination was performed using RFLP method.

Main results and the role of chance: Genotype frequencies among patients and controls were as follows in respect: E2/E3: 5% vs 8%, E3/E3: 66% vs 77%, and E3/E4: 28% and 15%. Statistical analysis revealed significant higher frequency for E3/E4 genotype in RIF group compared to control (P=0.038; OR=2.203; 95%CI =1.092 to 4.443). The allele frequencies in patients and controls were respectively as follows: E2: 2.5% vs 4%, E3: 82.5% vs 88.5% and E4: 15% vs 7.5%. Once again, statistical analysis showed significant higher frequency for E4 allele in RIF group compared to control (P = 0.026; OR=2.176; 95%CI =1.131 to 4.185). Our data supports the association of E4 polymorphism with recurrent implantation failure (E3/E3 vs E3/E4: P =0.031; OR = 2.177; 95%CI = 1.072–4.421, and E3 vs E4: P = 0.022; OR = 2.145; 95%CI =1.114–4.130). Due to significant higher frequency of E4 allele and E4 carrier genotypes in RIF patients, for the first time, we propose the *APOE*-E4 polymorphism as a potential risk factor for human embryonic implantation process.

Limitations, reasons for caution: Sample size of the study including patients and fertile controls was relatively small, because of inadequate accessible RIF patient samples. In addition, Ethnic backgrounds of participants were not considered in the study.
Wider implications of the findings: Evaluation in a larger cohort of RIF patients, APOE genotyping in ART success cases and RIF cases with different ethnic backgrounds can help to establish present data.
Trial registration number: not applicable

P-384 Bed rest following embryo transfer – Freedom of choice doesn't impair pregnancy rates

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Study question: Is there a difference in life birth rates in patients with bed rest following fresh/frozen embryo transfer?
Summary answer: There is no difference in life birth rates between patients with and without bedrest following fresh/frozen embryo transfer.
What is known already: Bed rest following embryo transfer has always been controversially discussed. While it has been recommended as a way to prevent embryo expulsion by gravity, a recent meta-analysis showed no benefit to improve clinical pregnancy and live birth. However, implantation rates were found to be reduced in patients choosing bed rest. The big heterogeneity in different study designs makes a general consensus very complex. Hence, we wanted to add further knowledge about the necessity and influence of bed rest after embryo transfer considering fresh versus frozen embryo transfer.
Study design, size, duration: The study was designed and conducted at the Kinderwunsch Institut Schenk GmbH (Dobl, Austria) between February 2017 and September 2018. 1000 embryos from patients undergoing IVF (*in vitro* fertilization) and ICSI (intracytoplasmic sperm injection) treatment were included and retrospectively analyzed. 535 patients (53.5%) had fresh embryo transfer, while 465 patients (46.5%) had frozen embryo transfer. Patients who decided to have bed rest following embryo transfer stayed in bed for 10-15 minutes.
Participants/materials, setting, methods: Embryos from women (aged 18 - 41) with good embryo quality, according to the Istanbul consensus criteria, cultured in time-lapse system were included in the study while embryos from patients with known genetic predispositions were excluded. Cryopreservation was performed using GAVI vitrification system (Merck). Embryo transfer (fresh and frozen) was performed on day 3 or day 5 taking into consideration the number of fertilized oocytes, patient age and embryo quality in previous attempts (if any).
Main results and the role of chance: Overall, embryo transfers (fresh and frozen) resulted in a life birth rate of 29.9% in patients with subsequent bed rest and 30.1% in patients who were immediately discharged after transfer. Pregnancy termination after positive beta-hCG was equal in both groups (10.87% versus 10.94% respectively). In detail, patients with bed rest achieved life birth with fresh transfer in 30.1% of all cases, while 29.7% achieved pregnancy with frozen transfer. Without bed rest pregnancy with fresh transfer was achieved in 28.9% of all cases while 31.6% achieved pregnancy in the frozen embryo transfer group. Overall, there were no statistical differences in the above-mentioned parameters between the two groups.
Limitations, reasons for caution: The retrospective data analysis may be seen as study limitation. Nonetheless, results should be confirmed with a higher sample size in future studies.
Wider implications of the findings: The data show no difference in life birth rates with or without bed rest following embryo transfer, independent from fresh or frozen cycles. It is tempting to speculate that the empowerment of the patients to decide about their post-transfer routine is the best advice for a successful treatment outcome.
Trial registration number: not applicable

P-385 OUTCOMES OF A LARGE TRIAL TO DEVELOP A LOW COST, MASS MARKET, NON-INVASIVE, URINARY, PRENATAL DIAGNOSTICS TEST FOR TRISOMIES AND PREECLAMPSIA FOR THE CHINESE MARKET

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Study question: Is it possible to apply a computational-based mass spectral data analysis workflow previously developed for UK, to urinary prenatal screening in a Chinese population?
Summary answer: The automated computational workflow made it possible to rapidly and affordably screen urine-derived mass spectral data for prenatal diagnosis in a large Chinese population.
What is known already: Prenatal testing is rapidly changing the way in which early pregnancies are being managed. However, with the exception of serum marker assays and confirmatory amniocentesis, prenatal testing is still only offered to the privileged few or those in developed countries with national health systems. Despite the rapid growth in NIPT, the high cost associated with blood based DNA testing has meant that pregnancies in low/middle income countries, including China, rely on more invasive methods, late screening (3rd trimester), or not at all. This puts patients at risk and leads to uninformed, underprepared healthcare decisions and provision for pregnant women.
Study design, size, duration: A screening test study to analyse the urine from 4751 pregnant women collected in an 11 month period from two hospitals in the Jiangsu province in China. 8692 analyses were performed on two different MALDI instruments which were benchmarked against each other to determine if the Chinese manufactured instrument could match the performance of the established UK manufactured instrument. The data was systematically analysed for quality and normalized using our currently available pre-processing bioinformatic pipeline.
Participants/materials, setting, methods: The analysis of urine using MALDI mass spectrometry was developed in the UK and applied in this study to determine the potential sensitivity and specificity of detecting Downs syndrome, T18 and preeclampsia in this large cohort. The average gestational age was 13 weeks and maternal age 29 years. Quality control assessment, pre-processing and data analysis of all samples from UK and Chinese instruments was conducted respectively using an automated pipeline which was implemented in Python.
Main results and the role of chance: A subset of samples was randomly selected from a pre-processed data set and their peak positions and intensities were systematically compared within a range of 50 m/z windows using a previously developed computational workflow. Mass spectra from both MALDI instruments had patterns of peak enrichment and intensity differences in urine samples for aneuploidies (T21, T18) and preeclampsia pregnancies when compared with a representative set of non-aneuploids or with non-preeclamptic outcomes. The identified patterns from selected representative samples (training data sets) were used to develop 12 predictive models to identify T21, T18 and preeclampsia in pregnancies. The models were validated using a larger testing data sets, which were not used for model development. Data was analysed and validated in sets grouped by gestational age to eliminate bias due to changes occurring with gestational age. ROC analysis showed that all predictive models developed for identification of T21 and T18 have good performance with 100% sensitivity and false positive rates between 0 and 20%. For preeclampsia the best performing algorithm gave 83.3% sensitivity for 35% FPR.
Limitations, reasons for caution: The number of trisomy outcomes analysed in the cohort was small, even though this was representative of the true population rate. Furthermore, urine samples were frozen prior analysis and in some cases more than one freeze thaw cycle, poor spectra resulting from this process were not used in the study.
Wider implications of the findings: Earlier, cheaper, urine-based testing can be implemented to increase the ease and availability of prenatal testing in low/medium income countries like China, enabling better informed healthcare decisions earlier in a pregnancy without having to rely solely on NIPT or traditional biomarker screening tests which require advanced and expensive infrastructure.
Trial registration number: N/A

P-386 Evaluating the risk of ectopic pregnancy and spontaneous abortion in early pregnancy using a serum hCG and CA125 measurement algorithm

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Study question: Can a mathematical algorithm based predictive model, utilising serum biomarkers, be a viable test to predict risk of ectopic pregnancy or spontaneous abortion?
Summary answer: Single point hCG and CA125 combined measurement gives an accurate risk score, detecting ectopic pregnancy or spontaneous abortion in the second month of gestation.
What is known already: About 10–15% (increasing to 18-30% for IVF patients) of all spontaneous pregnancies end in recognised spontaneous abortion and 1-2% result in ectopic pregnancy. Currently, diagnosis is based on repeat hCG measurement and ultrasound scan and results are often inconclusive, requiring repeat follow up causing patient anxiety and increased risk of tubal rupture. Both hCG and CA125 have been used extensively in management of viable pregnancies, spontaneous abortion and ectopic pregnancy but not in combination with mathematical modelling following single point measurement to predict risk.
Study design, size, duration: This was a diagnostic test study including serum samples from 380 pregnancies. Serum samples were collected from the A&E and EPAU at London Hospital from pregnant women (average gestational age: 7 weeks) presenting with lower abdominal pain and/or vaginal bleeding between 2002 and 2003. Serum hCG and CA125 of 137 samples were included in the study. Outcomes were confirmed by the follow up. Analysis of data using supervised machine learning was performed in 2017.
Participants/materials, setting, methods: There were 13 cases with 124 controls for ectopic pregnancy and 62 cases with 75 controls for spontaneous abortion models. Multiple algorithms were built and tested from the data which were then validated by ten-fold cross-validation to ensure reproducibility and reliability. Quadratic and linear discriminant analysis algorithms based predictive models were built on a log normalised data. Data analysis was performed in R.
Main results and the role of chance: Two separate models were developed which combined serum measurements of hCG and CA125 to calculate the relative risk for either ectopic pregnancy or spontaneous abortion. Ectopic pregnancy test performance was 100%, 73% and 77% for sensitivity, specificity and accuracy respectively and an ROC of 0.87. The ROC for spontaneous abortion was 0.74 with sensitivity of 63% and specificity of 72%. This diagnostic test reports the relative risk comparing an adjusted risk based on the results of the general risk. Five categories of risk were modelled for both ectopic pregnancy and spontaneous abortion which included: low, normal, moderate, elevated and high risk.

Following the cross validation, the study resulted in a user friendly, clinical decision making support system being created. The system requires simple data input of serum marker values and patient information to rapidly provide a calculated risk score. This risk score can enable clinicians to make informed decisions regarding patient management and pregnancy advice.
Limitations, reasons for caution: This study is limited by the cohort size and the limited number of ectopic pregnancy cases.
Wider implications of the findings: This study has shown that simple, single point measurement test can be used quickly at the most critical time to identify women with high risk for ectopic pregnancy or spontaneous abortion when compared to serial measurement and ultrasound scan alone.
Trial registration number: not applicable

P-387 Evaluating the clinical utility of endometrial biopsy for chronic endometritis screening in patients who experience implantation failure following euploid embryo transfer

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Study question: Does endometrial biopsy (EMB) and chronic endometritis (CE) screening offer clinical utility in patients following euploid embryo implantation failure?
Summary answer: Patients, regardless of endometrial biopsy findings, have similar IVF outcomes on a subsequent treatment cycle as compared with patients who do not undergo endometrial biopsy.
What is known already: Chronic endometritis is a local inflammatory disease characterized by plasmacyte infiltration within the endometrial stroma and is found within 8-72% of tested infertile patients (Kitaya K, 2018). Recent studies showed that CE may exert a negative effect on embryo implantation through impairing decidualization and altering the expression of proteins involved in endometrial receptivity (Wu D, 2017). Further study findings have suggested that antibiotic treatment for CE can improve IVF success rates. Clinical evidence is still scarce regarding the value of performing an endometrial biopsy for screening CE in patients with a history of failed implantation after a euploid blastocyst transfer.
Study design, size, duration: A retrospective, cohort study included infertile patients who failed a euploid embryo transfer and thereafter underwent an endometrial biopsy for CE screening, received antibiotic treatment (if indicated), and had a subsequent single, euploid frozen embryo transfer from January 2016 to December 2018. A sample size of 70 patients per group was calculated to be necessary to detect a 15% difference in clinical pregnancy rates with 80% power. Alpha=0.05.
Participants/materials, setting, methods: Cohorts were segregated into two groups (Group 1: patients who had a failed euploid embryo implantation that underwent EMB, this group also included patients in which EMB was negative for CE. Group 2: Control patients who had an unsuccessful euploid embryo implantation and did not undergo EMB). For EMB cases, CE was diagnosed using CD138 immunohistochemistry staining. All patients in the study underwent a subsequent single, euploid frozen embryo transfer (FET).
Main results and the role of chance: Group 1 included 241 patients of which 42% (n=90) were diagnosed with CE and received antibiotic therapy; while the remaining 124 patients were found to be negative for CE. Group 2 included 985 control patients.

On an unadjusted comparison, no differences were found in age, baseline FSH, baseline AFC, previous number of stimulation/IVF cycles, and embryonic quality among cohorts. However, a significant difference in AMH levels (3.3±2.9 vs 4.0±4.7, p=0.02), BMI (24.5±4.6 vs 23.5±4.2, p=0.002), and endometrial thickness (8.6±1.3mm vs 9.0±1.6mm, p=0.03) was observed. When analyzing a subsequent FET cycle, no differences were found in clinical pregnancy (CPR) rates 76% vs 81.1%(p=0.28), ongoing pregnancy rate (OPR) 78.8% vs 85.9(p=0.36) clinical loss rate (CLR) 10.6% vs 17.1%(p=0.09) or multiple pregnancy rate (MPR) 0.06% vs 1.6%(p=0.41) among Group 1 vs Group 2 respectively.

Using a logistic regression analysis fitted with a GEE model, and after adjusting for age, BMI, endometrial thickness at FET, embryo quality, and day of embryo biopsy, there was no positive association to performing an EMB and FET cycle outcomes (CPR (OR 1.01 CI95% 0.7-1.4,p=0.9), OPR (OR 1.16 CI95% 0.8-1.6, p=0.3), CLR (OR 0.6 CI95% 0.3-1.3, p=0.3) and MPR (OR 0.6 CI95% 0.05-3.3, p=0.4)).
Limitations, reasons for caution: This study is limited by its retrospective nature. Also, there is limited evidence and differences in worldwide standardization for the ideal time to perform an endometrial biopsy, methodology for CE diagnosis, and treatment protocols. These caveats might reduce this study findings generalizability. Future randomized controlled trials are needed.
Wider implications of the findings: Understanding the clinical utility of CE screening and the potential therapeutic advantages on embryo implantation is of critical importance for guiding patients during ART treatment. To date, there is limited evidence to support performing endometrial biopsies in patients with a history of failed implantation following euploid embryo transfer.
Trial registration number: This study was approved by the Western Institutional Review Board (Study Number: 1167398).

P-388 Mancozeb but not its metabolite ethylene thiourea (ETU) affects the embryo implantation process via modulating trophoblast invasion in vitro models

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Study question: Will Mancozeb and its metabolite ETU play a novel regulatory role in the invasion and migration of trophoblast cells? What is the underlying mechanism of Mancozeb?

Summary answer: Mancozeb but not ETU suppresses the invasion and migration through down-regulation of MMP-2 via ERK1/2 signaling pathway in trophoblastic cells.

What is known already: Mancozeb is a zinc/manganese-containing fungicide belonging to the subclass of ethylene-bis-dithiocarbamate (EBDC), which is largely used in agriculture. Mancozeb is classified as an endocrine disrupting chemical (EDC) modulating human reproductive and endocrine systems. Ethylene thiourea (ETU) is the main metabolite of Mancozeb with higher water-solubility and longer half-life period. Mancozeb and ETU were found to have teratogenic, mutagenic and carcinogenetic effects in vivo models. Successful pregnancy requires optimal trophoblast cell invasion into the uterine decidua soon after embryo implantation. However, the roles of Mancozeb and ETU on trophoblast invasion and migration remain largely unknown.

Study design, size, duration: The roles of Mancozeb and ETU on trophoblast biological function were investigated with human trophoblast cell lines JEG-3 and BeWo. The expression of metalloproteinases (MMPs) and pERK/ERK after Mancozeb treatment was analyzed. The effects of Mancozeb and its metabolites ETU and zinc salt on MMP-2 expression and activity were compared.

Participants/materials, setting, methods: Cell viability was quantified with CyQUANT NF cell proliferation assay. Matrigel-coated transwell assay and wound healing assay were used to study trophoblast invasion, outgrowth and migration after Mancozeb or ETU treatment. The expression and activity of MMP-2 in JEG-3 and BeWo cells were detected by qPCR, Western blotting, and gelatin zymography, respectively. Differential expression of MMP-9, TIMP1, TIMP3 and ERK/pERK in the JEG-3 cells after Mancozeb treatment were measured by qPCR and Western blotting.

Main results and the role of chance: Mancozeb and ETU strongly reduced cell viability at a concentration higher than 10μg/ml and 5000μg/ml after 48h treatment, respectively. Mancozeb at 3μg/ml significantly inhibited trophoblast invasion, outgrowth and migration of JEG-3 cells, but ETU had no effect at the same concentration. Mancozeb but not ETU decreased MMP-2 expression in both JEG-3 and BeWo cells, as well as MMP-2 activity in the conditioned culture medium. Furthermore, Mancozeb at 3μg/ml induced ERK1/2 phosphorylation from 15min to 1h and reduced ERK1/2 phosphorylation from 2h to 48h in the JEG-3 cells. No change was observed for the expression of MMP-9, TIMP1 and TIMP3. Our results show that Mancozeb induces cytotoxic effect at concentrations 100-fold lower than ETU. The inhibition effect of Mancozeb on the invasion and migration of trophoblast cells is not through its metabolite ETU and zinc salt. Mancozeb suppressed the invasive ability of JEG-3 cells via inhibiting MMP-2 expression and reducing MMP-2 enzyme activity trough modulating ERK1/2 phosphorylation.

Limitations, reasons for caution: The present study was based on in vitro models established with JEG-3 and BeWo cells which belong to human choriocarcinoma cell lines. Human primary trophoblast cells and in vivo studies will be carried out in our future Mancozeb investigation.

Wider implications of the findings: This study first reports that Mancozeb is more cytotoxic than ETU. Mancozeb is often applied to the plant through aerial spraying, which elevates the potential acute exposure to pregnant women nearby. Because Mancozeb has a short half time, measures should be taken to decrease acute environmental and occupational exposures.

Trial registration number: N/A

P-389 Differentiated approach to immunotherapy in patients with recurrent implantation failure.

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Study question: Personalized immunotherapy: will it improve the chance of pregnancy occurring in women with recurrent implantation failure (RIF)?

Summary answer: In RIF patients were 3 types of immunogram. Personalized approach to choosing medication for particular patient improves frequency of implantation, onset and outcome of pregnancy**.**

What is known already: The process of preparing the endometrium for implantation is determined by the coordinated interaction of the endocrine and immune systems. Hyperestrogenism, which occurs during Controlled Ovarian Stimulation (COS), affects the function of immunocompetent cells. Although the main events of the immunological dialogue between the embryo and the endometrium occur at the implantation site, changes in the peripheral immune system (blood) to some extent reflect the state of endometrial immunity. The role of the immunological factor in RIF is a subject of numerous discussions. Randomized controlled clinical trials in this area were disappointing, but they lacked a personalized approach.

Study design, size, duration: Randomized controlled trial of 143 patients with RIF, IVF / ICSI program (June 2013- April 2018). 3 months before ART, general and local immunity was studied. Women with endometrial pathology were excluded. 1. Inhibition of phagocytosis and / or T-cell immunity was detected in 74 (51.75%) patients. 2. Increased NK activity - in 70 (48.95%). 3. The presence of various autoantibodies in 21 (14.61%) women. Imbalance of local immunity in 65 (45.48%).

Participants/materials, setting, methods: 74 RIF and reduced immunity indicators patients were randomized into 2 groups. Intervention group (IG) - n = 37 received marine mollusks hydrolyzate preparation two months before ART for 20 days according to manufacturer's scheme. Afterwards, a control immunogram was performed. Control group (CG) - n = 37 received only folic acid preparations. Outcomes measured were the implantation rate and clinical pregnancy rate. No adverse effects of the marine mollusks hydrolyzate were observed.

Main results and the role of chance: Groups were comparable in age, body mass index, reproductive and gynaecological history, endocrine and somatic morbidity. The average number of IVF attempts was (2.53 ±0.5) in both the groups, minimum and maximum of 2 and 3 previous failed IVF. **Reduction:** absolute leukocyte count in 12 (32,43%) patients of **IG** and 13 (35,14%) patients of **CG** (P>0,05); **absCD3** in 18 (48,65%) and 16 (43,24%) respectively; **absCD4** in 24 (64,86%) women in both groups; **absCD8 –** 19 (51,35%) **IG** and 16 (43,24%) **CG** (P>0,05) respectively; Reduced immunoregulatory index in 25 (67,57%) and 26 (70,27%) (P>0,05); neutrophil phagocytic activity was reduced in 6 (16,22%) patients of both groups.

After immunocorrection, before IVF protocol incusion, leukocytes in **IG** amounted to - 6,83±0,21 G /l in **CG** 5,87±0,03 gig/l (p<0,05); CD3- 1, 39±0,03 and 1,03±0,02 G /l (p<0,05) ; **CD4-**0,81±0,02 and 0,58±0,01 gig/ /l (p<0,05); **CD8 -** 0,65±0,01 and 0,50±0,01 (p<0,05); Clinical pregnancy rate as determined by sonographic fetal cardiac activity was 18 (48,65 %) and 8 (21,62%) in IG and CG (p<0.05). In IG, 16 pregnancies ended in timely deliveries (88.89% of pregnancies; 48.65% of all women in the group); In CG, childbirths were in 3 women (37.50% and 8.11%, respectively) (p <0.05).

Limitations, reasons for caution: This work presented the results of treatment of only one of the three types of identified immunity disorders

Wider implications of the findings: This study shows a statistically significant increase in implantation rate and clinical pregnancy rate in women with prior implantation failure after IVF/ICSI receiving personalized immunotherapy, with no adverse effects.

Trial registration number: Not trial; this study was approved by Ethics Committee of the Reproductive Health Institution

P-390 Galectin-14 promotes trophoblast migration and invasion, and may participate in the pathogenesis of early pregnancy loss

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Study question: Is galectin-14 involved in early pregnancy loss (EPL), and what are its effects on trophoblast function?

Summary answer: The expression of galectin-14 was dramatically decreased in the villi of EPL, over-expression of galectin-14 in HTR-8/SVneo promoted cell migration and invasion.

What is known already: EPL is a common kind of pathologic pregnancy, and is closely associated with abnormal trophoblast function. Galectin-14 is a low-molecular-mass protein, specifically expressed in placenta. It was found highly expressed in extravillous trophoblasts. However there is few study on the effects of galectin-14 on trophoblast function. TGF-β1 can inhibit trophoblast invasion. Vasorin can inhibit TGF-β signaling, and promote cell migration in hepatocarcinoma.

Study design, size, duration: The study was approved by the local ethics committee. Villi from 20 cases of early pregnancy loss and 20 cases of induced abortion with similar gestational age were collected. The expression of galectin-14 was compared by western-blot. HTR-8/SVneo were artificially expressed with galectin-14. The migration and invasion ability of HTR-8/SVneo were measured. Than a RNA-seq was performed on HTR-8/SVneo , to find the underlying mechanisms.

Participants/materials, setting, methods: We collected villi from 20 cases of early pregnancy loss and 20 cases of induced abortion with similar gestational age. The expression of galectin-14 was analyzed by western-blot. The over-expression vector of galectin-14 gene was constructed and transformed into HTR-8/SVneo. Transwell assay was used to study migration and invasion ability of HTR-8/SVneo. RNA-seq was performed on HTR-8/SVneo.

Main results and the role of chance: Compared with normal control, the expression level of galectin-14 significantly decreased in villi from early pregnancy loss ($p<0.001$). The over-expression of galectin-14 in HTR-8/SVneo promoted cell migration ($p<0.05$) and invasion ($p<0.05$). The result of RNA-seq showed the expression level of VASN (vasorin) increased in galectin-14-overexpressed HTR-8/SVneo.

Limitations, reasons for caution: Since no endogenously galectin-14-expressed trophoblastic cell line has been found, a further study in primary trophoblasts can help us further verify these results.

Wider implications of the findings: Our results indicated galectin-14 might play an important role in the function of trophoblasts, thus might participate in the pathogenesis of early pregnancy loss.

Trial registration number: No.

P-391 Polo-like kinase 4, PLK4, is not associated with recurrent pregnancy loss caused by embryonic aneuploidy

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Study question: Is *PLK4* associated with recurrent pregnancy loss (RPL) caused by embryonic aneuploidy?

Summary answer: *PLK4* (rs2305957) was not associated with RPL caused by embryonic aneuploidy.

What is known already: No genetic association with RPL caused by embryonic aneuploidy has been found, although it is well-known that it is the most frequent cause of miscarriage and that the oocyte aneuploidy increases according to the maternal age. Recent studies have indicated that a common genetic variant, rs2305957, encompassing the *PLK4* gene contributed to mitotic-origin aneuploidy risk during human early embryo development and it was associated with early RPL. However, the previous study did not examine the aborted concepti in patients with RPL.

Study design, size, duration: Our case control study included 184 patients with RPL whose previous aborted conceptus was ascertained to be an aneuploidy embryo and 190 fertile control women without a history of miscarriage. The mean (SD) number of miscarriage was 3.09±1.13. Samples were mainly collected from 2007 to 2018 at Nagoya City University Hospital.

Participants/materials, setting, methods: We performed a genetic association study to examine the genotype distribution at rs2305957 of patients with RPL caused by aneuploidy compared with controls. Patients whose previous aborted concepti were normal, polyploidic or 45, X-positive were excluded.

Main results and the role of chance: Minor allele frequencies (MAF) were 0.361 for patients and 0.367 for controls. The frequencies of AA, AG and GG were 12.6%, 46.8%, and 40.5% in patients and 15.8%, 41.8%, and 42.4% in the controls.

No significant differences in MAF or the distribution in the dominant model, recessive model or additive model of rs2305957 (G>A) were found between the patients and controls.

Limitations, reasons for caution:

Whether there is an association between RPL and 45, X is unclear because we excluded patients whose previous aborted concepti were 45,X-positive.

Wider implications of the findings: Maternal rs2305957 spanning *PLK4* was not associated with RPL caused by embryonic autosomal aneuploidy. It is speculated that the *PLK4* dysfunction might cause mosaic aneuploidy. Mosaic aneuploidy except with 45, X is rare in RPL. Further study is needed to examine why rs2305957 is associated with early RPL.

Trial registration number: not applicable

P-392 investigation of inflammatory and oxidative stress factors in recurrent pregnancy loss (RPL) patients with and without metabolic syndrome

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Study question: How are the changes in inflammatory cytokines and oxidative stress factors among patients with Recurrent Pregnancy Loss (RPL) with and without Metabolic Syndrome (MetS)?

Summary answer: Significant increase in inflammatory cytokines and oxidative stress factors were seen in RPL patients with MetS compared to RPL patients without MetS and healthy controls.

What is known already: Recurrent miscarriage is defined as three or more consecutive pregnancy losses prior to the 20th week of gestation. Exaggerated maternal immune response and oxidative stress status have been proposed to be the main underlying mechanisms for RPL. MetS is identified to be the accumulation of insulin resistance and cardiovascular risk factors, along with abnormal glucose tolerance, atherogenic dyslipidemia, visceral obesity and hypertension and is caused by several situations like increased free radicals and inflammatory cytokines secretion. Accompaniment of RPL with metabolic syndrome may lead to excessive secretion of inflammatory cytokines, oxidative stress factors and more complications of the disease.

Study design, size, duration: This study designed based on the alterations of effective factors in RPL patients group with and without MetS.

28 RPL patients without Mets and 21 RPL patients with MetS were included. In parallel, 42 healthy women in reproductive age were assigned as the control group. All of the participants signed an informed consent at Al Zahra Hospital of Tabriz University of Medical Science (TUOMS) from February to November; 2017.

Participants/materials, setting, methods: Healthy pregnant women and RPL patient with and without MetS were included. Blood samples were collected and serum levels of IL-1β, IL-6, IL-17, TNF-α, CCL-2, CXCL-8, levels of TAS, TOS, NO, CAT, SOD, AOPP, MPO, gene expression of IL-1β, IL-6, IL-17, TNF-α, CCL-2, CXCL-8, NF-κB, AP-1, miR-21, miR-146-a, miR-223 and the frequency of Th17 and T-reg cells were evaluated by ELISA, spectrophotometry, Real-Time PCR, and flow cytometry, respectively.

Main results and the role of chance: Our results showed statistically significant increase in the gene expression levels of IL-1β, IL-6, IL-17, TNF-α, CCL-2, CXCL8, NF-κB, AP-1 and miR-21 in RPL patients with MetS ($p< 0.05$ was considered to be statistically significant). We also demonstrated significant decrease in the gene expression levels of FoxP3, miR-146-a and miR-223 in RPL patients with metabolic syndrome. Serum levels of IL-1β, IL-6, IL-17, TNF-α, CCL-2, CXCL-8, NO, MPO and TOS were found to be higher in RPL patients with MetS when compared to the other two groups (*P* values of noted cytokines level in RPL patients with MetS in comparison to RPL without MetS and healthy pregnant women were, IL-1β: 0.0004 and <0.0001, IL-6: 0.048 and <0.0001, IL-17: 0.0005 and <0.0001, TNF-α: 0.0004 and <0.0001, CCL-2: 0.0077 and <0.0001, CXCL-8: 0.0069 and 0.0001, NO: 0.0037 and 0.0001, MPO: 0.001 and <0.0001 and TOS: 0.0202 and 0.0038 respectively). In contrast CAT and SOD levels in RPL patients with MetS were decreased. Also, the RPL patients with MetS had higher and lower frequency levels of Th17 cells (*P*=0.0052 and <0.0001) and T-reg cells (*P*=0.0207 and <0.0001) when compared to the other groups, respectively.

Limitations, reasons for caution: Small sample size and unknown genetic background were the most important limitations. Due to the genetic variations in different population, it is necessary to conduct comprehensive studies with large sample size to find exact etiology, clinical diagnoses and best therapy for this disease.

Wider implications of the findings: The results of this study were somewhat in line with previous literature and open new insights in the etiology of RPL. This finding might be valuable as a new therapeutic potential in the prediction or risk assessment of RPL.

Trial registration number: Not Applicable

P-393 Study of 109 triplet pregnancies after single embryo transfer in the Japanese national ART registry

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Study question: To identify the cases of triplet pregnancies after single embryo transfer (SET) in Japan.

Summary answer: Most patients underwent ART with frozen–warmed embryo transfer (ET), blastocyst culture and assisted hatching (AH) as possible risk factors for zygotic splitting.

What is known already: SET is considered to decrease the risk of multiple pregnancies. However, we reported a 1.60% (4,419 pregnancies) prevalence of multiple pregnancies, including 109 triplets, which is substantially higher than the 0.3%–0.5% rate of spontaneous multiple pregnancies according to 937,848 SET cycles in Japan. Regarding monozygotic triplets after SET, only six sets of pregnancies have been reported worldwide to date. We reported that the possible risk factors for zygotic splitting after SET include frozen–warmed ET, blastocyst culture and AH.

Study design, size, duration: A retrospective observational study was conducted based on 274,605 pregnancies after 937,848 SET cycles in registered ART data from the Japan Society of Obstetrics and Gynaecology (JSOG) between 2007 and 2014. Our study was approved by the Registration and Research Subcommittee of JSOG and our hospital's ethics committee.

Participants/materials, setting, methods: Triplet pregnancy was defined as one or two gestational sacs (GSs) with three foetuses or three GSs with 0–3 foetuses. SET was defined as one ET in one cycle. To analyse data on triplets, we extracted data including age, methods of controlled ovarian stimulation, fertilisation procedures, frozen–warmed/fresh embryos at ET, number/grade of transferred embryos, AH, number of GS/foetus, pregnancy courses and number/findings of liveborn infants from the registered ART database.

Main results and the role of chance: The Japanese ART national registry database demonstrated 109 triplet pregnancies (0.04%), including 37 monochorionic (1 GS), 17 dichorionic (2 GSs) and 55 trichorionic triplets (3 GSs). In trichorionic triplets, 9, 11, 8 and 27 cases showed three GSs with 0–3 foetuses, respectively. Mean ages of women with mono-, di- and trichorionic triplets after SET were 36.1 ± 3.3, 32.0 ± 3.7 and 35.3 ± 4.7 years, respectively. Regarding the risk factors for zygotic splitting, the ratios of ART use were 78.4% (29/37 triplets), 76.5% (13/17) and 76.4% (42/55), respectively, for frozen–warmed ET; 91.9% (34/37), 70.6% (12/17) and 81.8% (45/55), respectively, for blastocyst culture and 67.7% (21/31), 71.4% (10/17) and 76.1% (35/46), respectively, for AH. Most triplet patients conceived after ART using frozen–warmed ET, blastocyst culture and AH. Livebirth (defined as at least one baby born) rates were 57.1% (20/37), 70.6% (12/17) and 71.7% (33/46) with 1–3 foetuses, respectively. Monochorionic triplets had a higher incidence of foetus mortality than the other triplet types, but there was no significant difference ($P = 0.23$).

Limitations, reasons for caution: In the current Japanese ART registry system, data regarding frozen–thawed ET do not include information about ovarian stimulation and fertilisation methods. Moreover, registration for AH started only in 2010.

Wider implications of the findings: Monozygotic triplets occur rarely. After SET was first recommended in Japan in 2008, the SET rate reached 80% for all ET cycles in 2015. Elective SET using frozen–warmed ET, blastocyst culture and AH might result in an increased prevalence of triplet pregnancies iatrogenically.

Trial registration number: None

P-394 The use of autologous immune cell by intrauterine inoculation enhances clinical pregnancy rates in case of recurrent implantation failure following intracytoplasmic sperm injection (ICSI)

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Study question: To assess the effect of the use of autologous lymphocyte followed by intrauterine inoculation on cases in couple's failure to achieve pregnancy following several intracytoplasmic sperm injection treatment cycles.

Summary answer: These findings indicate that intrauterine administration of autologous AL may promote implantation rates in patients after failure repeated ICSI cycles.

What is known already: Some couples have repeated implantation failures during assisted reproductive technology (ART) programs. Indeed embryo implantation represents a critical step that determines reproductive success. Several factors can influence this implantation rate like the endocrine system that plays a main role to regulate endometrial differentiation. The immune system can also affect endometrial differentiation at the peri-implantation period by some populations of immune cells and support embryo implantation.

Study design, size, duration: In the present study a total of 135 ICSI cycles were included and classified into AL-treated (68 cycles, 50.3%) and non-treated (control) groups (67 cycles, 49.6%). All patients had experienced four or more failures of ICSI–embryo transfer therapy. All females were stimulated using a gonadotropin releasing hormone agonist or antagonist and human FSH. Human chorionic gonadotropin was administered when optimal follicle development was achieved.

Participants/materials, setting, methods: On the day of HCG injection, 5ml of peripheral blood was collected and AL were isolated using Ficoll-Hypaque centrifugation. AL was collected from interphase layer and washed four times with RPMI 1640. AL ($1x10^6$ cells/ml) supplemented with 10% Serum Protein Substitute were incubated in the presence of HCG (5IU/ml) for 72h at 37°C. After culture and centrifugation, the pellet was suspended culture media. The AL suspension was injected in the uterine cavity using insemination catheter

Main results and the role of chance: A total of 972 oocytes were retrieved and 850 metaphase II oocytes were microinjected, 424 for control group (mean 6.3) and 426 for AL-treated group (mean 6.2). The fertilizing rate was 66.9% (284/482) in control group and 66.1% (282/490) in AL group. A total of 67 transfers with a mean of 2.1 embryos replaced in control group and 65 transfers with a mean of 2.2 embryos replaced in AL group. The clinical pregnancy rate per cycle in non-treated groups and AL group was 14.3% and 38.2% respectively. The deliveries rate per transfer in control group and AL group was 5.0% and 29.0% respectively.

Limitations, reasons for caution: The reduced number of patients and the precise mechanism of intrauterine inoculation of lymphocytes that remains unknown may be the limit of this study.

Wider implications of the findings:

This work provides a simple method for improved pregnancy rates for cases of implantation failure in ART programs.

Trial registration number: no trial registration number

P-395 Likelihood of subsequent successful pregnancy outcome after evaluation and management of women with a history of idiopathic recurrent early pregnancy loss (REPL)

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Study question: What is the likelihood of a successful pregnancy outcome after evaluation and management of women with a history of idiopathic REPL?

Summary answer: In this cohort of women with idiopathic REPL, the live birth rate (LBR) per index pregnancy was 58% (236/410), and cumulative LBR was 74% (201/271).

What is known already: REPL impacts ~5% of couples who are trying to have a child(ren). Despite evaluation for parental translocations, endocrine, uterine, and autoimmune factors, ~40% of couples are classified as idiopathic REPL (Stephenson, 1996).

Clifford et al. (1997) reported a 74% subsequent LBR/index pregnancy in idiopathic recurrent miscarriage (IRM) with close monitoring/without medication. Brigham et al. (1999) reported 75% LBR; >95% seen ≥6 weeks', close monitoring provided/medications unknown.

Since the 1990's, pregnancies are documented <6 weeks', terminology has changed, and pregnant women are generally older. Updated LBRs need reporting for counseling of couples with idiopathic REPL. Empiric medications also need to be addressed.

Study design, size, duration: Observational cohort study using prospectively collected data of 271 women seen in an academic RPL Program, 2004-2012. Inclusion criteria: History of REPL, defined as ≥2 pregnancy losses <10 weeks excluding aneuploid/polyploid miscarriages; Negative REPL evaluation, ≥1 subsequent pregnancy, excluding ectopics and/or terminations, conceived without fertility medication. Timed intercourse to LH surge. Pregnancy defined as positive serum hCG at missed menses. Ultrasound q1-2 weeks between 6-12 weeks'. Subjects gave written informed consent for future REPL research.

Participants/materials, setting, methods: Primary outcome was LBR/subsequent pregnancy. Secondary outcomes were cumulative LBR and LBR/subsequent index pregnancy (aneuploid/polyploid miscarriages excluded) with subanalysis according to empiric medications, time to conception, and descriptive analysis of maternal and neonatal complications.

Continuous variables reported as mean (standard deviation; range). Categorical variables reported as numbers with percentages. Chi-square was used for comparison of categorical variables.

Main results and the role of chance: The cohort consisted of 271 patients, with 410 subsequent pregnancies. Caucasian 85%, non-Hispanic 93%. Mean number of prior pregnancies 3.9 (1.9; 1-14); Mean number of prior pregnancy losses 2.9 (1.4; 2-12); Mean number of prior live births 0.75 (0.5; 0-4), Mean prior cycles to conceive 3.4 (6.1; 1-96).

There were 410 subsequent pregnancies with 236 subsequent live births. Empiric medications included: luteal start progesterone suppositories (prog) (n=59 pregnancies); acetylsalicyclic acid (LDA) (n=34); prog/LDA (n=51); prog/LDA/prophylactic heparin (n=1); prog/levothyroxine (n=14); IVIG (n=6); placebo (n=7); other (n=32), and close monitoring only (n=32).

LBR/subsequent pregnancy was 58% (236/410) with 8% (19/236) preterm births. Mean number of cycles to conceive was 2.2 (2.0; 1-12). Cumulative LBR was 74% (201/271 women). LBR/subsequent index pregnancy was 64% (141/221).

Cesarean section rate was 29% (68/236); mean birthweight was 3,328 grams (613; 1,350-4,819). Other outcomes included hypertensive disorders 13% (13/236), gestational diabetes mellitus 2% (5/236), thromboses 0% (0/236), postpartum hemorrhage 3% (8/236), and Neonatal Intensive Care Unit admission 8% (18/236).

Use of any empiric medication resulted in higher LBR/subsequent index pregnancy compared to close monitoring only, 60% (204/342) vs. 52% (32/61) , *P*=0.002.

Limitations, reasons for caution: Protocol-driven evaluation/management in an academic RPL Program by a single provider should provide consistent results, but may not be generalizable. Maternal/fetal morbidity may have been under reported; based on subject recall if medical records unavailable. Randomized controlled trials are needed to determine if medications are beneficial.

Wider implications of the findings: Despite history of idiopathic REPL, subsequent live birth rate, based on diagnosis of pregnancy at missed menses, are encouraging, along with short time to conception. In addition to first trimester close monitoring/supportive care, data suggests medications may be of benefit for idiopathic REPL, although further studies are warranted.

Trial registration number: not applicable

P-396 HCG activates Wnt signaling pathway through miR-125a-3p in Ishikawa cells to enhance Jeg3 spheroid attachment

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Study question: To investigate how blastocyst derived hCG regulates embryo implantation through endometrial miR-125a-3p

Summary answer: **hCG promoted spheroid attachment through activation of Wnt signaling pathway via increasing miR-125a-3p expression in endometrial cells**

What is known already: Accumulating evidence showed that human chorionic gonadotropin (hCG) derived from embryo has paracrine effect on endometrium to modulate endometrial receptivity favorable for implantation. Recent findings suggested that hCG can alter miRNA expression in endometrial cells. We hypothesize that hCG regulated endometrial cell receptivity through regulating miRNA expression.

Study design, size, duration: Ishikawa cell was used as endometrial epithelial cell model. JEG3 cells were used to form spheroids to mimic human blastocysts. Endometrial biopsies were performed to collect hCG+7/8 samples and LH+7/8 samples. hCG (1IU/ml, 5IU/ml 10IU/ml) were used to stimulate Ishikawa cells in for 6h, 24h or 48h *in- vitro*. MiR-125a-3p force-expressed Ishikawa cells was used to identify the miRNA target and to demonstration its role in Wnt signaling.

Participants/materials, setting, methods: miRNAs were isolated from Ishikawa cells that treated with 1,5 or 10IU/ml of hCG for 6 hours. Real-time PCR (Q-PCR) was used to measure the expression levels of miR-125a-3p. Western blotting was used to detect expression of Wnt signaling target

β-catenin and miR-125a-3p target CTNNBIP1in the miR-125a-3p force-expressed Ishikawa cells. Immunostaining was used to observe β-catenin expression under hCG stimulation. Spheroid attachment assay was performed to assess the function of miR-125a-3p in early embryo implantation.

Main results and the role of chance: Q-PCR results showed that hCG had dose-dependently increased miR-125a-3p expression in Ishikawa cells. The expression of miR-125a-3p was significantly increased by 3.04±0.36-fold and 3.89±0.54-fold when stimulated with 5IU/ml and, 10IU/ml of hCG respectively. HCG+7/8 biopsies have significantly higher miR-125a-3p (6.26±1.19-fold) when compared with LH+7/8 biopsies. The expression of active β-Catenin presented dose-dependent increase upon hCG stimulation in Ishikawa cells, which was significantly increased under 10IU/ml hCG treatment. Immunostaining revealed nuclear expression of β-Catenin in Ishikawa cells and β-Catenin signal intensity increased with dose of hCG. Western blotting showed that the active β-Catenin were significantly reinforced by 1.89±9.53-fold in the pre-miR-125a-3p force-expressed cells when compared to the scramble group. ***In-silicon*** analysis identified CTNNBIP1 is a putative target of miR-125a-3p. Western blotting showed the expression of CTNNBIP1 was significantly decreased (0.58±0.31-fold) upon force-expression of miR-125a-3p and significantly increased (2.05±0.32-fold) upon miR-125a-3p suppression. Spheroid attachment assay were performed on miR-125a-3p enriched Ishikawa cell layer with JEG3 cell spheroids. It was found the miR-125a-3p significantly increased spheroids attachment by 1.43±0.20-fold.

Limitations, reasons for caution: It is not ethical to investigate early embryo implantation event in humans, thus, only ***in-vitro*** investigation can be performed. How does ***in-vivo*** situation is different to ***in-vitro*** conditions is unknown.

Wider implications of the findings: We discovered that miR-125a-3p is a regulator for early embryo implantation in endometrial cells; this miRNA may potentially be used as a marker to identify endometrial receptivity.

Trial registration number: not applicable

P-397 Membrane expression of PDIA1 and PDIA6 regulated by steroid hormones affects spheroid attachment onto human endometrial epithelial cells

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Study question: Do steroids regulate membrane PDIA1/PDIA6 expression in endometrial cell lines which in turn govern the endometrial receptivity

Summary answer: PDIA1/PDIA6 expression is regulated by steroids and PDIA1/PDIA6 can regulate the receptivity of endometrial cell lines.

What is known already: Differentially expressed proteins at the receptive phase of endometrial epithelium play a pivotal role in embryo implantation. Protein Disulphide Isomerase (PDI) family contains 21 members and PDI function as chaperone protein with redox activity. Recent studies suggested a differential expression of PDI on the cell surface of endometrial epithelial cells RL95-2 and HEC-1A. Results from our mass spectrometry analysis of endometrial surface protein and spheroid-endometrial cells co-culture studies suggested differential expression of PDI in receptive and non-receptive endometrial cells responsible for spheroid attachment.

Study design, size, duration: We used receptive (Ishikawa and RL95-2) and non-receptive (HEC1-B and AN3CA) human endometrial cells lines and Jeg-3 trophoblastic cell line for co-culture study. Ishikawa cells were treated with steroid hormones for 24 hours. Total protein, membrane protein and cytosolic protein were extracted and specific antibodies were used to analyze the enrichment of proteins in different fractions.

Participants/materials, setting, methods: The expressions of PDIA1and PDIA6 in the membrane or cytoplasmic proteins were studied. PDI inhibitors (Bacitracin, 16F16 and LOC14) were used in AN3CA cells and co-culture studies were conducted to confirm the functional role of PDI proteins. The receptive Ishikawa cells were treated with estrogen (0.01-100 nM) and progesterone (0.01-1uM) and the combination of estrogen (743 pM) and progesterone (52.6 nM) for 24 hours. The expressions of PDIA1/PDIA6 were studied by Western blotting and immunohistochemistry.

Main results and the role of chance: The expression of PDIA1 and PDIA6 is high in the 4 endometrial epithelial cell lines; while a high membrane expression of PDIA1 and PDIA6 was found in non-receptive AN3CA cells. No significant difference was found in the PDIA1 and PDIA6 expression between total protein and cytosolic protein fractions of Ishikawa, RL95-2, AN3CA and HEC1B. Interestingly, the spheroid attachment rate was significantly higher in receptive (Ishikawa and RL95-2) than non-receptive (HEC1-B and AN3CA) endometrial cells. The expression of steroid hormone receptors (ERα, ERβ, PRA, and PRB) was found in receptive cells (Ishikawa, RL95-2). Estrogen (0.1nM-100nM) up-regulates membrane PDIA1/PDIA6 expression; while progesterone (0.1uM-1uM) down-regulates PDIA1/PDIA6 expression in Ishikawa cells. Estrogen (743pM) plus progesterone (52.6nM) reduces membrane PDIA1/PDIA6 expression. Inhibition of PDI by Bacitracin (1mM), LOC14 (1uM) or 16F16 (1uM) increased the attachment rate of Jeg-3 spheroids in AN3CA cells when compared with vehicle control. Immunohistochemical staining localized PDIA1/PDIA6 in glandular and luminal epithelium of human endometrial samples.

Limitations, reasons for caution: As PDI family has 21 members, the function of other members in terms of endometrial receptivity is largely unknown. Whether the compensatory role of PDI family members on spheroid attachment exist needs to further investigation.

Wider implications of the findings: Taken together, steroid hormones regulate PDIA1/PDIA6 expression on the endometrial surface, and suppression of membrane PDI expression may increase spheroid attachment and endometrial receptivity to the pre-implantation embryo. Modulation of PDI function with inhibitors may provide a new approach to enhance implantation rate and pregnancy outcome in IVF patients.

Trial registration number: N/A

P-398 Transient suppression of immune checkpoint Tim-3 in peripheral NK cells 3 days after blastocyst transfer is a marker of successful implantation

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Study question: Is successful implantation associated with a significant change in immune checkpoint Tim-3 and its ligand Galectin-9 over a nine-day period after blastocyst transfer?

Summary answer: Transient, significant reduction of Tim3+ pNK cells occurred 3 days after blastocyst transfer was correlated with successful implantation.

What is known already: Various immune cells are involved in successful implantation. Several earlier studies suggested that immune cell derangement is an underlying cause of miscarriage; specifically, the role played by Tim-3 has recently received increasing attention because it serves as an immune checkpoint with essential immunological function in many physiological and pathological conditions in Galectin-9 dependent pathway. It has been found that reduction of Tim-3+ pNK cells induce Th1 immune response, leading to influx of pro-inflammatory cytokines, predisposing to immune rejection and leading to early miscarriage. However, the role of Tim-3/Galectin-9 at the time of embryo implantation, has not been previously examined.

Study design, size, duration: Serial blood samples were obtained from 20 women on the day of blastocyst transfer, and repeated 3, 6 and 9 days after for measurement of Tim-3+pNK cells and Galectin-9 levels. Serum β-hCG was measured 9 days after blastocyst transfer; in women who was tested positive for β-hCG, a transvaginal ultrasonography was performed 23 days after embryo transfer to confirm viability. The study was approved by the local ethics committee.

Participants/materials, setting, methods: A total of 20 women were recruited from women undergoing embryo transfer in an IVF unit of a University hospital in Hong Kong over a 4 months period. 13 women did have successful implantation and the remaining 7 did not conceive. Flow cytometry was employed to measure the percentage of Tim-3+ pNK cells and commercial ELISA kits were used to measure the concentration of serum Galectin-9.

Main results and the role of chance: The peripheral blood Tim-3+pNK cells (33.9±9.8) and galectin-9 (4.6±2ng/ml) levels in the pregnant group were not different from that of the non-pregnant group (36±8.1, 3.9±1.2ng/ml) on the day of embryo transfer. In the non-pregnant group, there was no change in the level of Tim-3+pNK cells between baseline (day of blastocyst transfer) and the subsequent time points (ET+3, 39.9±12.1; ET+6, 41±8.9; ET+9, 46.9±13.7). However, in the pregnant group, there was a significant drop in Tim-3+ pNK cells from the baseline to 23.5±10.1 on day ET+3; the level partially recovered to 29±8.9 on day ET+6 and back to the baseline level (43±11.6) on day ET+9. Furthermore, the percentage of Tim-3+ pNK cells was significantly lower in the pregnant group when compared with non-pregnant women on ET+3 (p=0.01) and ET+6 (p=0.03) but not on ET+9 (p=0.59). On the other hand, the concentration of serum Galectin-9 did not show any significant fluctuation between the several time points within each group and between the pregnant and non-pregnant groups.
Limitations, reasons for caution: The number of subjects included in this study was relatively small but the finding of significant difference in a small sample size suggests that the result is likely to be clinically important. Nevertheless, we are continuing the recruitment to increase the sample size.
Wider implications of the findings: The transient and significant reduction of Tim-3 level in pNK cells observed in this study had already returned to baseline level by the time pregnancy was confirmed 9 days after embryo transfer. Tim-3 level in pNK cell may be an important early prognostic marker for successful implantation.
Trial registration number: nil

P-399 An evaluation of first subsequent pregnancy outcomes among women with recurrent miscarriage: a retrospective cohort study.

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Study question: To investigate pregnancy outcomes among women with recurrent miscarriage (RM) and the potential epidemiological and clinical factors associated with the outcome.
Summary answer: More than 60% of our sample had a livebirth after RM. Advanced maternal age and primary RM increased the risk of a subsequent miscarriage.
What is known already: Contradictory evidence has been published regarding the influence of previous reproductive history among women with RM and the likelihood of having a further miscarriage in subsequent pregnancies. Some studies have shown that women with RM have a 40% increased risk of having a miscarriage in subsequent pregnancies; others have reported that previous live births were not associated with a positive prognosis for further subsequent pregnancies. Only a few epidemiological studies have proved positive prognosis for pregnancy outcomes after RM. Therefore, more research is needed to clarify the influence of past reproductive history, investigations and management and subsequent pregnancy outcomes.
Study design, size, duration: A retrospective cohort study was conducted in a Pregnancy Loss Clinic (PLC) at a large, tertiary maternity hospital in Ireland. It is a dedicated clinic to investigate causes of RM and to provide support to parents with RM. The clinic facilitates the recruitment of a reliable RM cohort. Epidemiological and clinical information was gathered from medical records from 2008 to 2016. Outcomes data included livebirth, miscarriage, other pregnancy loss and loss of follow-up.
Participants/materials, setting, methods: All women who attended the PLC and who had first trimester RM were identified using medical records. A total number of 508 women with RM were included in the analysis. Of these, outcome data were available for 74.4% (n=378) on the first subsequent pregnancies, 10.4% (n=53) on the second subsequent pregnancy and less than 1% (n=4) on the third. Data were analysed using descriptive and inferential statistics.
Main results and the role of chance: In the first subsequent pregnancy after RM, 63% (n=232) had a livebirth and 37% (n=136) had miscarriage. Women who had a livebirth in the first subsequent pregnancy were significantly younger than women who had a miscarriage (34.7 versus 35.8 years respectively; p-value 0.05). Women aged 40 or older had almost four times more risk of having a miscarriage than women aged 29 years or younger. Women with secondary RM (i.e. previous livebirth) had less risk of having a miscarriage than women with primary RM (34.3% versus 38.6%; p-value 0.03). Among women whom cause of RM at recruitment was fetal abnormalities, 37% (n=27) had a livebirth and almost 30% (n=21) had a miscarriage in the first subsequent pregnancy. Almost 41% (n=9) of women who had a metabolic or endocrine factor in the previous RM had a miscarriage in the first subsequent pregnancy, and 32% (n=7) had a livebirth. Causes of RM at recruitment were unexplained or unknown in 26% (n=43) of women. Among these, almost 50% (n=20) had a livebirth in the first subsequent pregnancy and 26% (n=11) had a further miscarriage.
Limitations, reasons for caution: Data were obtained from a single hospital, in a dedicated unit, and therefore, the results might not be generalisable to another countries or populations. Nonetheless this is a large cohort managed by the same clinical team in a standardised manner.
Wider implications of the findings: The proportion of women who had a successful pregnancy after RM was higher in our study population. Our findings illustrate potential factors associated with pregnancy outcomes for women with RM. These findings help inform clinicians when counselling women who present with RM about the prognosis for subsequent pregnancies.
Trial registration number: not applicable

P-400 Does exogenous progestogen administration affect placental steroid production in early pregnancy?

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Study question: Is the onset and magnitude of endogenous, placental progesterone production in early pregnancy associated with the dose of exogenous dydrogesterone administered in programmed FET cycles?
Summary answer: Progestogen from exogenous administration is potentially associated with progesterone production of the early placenta.
What is known already: The onset of trophoblastic progesterone production in early pregnancy is termed the luteo-placental shift (LPS). Recently, the kinetics of serum progesterone levels of trophoblastic origin have been described by utilization of dydrogesterone (which is not cross-reacting with endogenous progesterone in the ELISA) in programmed FET cycles where no corpus luteum is present. To date, a modulatory effect of exogenous progestogens or endogenous progesterone released by the corpora lutea after ovarian stimulation on placental activity and growth has not been sufficiently studied. However, early placental development may have far-reaching consequences on the course of pregnancy and the health of the child.
Study design, size, duration: Prospective study of singleton pregnancies resulting from programmed FET cycles with oral E2 (6mg/d for minimal intake 13 days) followed by E2 (6mg/d) and 30mg/d oral dydrogesterone (NCT03507673). On day of a positive pregnancy test (day 9-15 after embryo transfer, patients either continued intake of 30mg (during phase I of the study) or increased dydrogesterone dose to 50mg daily (phase II) till +37d-43d post transfer. The sample collection started in 7/2015 and is currently ongoing.
Participants/materials, setting, methods: Progesterone, E2 and hCG were measured in serum samples by the Roche Elecsys ELISA before dydrogesterone intake on day 13-15 (to confirm absence of ovulation) and weekly in early pregnancy. Progesterone levels are expressed in µg/l (±standard deviation). A two-way repeated measures ANOVA was conducted for statistical analysis.
Main results and the role of chance: Endogenous serum progesterone was similar in both groups on first blood analysis on day +9-15 days following ET (patients later on 50mg/d: 0.26±0.19; n=22; patients continuing on 30 mg/d: 0.28±0.18; n=18) and increased only marginally +16-22d post ET in patients increasing dydrogesterone to 50 mg/d contrary to the group of patients continuing 30 mg/d (patients 50mg/d: 0.35±0.15; n=21; patients 30mg/d: 0.51±0.31; n=17; ANOVA repeated measures P=0.09).

The between group difference in serum progesterone levels was pronounced +23-29d post ET (patients 50mg/d: 1.82±1.5; n=22; patients 30mg/d: 2.22±2.09; n=18;), +30-36d post ET (patients 50 mg/d: 5.07±3.03; n=18; patients 30 mg/d: 4.77±3.32; n=20;) and reached its peak 37-43d post ET (patients 50mg/d: 8.37±2.73; n=20; patients 30mg/d: 9.35±3.99; n=17;). On average, serum progesterone levels were reduced by 16±11% from +16-22d - 37-43d post ET.

Limitations, reasons for caution: Differences in serum progesterone levels do not reach statistical significance yet due to insufficiently large group sizes. Moreover, the observed differences are small and close to the ELISA quantification limits.

Wider implications of the findings: Modulating the progestogen exposure of the early conceptus by exogenous administration or by creating multiple corpora lutea via ovarian stimulation may influence placental growth and activity, which may have far-reaching consequences on fetal growth and well being.

Trial registration number: NCT03507673

P-401 Serial Endometrial Volume Assessment as a predictor of Endometrial Receptivity in Assisted Reproductive Technology Cycles

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Study question: Investigate the ultrasonographic value of sequenced endometrial volume analysis in ART cycles as a indicator of endometrial receptivity and embryo implantation.

Summary answer: Adjusted endometrial volume, and the pattern of increment volume in a continuous analysis showed significant impact in predicting endometrial receptivity and embryo successful implantation.

What is known already: There is no controversy regarding the importance of precise and specific endometrial maturational development in allowing implantation following assisted reproduction treatment. Adequate proliferation and differentiation during the proliferative phase must be followed by timely secretory changes during luteal phase with stroll decidualization. However there has been many conflicting results in regards to the role of endometrial assessment by ultrasonographic parameters. Although ultrasonographic parameters of endometrial receptivity have a strong negative value in setting some minimum criteria, their prognostic value for implantation following embryo transfer is yet scarce.

Study design, size, duration: Prospective longitudinal study. Continuous, sequenced ultrasonographic analysis from basal point prior to ovarian controlled stimulation to continuous analysis throughout duration of treatment, trigger day with hCG and embryo transfer. From initial 200 recruited subjects, complete full evaluation of 169 subjects that completed treatment and had a successful embryo transfer.

Participants/materials, setting, methods: Short term antagonist ovarian controlled stimulation to all participants. Assessment of Endometrial Thickness, Endometrial pattern, Endometrial Volume and Adjusted Endometrial Volume (in ratio with Uterine Volume for each participant). Excluded patients with clinical risk for ovarian hyper stimulation syndrome and those without viable good qualitiy day 3 double embryos for transfer. Evaluation of remaining 169 subjects complete and sequenced in time.

Main results and the role of chance: There was no statistical difference between groups with and without successful implantation in demographics, cause of infertility, duration and total dose of gonadotropins and treatment course used. Statistical significant difference was found in terms of Endometrial volume between the group with successful implantation versus the one without implantation, not at base line but after day 10 of controlled ovarian stimulation (Mean value at base line , day 6 and day 8 after controlled ovarian stimulation for the non implantation group of 2,52; 3.08 and 3,91 respectively versus 2,77; 3,33 and 4,40 on the implantation group and Mean value at Day 10 and embryo transfer day for non implantation group of 4,12 and 4,83 versus 4,91 and 5,59 on the implantation group). The continuous analysis of endometrial patterns showed statistical difference between the two groups after day 10 of the ovarian controlled stimulation with p values <0.001. With adjusted volume scores the difference between two groups is shown and sustained in all moments except at base line (Mean values at baseline for non implantation versus implantation group 4,60 versus 5,53 and Mean Values of 5,63; 7,23 ; 7,59 and 8,31 versus 6,70; 9,05; 9,92 and 10,77 with p value <0.001).

Limitations, reasons for caution: Differences in ultrasonographic examination techniques do exist, but the use of a rigid protocol and single operator in all evaluations reduces the possibility of conflicting evaluations. The use of high resolution and 3-D analysis further reduces the possibility of inaccurate evaluation.

Wider implications of the findings: The presence of a receptive endometrium is a major factor in deterring implantation and success in reproductive medicine. The possibility of a non invasive ready to use ultrasonographic parameter would allow predicting more accurately the outcome.

Trial registration number: NONE

P-402 Chromosomal testing at the blastocyst stage reduces miscarriages, aneuploid gestations and provides similar cumulative live birth rate per cycle: a large public health-care provider experience.

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Study question: Is aneuploidy testing at blastocyst stage effective in reducing miscarriages, aneuploid gestations and time-to pregnancy in advanced reproductive age (ARA) women?

Summary answer: Preimplantation genetic testing for aneuploidies (PGT-A) at the blastocyst stage is effective in reducing miscarriages, aneuploid gestations and time to pregnancy in selected IVF populations

What is known already: PGT-A allows the detection of chromosomal abnormalities in preimplantation embryos. However, it is known to be challenging to quantify the benefits that such program brings to the patients treatment outcome. Particularly, long-term clinical data are lacking from single, large IVF centres where inclusion/exclusion criteria for accessing treatment options (i.e., PGT, blastocyst transfer) are standardized for all patients. In this study, we combined laboratory and clinical outcomes, preimplantation and prenatal testing genetic results with detailed effects on pregnancy and neonatal follow-up from a large, public healthcare provider without commercial interest in genetic analysis

Study design, size, duration: Observational cohort study performed between January 2015 and May 2017 at an Academic tertiary care healthcare provider. Couples with female age >38 were counselled for PGT-A before ovarian stimulation. Consenting couples dropped out from PGT-A if <4 fertilized eggs were available after ICSI. In these cases, day3 embryo transfers were performed (DROP-OUT group). All non-consenting couples were included as controls (Standard IVF group). Laboratory, clinical, follow-up and genetic analysis outcomes were compared across all groups.

Participants/materials, setting, methods: This study compares data from groups: PGT-A (273 couples, 312 cycles), DROP-OUT (101-couples, 106-cycles) and Standard IVF (2,168-couples, 2,905-cycles). Confounders-adjusted multivariate analysis was used to compare main outcome measures: miscarriage rate, aneuploid gestation rate, live-birth rate per cycle (LBR) with one year minimum follow-up. Kaplan-Meier and Cox regression were used to compare time-to-pregnancy(TTP) in PGT-A and Standard IVF using as primary outcome the cumulative live birth rate in function of number of transfers required

Main results and the role of chance: Mean female age was higher (P<0.001) in DROP-OUT (41.13 ± 3.03 vs 40.38 ± 3.33 in PGT-A and 39.72 ± 2.37 in Standard-IVF), BMI, infertility duration, FSH and AMH levels, oocyte maturation rate, fertilization and cleavage rates were similar across groups (NS). Euploidy rate in PGT-A group was 56% (306/542). In total, 188 (100% blastocysts), 117 (cleavage-stage) and 3573 (32.1% blastocyst stage) embryo transfers were performed in PGT-A, DROPOUT, Standard-IVF, respectively. PGT-A group showed significantly superior transfer clinical outcomes (P<0.01). Particularly, ongoing implantation (43.1% PGT-A, 18.4% Standard IVF; 10.8% DROPOUT), miscarriage (8.6% PGT-A, 32.0% Standard-IVF; 50% DROPOUT), ascertained

aneuploid gestation (4.3%, 1/23 for PGT-A versus 19.2%, Standard-IVF) and multiple pregnancy rates (0% in PGT-A and 15% in Standard-IVF) were all significantly favourable after euploid embryo transfer. Worst miscarriage rates were recorded in the DROP-OUT group (50%). Multivariate analysis showed no difference in LBR per started cycle between PGT-A and Standard-IVF (23.7% and 23.5%, respectively; OR:0.78; 95%CI=0.57-1.07). Among patients reaching transfer, PGT-A provided a shorter time to live birth. All neonatal outcomes were similar across the groups except low birth weight, which was more frequent in Standard-IVF as a consequence of multiple gestations (14.4% and 4% for Standard-IVF and PGT-A, respectively)

Limitations, reasons for caution: Lack of randomization for patients allocation to PGT-A and control group was the main limitation of this study. However, the large IVF dataset, completed with extensive prenatal and neonatal follow-up, provided reliable and meaningful comparisons.

Wider implications of the findings: Euploid embryo transfer showed to significantly reduce the incidence of aneuploid gestation and miscarriage rate in a public healthcare provider while preserving the cumulative LBR. Shorter time-to-pregnancy was observed when a euploid embryo was transferred. Patients' drop-out from aneuploidy testing due to poor response/prognosis should be carefully considered

Trial registration number: none

P-403 HLA-E is involved in the invasion of trophoblast cell line JEG-3 induced by progesterone

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Study question: This study was performed to determine if HLA-E plays a role in the regulation of invasive ability of trophoblast cells.

Summary answer: HLA-E is involved in the stimulatory effects of progesterone on JEG-3 invasion.

What is known already: Progesterone has been considered to be an essential hormone in maintaining pregnancy although its function is not fully elucidated. HLA-E, one of the MHC-Ib molecules, contributed to the establishment of an immune tolerance at maternal-fetal interface during pregnancy. In our previous study, progesterone was found to up-regulate HLA-E expression in JEG-3 cells through the pathway mediated by progesterone receptor, and increase the invasive ability of JEG-3. However, if HLA-E plays a role in the regulatory effect of progesterone on JEG-3 cell invasion has not been studied.

Study design, size, duration: In this study, the human choriocarcinoma JEG-3 cells (Cell Resource Center, IBMS, CAMS/PUMC), a well-established cellular model for extravillous trophoblasts (EVTs), was used to investigate the possible role of HLA-E in the regulation of trophoblasts invasion.

Participants/materials, setting, methods: The expression of HLA-E in JEG-3 was silenced using RNAi technology to examine its role in the regulation of JEG-3 invasion. Transwell invasion assay was performed to evaluate the invasion of JEG-3 48h after the transfection.

Main results and the role of chance: JEG-3 cells were subjected to different treatments including addition of culture medium (group 1, the blank control), progesterone treatment (group 2), transfection of lentivirus carrying siRNA targeting HLA-E before progesterone treatment (group 3), transfection of lentivirus carrying siRNA targeting HLA-E (group 4), and transfection of the negative control siRNA (group 5). Transwell invasion assay showed that the number of invaded cells attached to the lower surface of the filters was significantly increased following treatment of progesterone (group 2)

while decreased after transfection of lentivirus carrying siRNA targeting HLA-E (group 4), compared to the control groups. In group 3, the HLA-E in JEG-3 was blocked by transfection of siRNA targeting HLA-E before progesterone treatment. Data showed that the number of invaded cells was significantly smaller than that of group 2, but larger than group 4, indicating that the up-regulatory effect of progesterone on invasion of JEG-3 was at least partly inhibited by HLA-E silence.

Limitations, reasons for caution: Although JEG-3 is a commonly used in vitro model for EVT, further investigation is required to see whether the changes of JEG-3 invasion observed here also occurs in trophoblasts in placenta.

Wider implications of the findings: The findings in this study might give a new clue to the immunological mechanism supporting placental development.

Trial registration number: Non-clinical trials.

P-404 Relevance of next generation sequencing (NGS) as a tool for chromosomal analysis of products of conception (POC) compared with conventional karyotyping (G-banding).

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Study question: Is NGS valuable as a tool for chromosomal analysis of POC compared with conventional karyotyping?

Summary answer: NGS testing improved diagnostic accuracy compared with conventional karyotyping and the ratio for 46,XX to 46,XY dropped from 8.50 to 2.53.

What is known already: Conventional karyotyping is known to be labor-intensive and time-consuming, requiring reliable cell culture with possible maternal cell contamination leading to failed analysis. While NGS is characterized as a technique demanding smaller specimen with a lower failure rate, higher resolution, and shorter turnaround time than conventional karyotyping when used for the genetic analysis of miscarried fetal tissue and/or chorionic villi.

Study design, size, duration: This retrospective study was conducted between March 2017 and December 2018 at a single reproduction center. All patients involved gave written consent, and institutional review board approval was granted. This study included 160 women with first-trimester miscarriage, wished for POC analysis.

Participants/materials, setting, methods: Patients undergoing dilation and curettage (D&C) after diagnosis as miscarriages the first trimester were involved. Conventional karyotyping (G-banding) was performed in 50 cases, and NGS testing was performed in 110 cases. Among 110 cases, 7 were assessed by both of NGS and conventional karyotyping.

Main results and the role of chance: In G-banding, 11 out of 50 cases (22.0%) were not conclusive due to cell culture failure. In 39 cases analyzed, 17 (43.6%) were 46,XX, 2 were 46,XY, 2 were 45,XO, 1 was triploid, and the remaining 18 (46.2%) were autosomal single or multiple trisomy. In NGS groups, results were obtained in all 110 cases. There were 18 cases where chorionic villi or fetal tissue were not confirmed by dissecting microscopy, the results were all 46,XX. Among 92 cases in which chorionic villi could be detected by dissecting microscopy, 15 cases (16.3%) were 46,XX, 13 (14.1%) were 46,XY, 7 were 45,XO, one was 47,XXY,and the remaining 56 (60.9%) were autosomal single or multiple trisomy. The ratio for 46,XX to 46, XY was 8.50 (17/2) after G-banding, while it was 2.53 (33/13) after NGS. If excluding 18 cases where chorionic villi or fetal tissue was not be detected, the ratio after NGS was 1.15 (13/15). The rate of chromosomal abnormalities detected by NGS was higher than G-banding (69.6% vs 51.3%).

In 7 cases, assessed by both of NGS and G-banding, one was 46,XX in G-banding but 46,XY in NGS, and one was 46,XO,+11 in NGS but culture frailer in G-banding.

Limitations, reasons for caution:

This was a retrospective study. Maternal cell contamination was not definitely confirmed. In the NGS method, balanced reciprocal translocation cases could not be detected. The participants were limited to Japanese women only.

Wider implications of the findings: Because NGS does not require cell/tissue culture, chromosomally abnormal cells that may not survive culture, can be detected by NGS method. Thus, it can eliminate possible maternal cell contamination more effectively than conventional karyotyping, leading to more accurate and conclusive analysis.

Trial registration number: N/A.

P-405 Endometrial receptivity status and live birth rate in patients with a thin endometrium and repeated implantation failures : new considerations

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Study question: To assess the endometrial receptivity and pregnancy outcome in repeated implantation failure patients (RIF) according to the endometrial thickness during the periovulatory period

Summary answer: There were no difference in endometrial receptivity status and live birth rate (LBR) between patients with an endometrial thickness above or under 7 mm

What is known already: To date, the number of molecular diagnostic tools available to characterize the implantation window is very limited. In addition, classical ultrasound parameters such as endometrial thickness have low predictive value in determining endometrial receptivity and IVF outcome. Using an innovative genomic testing that allows to characterize an endometrial biopsy obtained during the implantation window as receptive, partially receptive (ongoing endometrial receptivity acquisition) or non-receptive, we investigated the endometrial receptivity status and reproductive outcome according to the endometrial thickness (< 7 mm *vs.* ≥ 7 mm).

Study design, size, duration: Endometrial biopsies were performed during the implantation windows 6-9 days after the LH surge or 5-9 days after progesterone administration under natural cycles and hormone replacement therapy (HRT) respectively in preparation for frozen embryo transfer. According to a genomic testing result, the transfer strategy was: blastocysts transferred at the specific day where endometrium is identified as 'receptive'; D2/D3 cleavage stage embryos transferred 72/48 hours before the specific cycle day where endometrium is identified as receptive.

Participants/materials, setting, methods: 21 RIF patients with several unsuccessful fresh and/or frozen embryo transfers were included. Endometrial thickness was assessed by ultrasound during the periovulatory period. Genomic testing of endometrial biopsies was performed under natural cycle or HRT. RNAs from biopsies were extracted and mRNA expression levels of specific genes predictive of receptivity were established using RT-qPCR. LBR was recorded after personalized embryo transfer using the genomic testing result and assessed according to endometrial thickness cut-off.

Main results and the role of chance: In RIF patients 12 had an endometrial thickness ≥ 7 mm and 9 patients < 7 mm (mean $\pm$ SD: 10.1 $\pm$ 1.5 vs. 6.2 1.1, p-value < 0.0001). There were no significant differences between the two groups in regard to the average age (mean $\pm$ SD: 38.8 $\pm$ 4.7 *vs.* 37.6 $\pm$ 4.4 yrs, p-value = 0.54), the number of previous failed attempts (mean $\pm$ SEM: 3.9 $\pm$ 0.3 *vs.* 3.4 $\pm$ 0.4, p-value = 0.34) and the number of previous non-implanted embryos (mean $\pm$ SEM: 5.4 $\pm$ 0.7 *vs.* 5.3 $\pm$ 0.6, p-value = 0.93). For each patient, the receptivity window was identified and personalized embryo transfer according to the specific cycle day where endometrium is considered receptive demonstrated no significant difference in live birth rates between the two groups (41.7 vs. 33.3 %, p-value = 0.72).

Limitations, reasons for caution: These results must be validated in a larger cohort of patients.

Wider implications of the findings: RIF patients with a thin endometrium show a well defined receptivity window, comparable to patients with a standard thickness. Personalized embryo transfer according to the specific cycle day where endometrium is said receptive improves life birth rate irrespective of the endometrial thickness.

Trial registration number: not applicable

P-406 Comparable clinical outcome but different hCG values after different post-thaw culture duration in single blastocyst transfer cycles: a single center retrospective study

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Study question: Whether different cultivation duration after thawing leads to different hCG values on the day of pregnancy test, and whether longer cultivation duration affects pregnancy outcome.

Summary answer: Although different post-thaw culture duration doesn't affect pregnancy outcome. Longer post-thaw culture duration still leads to higher hCG value.

What is known already: Blastocysts regain viability 4～6 hours after thawing and resume mitosis within 24 hours, therefore, blastocysts undergoing long culture may associate with earlier embryo recovery in vitro and earlier implantation when transferred into uterus. Longer culture duration may allow for better observation of embryo quality and natural selection of the best embryo for transfer. hCG were detected to confirm pregnancy 10-15 days after embryo transfer. HCG was considered as a reliable factor for predicting pregnancy outcomes. Higher hCG values are associated with better outcomes such as ongoing pregnancies and live births, while low initial hCG levels indicate poor prognosis.

Study design, size, duration: A total of 2297 single frozen high quality blastocyst transfer cycles involving 707 short culture cycles and 1590 long culture cycles during June 2010 and July 2016 were extracted from the database of the Reproductive Medicine Research Center of the Sixth Affiliated Hospital of Sun Yat-Sen University. Embryos in short culture group were warmed 4 hours before embryo transfer (ET). While embryos in long culture group were thawed a day before ET and cultured overnight.

Participants/materials, setting, methods: Only patients undergoing their first or second embryo transfer cycle were recruited for the purpose of avoiding the influence of recurrent implantation failure. Student's t test or chi-square test Multivariate linear regression analysis, Logistic regression analysis were applied according to the nature of the data. Singleton live births, clinical pregnancy rate and hCG value were compared and regression analysis was run to explore whether different culture duration affect hCG value and clinical outcome.

Main results and the role of chance: The basic characteristics showed no difference. No difference was observed in total hCG positive rate (66.05% and 67.79%, P=0.411,short culture group at front and long culture group at last), clinical pregnancy rate (51.34% and 53.77%, P=0.281), implantation rate (57.99% and 62.01%, P=0.068) . The singleton live birth rate was comparable (44.27% and 47.48%, P=0.154). After analyzing the mean gestational age, neonatal gender ratio and mean birth weight of singletons, we found that there was no significant difference between these two groups. Multivariable logistic regression analysis indicated that duration of culture before embryos transfer was not correlated with clinical pregnancy (P=0.597).

In those 1427 hCG positive cycles, the mean hCG concentrations resulting from long duration culture after thawing were significantly higher than those from short culture group (915.51±637.66mIU/L and 735.35±481.21mIU/L, P<0.001). The differences were also observed when stratified by different pregnancy prognosis, including clinical pregnancies (1059.60±592.18mIU/L and 867.90±429.89mIU/L,P<0.001) and live births (1079.98±641.57mIU/L and 860.35±424.34mIU/L, P<0.001).

A multiple linear regression analysis model was run to assess the relationship between culture duration and hCG and other cofounders, including maternal age, BMI, AMH, the type of infertility and endometrial preparation protocols. The results showed that the difference in hCG values remained after adjusting the covariates (P<0.001).

Limitations, reasons for caution: This study was limited by its inherent shortcomings of retrospective studies.

Wider implications of the findings: Although similar outcomes regardless of different post-thaw culture duration, higher hCG values in long culture group reflects that difference may exist on the function and number of trophoblast cells as well as implantation time in embryos. But the mechanism still needed to be further discussed.

Trial registration number: not applicable

P-407 The molecular mechanism of human chorionic gonadotropin (hCG) increasing the endometrial Tregs in women with RIF.

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Study question: Did intrauterine perfusion of hCG increase the endometrial Tregs in women with RIF to increase the live birth rate and how did hCG take effect?
Summary answer: This study showed that intrauterine perfusion of hCG increased endometrial Tregs and hCG promoted Tregs migrate to endometrium by advancing the expression of chemokine CCL2.
What is known already: The pathogeny of repeated implantation failure was not clear so far. However, study showed that endometrial FoxP3$^+$ Tregs and peripheral FoxP3$^+$ Tregs in women with RIF significantly reduced (0.07% vs. 0.12%, $P<0.05$). And there had study showed that intrauterine perfusion of hCG improve the live birth rates of patients with repeated implantation failure (RIF) (26.97% vs. 17.22%). Meanwhile chemokine CCL2 can target the chemokine receptor of Tregs and migrate it.
Study design, size, duration: This study was aim to prove the effectiveness of intrauterine perfusion of hCG in women with RIF and explore the mechanism of hCG in regulating the immunological tolerance of uterus. And to study that hCG promoted Tregs migrate to endometrium by advancing the expression of chemokine CCL2. This research was included in vivo experiments, in vitro experiments and clinical experiments. And this study lasted for 18 months.
Participants/materials, setting, methods: Nine patients with RIF were included in this study, endometrial Tregs of these patients were analyzed by immunohistochemistry before and after hCG-treated. Meanwhile mice models which horn injected with hCG were included in this study and also tested by immunohistochemistry. The migration ability of Tregs was analyzed by cell migration assay and flow cytometry. The expression of CCL2 was analyzed by Western blotting and qPCR.
Main results and the role of chance: The expression of Foxp3 in women with RIF was significantly lower than other infertility patients (9.4±5.3 vs. 11.2±5.4, $P<0.05$). In this study, the expression of Foxp3 in mice which horn injected hCG was significant higher than that injected PBS (46±16.8 vs. 7±4.3, $P<0.01$). Meanwhile the expression of Foxp3 after intrauterine perfusion of hCG in women with RIF was significant higher than before (18.6±9.8 vs. 9.4±5.3, $P<0.05$). In vitro experiments showed that hCG could increase the quantity of migrated Tregs by enhancing the expression of CCL2 in human endometrium stromal cells (hESCs) (1115±670.7 vs. 2597±833.2, $P<0.05$). When down-regulated the expression of CCL2 in hESCs or add the antagonist of CCR2, the quantity of migrated Tregs decreased significantly. Meanwhile in women with RIF, the expression of CCL2 was higher after intrauterine perfusion of hCG (0.21±0.01 vs. 0.17±0.01, $P<0.001$).
Limitations, reasons for caution: In this study, we found that hCG could increase the expression of CCL2. However the mechanism of hCG increase the expression of CCL2 was not clear. And there have studies showed that hCG could increase Tregs in peripheral blood, the direct interaction between hCG and Tregs was need explore furtherly.
Wider implications of the findings: There have studies showed that intrauterine perfusion of hCG in women with RIF increased the live birth rate in IVF. And our study indicated the molecular mechanism of hCG increasing endometrial Tregs. This study proved the effectiveness of hCG and help doctors to apply intrauterine perfusion of hCG in clinic.
Trial registration number: not applicable

P-408 Analysis of the risk factors and treatment for recurrent implantation failure: OPTIMUM strategy

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Study question: What are the pregnancy outcomes after OPtimization of Thyroid function, IMmunity and Uterine Milieu (OPTIMUM) strategy in patients with recurrent implantation failure (RIF)?
Summary answer: Evaluation and treatment related to thyroid function, intrauterine environment and maternal immunity may improve pregnancy outcomes in patients with a history of RIF.
What is known already: Overcoming RIF in patients with a history of multiple high-quality embryo transfer (ET) cycles is considered difficult using common in vitro fertilisation (IVF) procedures. The risk factors for RIF include uterine inhibitory factors of implantation without embryo origin, such as impaired intrauterine circumstances and maternal immunological dysfunction. Persistent inflammation of the local endometrium (chronic endometritis) occurs in 30%–60% of women with RIF. Further, aberrant high T-helper (Th)1/Th2 cell ratio is detected in 50%–60% of such women. Vitamin D insufficiency is associated with high maternal Th1/Th2 cell ratio.
Study design, size, duration: This study was approved by the local ethics committee. Infertile women with RIF after ≥3 ET cycles underwent implantation testing, including hysteroscopy; endometrial biopsy for CD138 immunostaining; determination of interferon-γ-producing Th cell (Th1 cell), IL-4-producing Th cell (Th2 cell), serum 25-hydroxyvitamin D_3 and thyroid-stimulating hormone levels and thyroid peroxidase antibody testing, between April 2017 and August 2018. Preimplantation genetic testing for aneuploidy and endometrial receptivity array were not performed.
Participants/materials, setting, methods: The cornerstone of treatment is the remedy of detected causes by implantation testing. We treated chronic endometritis with antibiotics; aberrant high Th1/Th2 cell ratio with vitamin D and immunosuppressive drug (tacrolimus) supplementation and overt or subclinical hypothyroidism with levothyroxine therapy. Of 92 women with a history of RIF, 72 underwent 105 ET cycles after implantation testing. We conducted a questionnaire-based survey regarding pregnancy outcomes after the OPTIMUM strategy in IVF facilities.
Main results and the role of chance: Women with RIF were aged 38 ± 4 years and had an implantation failure history after 6 ± 3 ET cycles. The prevalence of RIF risk factors in patients aged <40 and ≥40 years was 72% (38/53 women) and 64% (25/39) in impaired intrauterine circumstances, 58% (31/53) and 38% (15/39) for aberrant elevated Th1/Th2 cell ratio, 85% (45/53) and 90% (35/39) for vitamin D insufficiency and 26% (14/53) and 38% (15/39) for thyroid disorder, respectively. Their cumulative clinical pregnancy rates were 60% (25/42) and 30% (9/30) at the first ET and 71% (30/42) and 50% (15/30) at the second ET, respectively. Further, cumulative ongoing pregnancy rates were 52% (22/42) and 17% (5/30) at the first ET and 64% (27/42) and 27% (8/30) at the second ET, respectively.
Limitations, reasons for caution: This study has some limitations. First, this was a retrospective study. Second, we did not compare pregnancy outcomes in patients without implantation testing. Lastly, the sample size was small, complicating data analysis using multivariable analysis.
Wider implications of the findings: Among <40-year-old RIF patients, 64% achieved ongoing pregnancy. However, cumulative ongoing pregnancy rates of ≥40-year-old patients were only 27% after two ET trials, suggesting chromosomal testing of embryos is required for improvement of pregnancy rate in RIF women of late reproductive age.
Trial registration number: not applicable

P-409 The evaluation of efficiency of the treatment of infertile women by elective single embryo transfer (eSET) in IVF programs: cohort study

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Study question: An evaluation of the effect of the treatment of infertile women by elective single embryo transfer (eSET) in InVitro Fertilization (IVF) programs.
Summary answer: eSET can be recommended for wider medical use in IVF cycles; this is an effective method, which reduces maternal and fetal risks.
What is known already: Initially, SET in the IVF cycle was proposed and implemented for patients with contraindications to carrying multiple pregnancies. The current global medical trend is the transfer of the best embryo. In some countries, however, several embryos are still transferred. Of course, SET is only applicable to a specific group of patients with high chances of succes. Various criteria have been proposed by different guidelines all over the world. In Russia, legally "no more than two embryos should be transferred into the uterine cavity". Currently, there is no unanimous opinion regarding that issue and no regulatory solution for the SET problem.
Study design, size, duration: The study included pro- and retrospective efficiency analysis of the treatment of 903 infertile women with normal ovarian reserve undergoing IVF in 2015–2017. In 143 women from the main group, elective single embryo transfer was performed. The comparison group included 760 women, which underwent double embryo transfer in IVF programs. The women in the groups were comparable in age, infertility duration, infertility factor, primary infertility proportion, and mean number of previous IVF cycles.
Participants/materials, setting, methods: The mandatory inclusion criterion in the main group was the presence of at least two embryos of excellent/good quality at the day of transfer. The following parameters were evaluated: number of oocytes obtained; fertilization rate; the number of embryos of good and excellent quality; pregnancy rate (PR); pregnancy termination; the labor number; the number of infants born; and the preterm labor incidence.
Main results and the role of chance: The difference in the efficiency of treatment in eSET and DET settings was not statistically significant. The PR was 55.9% in the main group and 51.9% in the comparison group ($p > 0.05$). However, the incidence of carrying pregnancy in the main group was significantly higher. The number of single pregnancies in the second group was 260 (65.8%). We didn't detect significant differences in pregnancy loss rate in both groups. The percentage of pregnancy loss was 18.8% in the main group and 27.1% in the comparison group ($p > 0.05$). The labor number per cycle was also similar in both groups (45.5% (65 cases) in the main group and 37.9% (288 cases) in the comparison group, $p > 0.05$). In the main group, no twin births were registered; in the comparison group, twin births were observed in 53 (18.4%) cases. We also analyzed perinatal outcomes. The number of preterm labor (22–37 weeks of pregnancy) was significantly lower in the main group than in the comparison group: 6.1 and 27.4% ($p < 0.001$). The incidence of carrying pregnancy until a term of more than 37 weeks was also higher in the main group: 93.9% vs. 72.6% in the comparison group.
Limitations, reasons for caution: The criteria for the exclusion of women from the study were the absence of indications for treatment with the IVF/ICSI method; acute somatic illnesses and/or exacerbations of chronic somatic illnesses; the low and/or decreased ovarian reserve; and the absence of two embryos of excellent/good quality at the day of transfer.
Wider implications of the findings: We propose that the eSET should become the "golden standard" of ART in infertile patients with normal ovarian reserve, and indications for its use should be extended. This will decrease the treatment costs and minimize pregnancy loss.
Trial registration number: not registrated

P-410 Comparison of two endometrial preparation methods for frozen-thawed embryo transfer in anovulatory PCOS patients: impact on miscarriage rate.

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Study question: The aim of this study is to determine which is the best protocol for endometrial preparation between artificial and stimulated cycle for PCOS patients undergoing a frozen embryo transfer (FET)
Summary answer: The two types of endometrial preparation did not influence FET issues, in particular miscarriage and live birth rates.
What is known already: In the literature, artificial cycles and stimulated cycles seem to be comparable for endometrial preparation in term of ongoing pregnancy rate and live birth rate. However, few studies evaluated early pregnancy loss rates, and had a specific interest on PCOS patients.

Yet, some authors suggested that PCOS patients are more at risk of miscarriage but this remains controversial. The association could be explained by several factors such as obesity, hyperinsulinemia and hyperandrogenism. These factors could have effects on PCOS endometrium. Furthermore, some studies suggest that FET may improve live birth rate and induce lower miscarriage rate in PCOS women.
Study design, size, duration: This is a monocentric retrospective cohort study including 255 cycles of frozen-thawed embryo transfer in 135 PCOS patients between 2011 and 2017 in an academic institution. Patients were under 35 years old and have received 2 types of treatment: artificial cycle (estradiol valerate 6mg and micronized vaginal progesterone 800mg daily) or stimulated cycle with hMG or recombinant FSH.

Statistical comparison was carried out by Mann-Whitney test or Chi-square test, when appropriate.
Participants/materials, setting, methods: PCOS was defined by at least two of the three following items: clinical and/or biological hyperandrogenia and/or oligo-anovulation and/or PCOM: ovarian volume > 10 mL and/or follicle number per ovary (FNPO) ≥ 19 and/or AMH ≥35 pmol/l., as previously published. Patients with endometriosis were excluded.

Clinical pregnancy, miscarriage and live birth rates were recorded.

Embryos were transferred at cleavage stage.
Main results and the role of chance: 118 FET were performed with an artificial cycle and 137 with a stimulated one. Our groups were comparable for the age, BMI, cigarette smoking, endometrial thickness and number of embryos transferred.

Implantation rate was similar in the two groups (23% in the stimulated cycle group, 26% in the artificial cycle one, NS).

There was no significant difference between artificial cycle and stimulated cycle in the PCOS patients in term of clinical pregnancy rate (respectively 37,3% versus 30%, NS), ongoing pregnancy rate (27% versus 23%, NS) , miscarriage rate (25% versus 22%, NS) and live birth rate (26,3% versus 23, 4%, NS).
Limitations, reasons for caution: Some bias may have occurred due to the retrospective design of the study.

We used strict criterias to select our PCOS population with home-made AMH and FNPO thresholds to define PCOM.

A prospective randomized study with a larger sample of patients should be realized to corroborate these data.
Wider implications of the findings: For PCOS patients, endometrial preparation doesn't seem to influence miscarriage rate and more generally FET clinical outcome.
Trial registration number: Not applicable

P-411 Medical management of early pregnancy failure (EPF) is highly effective in pregnancies following artificial reproductive techniques (ART)

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Study question: Does mode of conception have an impact on the success rate of uterine evacuation using mifepristone and misoprostol in EPF?
Summary answer: Medical management of EPF is highly effective in women getting pregnant through ART, showing no difference between fresh and frozen cycles

What is known already: Success rates, defined as no need for surgical intervention, for medical management using mifepristone and misoprostol exceed 85% and confirm that this is a safe and effective alternative to surgical evacuation in EPF. There is only one relatively small study from 2009 focusing on the impact of mode of conception onsuccess rate which showed a trend toward more favorable success rate in ART pregnancies. However, in contrast to our study only misoprostol was administered, resulting in a success rate of 77% for spontaneous pregnancies and 92% for ART pregnancies.

Study design, size, duration: This retrospective study evaluated all patients choosing medical management for EPF at our institution from March 2013 to January 2017. In pregnancies following ART we compared success rate in fresh vs. frozen cycles. Exclusion criteria included complete or incomplete abortion, multiple gestation, gestational age over 13 weeks by ultrasound, contraindication for mifepristone or misoprostol and pregnancies with an IUD in place.

Participants/materials, setting, methods: We performed a retrospective review of patients' medical records including sonographic and clinical findings at time of diagnosis and following treatment. Previous history as well as details regarding ART treatment were abstracted. According to our institutional protocol, patients received 200 mg mifepristone orally on an outpatient basis and were admitted 36 to 48 hours later for vaginal administration of misoprostol 800 mcg. Time to passage of pregnancy tissue and overall success rates were evaluated.

Main results and the role of chance: In total we identified 424 patients receiving medical management for EPF in the defined time span, of which 52 were pregnancies achieved following ART. The overall success rate was 92%. Subgroup analysis showed no difference in success rates comparing spontaneous (92%) and ART conceptions (94%), (p=0.524). Moreover, no significantly different success rate was found for ART pregnancies following fresh vs. frozen cycles (97% vs. 88%, p=0.218).

88% of patients reported passage of tissue during their day-clinic stay, with a mean time of 5.1±3.4 hours. If no passage of tissue or bleeding was observed after 6 hours or ultrasound suggested incomplete evacuation, an additional dosage of 400 mcg misoprostol buccally could be administered upon the treating physicians' choice. This was executed in 78 patients (18%) with no significant difference in ultimate success of treatment after performing subgroup analyses.

Of the 51 patients discharged without passage of tissue, the majority achieved a complete abortion. Surgical intervention was necessary in only 13 of these cases andno patient needed a blood transfusion, emphasizing the safety of this protocol.

Limitations, reasons for caution: The study is limited by its retrospective design. We collected data in a real life clinical setting prone to subjective influence by patients' wishes, treating physicians' decisions and preferences. However, this can also be seen as an advantage, reporting results for clinical daily routine, independent of restricted study protocols.

Wider implications of the findings: Up to 20% of pregnancies following ART end in pregnancy failure. Based on our results, we postulate that women should be routinely offered medical management as a highly effective and safe alternative to surgical management, when diagnosed with EPF following fresh as well as frozen ART cycles.

Trial registration number: not applicable

P-412 Multiscale 3D imaging: a useful approach for the study of endometrial glandular architecture in healthy women

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Study question: Can 3D imaging techniques be used for characterising endometrial glands and their component cells types?

Summary answer: Multiscale 3D imaging provides new insights into endometrial gland function during the window of implantation in healthy women.

What is known already: Endometrial glands contribute essential products to the uterine milieu, important for trophoblast implantation and early pregnancy. Previous immunohistochemistry and electron microscopy techniques have identified different cell types of the endometrial gland, including secretory cells and ciliated cells. However, the 3D ultrastructure of endometrial glands has not been characterised. Multiscale 3D architectural characterisation of healthy endometrial glands during the implantation window can provide important topographical insight into this crucial phase of endometrial development.

Study design, size, duration: An observational study on a cohort of healthy egg donor control patients.

Participants/materials, setting, methods: Endometrial samples were collected at the implantation period of the menstrual cycle (LH $^{+}$7-10). To study the spatial distribution of glandular cell types, formaldehyde fixed endometrial tissue was processed for whole-mount immunohistochemistry and imaged on the confocal microscope. To determine the 3D ultrastructure of the endometrial gland, glutaraldehyde fixed endometrial tissue was processed with heavy metals and imaged on the serial block face scanning electron microscope (SBFSEM). Finally, high-speed video observed endometrial glands live.

Main results and the role of chance: Multiscale 3D imaging techniques have been used to investigate endometrial glands. Confocal microscopy demonstrated the 3D spatial distribution of glandular cell types. SBFSEM allowed 3D reconstruction of the glandular luminal surface and high-resolution ciliated glandular epithelial cells. 3D reconstruction of the glandular lumen, shown by 3D video, provides informative insight into how glandular cell types interact in the lumen and suggested sites of microvesicle production. Characterising ciliated epithelial cells in endometrial glands has lead to a greater understanding of their interactive 3D roles during the window of implantation, which can be built upon for future research in infertility and miscarriage.

Limitations, reasons for caution: This is an observational study with a small sample size. 3D analysis of endometrial samples is currently time consuming in comparison to traditional 2D immunohistochemistry methods, therefore more applicable for research studies.

Wider implications of the findings: This 3D approach can potentially be applied to answer questions in relation to defective implantation.

Trial registration number: not applicable

P-413 The evaluation of the presence of CD56+ Natural Killer Cells in Patients with IVF failure.

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Study question: Does endometrial CD56+ Natural Killer cells play role in IVF failure.

Summary answer: Cases of uterine CD56 + NK cells may play significant role in the etiology of recurrent IVFf failure.

What is known already: Uterin natural killer cells plays important role in implantation and vascularization.

Study design, size, duration: Study had been designed as prospective randomized case control study. It was conducted between January 2012 and December 2017. 70 women were randomly selected 35 in control grup and 35 case group. Inclusion criterias for case group were minimum 2 IVF failure, Inclusion criterias for control group were minimum 1 live birth without receiving any infertility treatment,

Participants/materials, setting, methods: Endometrial biopsies were taken 21 th or 24 th day of menstural cycle. Endometrial samples were stained with CD 56 antibodies (Novocastra Laboratories Ltd. NCL-L-CD56-1B6).

Main results and the role of chance: Mean age in control and case group were 34.4 and 33.5 respectively. There were no gravid, parity or abortus in case group. Mean parity, abortus were 3.7 and 1.7 respectively. There were significant positive correlation between abort number and uterine natural killer count in control group $p<0.005$. There significantly low uterine natural killer count in case group $p<0.005$.

Limitations, reasons for caution: Other surface antigens of uterine natural killer cells may be studied in further studies.

Wider implications of the findings: In literature studies reported that more natural killer cells in patients with habitual abortus but We observe lesser uterine natural killer cells in patients with IVF failure.

Trial registration number: not applicable.

P-414 The impact of serum estradiol level prior to progesterone administration on live birth rate in single frozen-thawed blastocyst transfer cycles

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Study question: Does serum estradiol (E2) level monitoring on the day of progesterone administration affect live birth rate for artificial frozen-thawed (FT) single blastocyst transfer cycles?

Summary answer: Serum E2 level monitoring on the day of progesterone administration does not appear to be predictive for live birth rate.

What is known already: Estradiol is critical to endometrial and placental development. However, excess E2 may adversely affect placentation and endometrial receptivity, which may result in decreased implantation rates.Although much work has focused on identifying the optimal duration of exogenous E2 use, its dosage and route of administration, only few studies have addressed the predictive value of serum E2 level on the day of progesterone initiation for live birth rates. No consensus has been reached on E2 level monitoring on the day of progesterone initiation and previous studies have provided conflicting results.

Study design, size, duration: This study analyzed 468 FT single blastocyst transfer cycles performed between January-2015 and January-2018. All FTs were performed in patients aged between 20-37 years with unexplained or tubal infertility who had a top quality embryo. We excluded patients with repeated implantation failure, diminished ovarian reserve, a known structural chromosomal abnormality, severe male factor, recurrent pregnancy loss, hydrosalpinx and/or any uterine factor and those with progesterone level>1.5ng/mL or endometrial thickness<7mm on the day of progesterone initiation.

Participants/materials, setting, methods: This study was conducted in Bahceci Women's Health Center in Istanbul in Turkey. The patient population was stratified into four groups based on the percentiles of E2 levels measured on the first day of progesterone administration; group 1 (<25th percentile), group 2 (25-50th percentile), group 3 (50-75th percentile) and group 4 (>75th percentile). The impact of E2 level percentiles on clinical pregnancy and live birth rates were analyzed.

Main results and the role of chance: A total of 468 patients were included in the study. Data was obtained from the first single top quality blastocyst transfers of these patients, and thus 468 FT embryo transfer cycles were included in all calculations. The patients' median age, median body mass index, and median duration of infertility (range) were 31 years (20-37), 25 kg/m^2 (18-38), and 3 years (1-9), respectively. On the day of progesterone initiation day, the patients' median endometrial thickness, median E2 level and median progesterone level (range) were 9.8mm (7-13), 257pg/mL (64-1560) and 0.2ng/mL (0.01-1.45), respectively. Based on E2 level percentiles measured on the first day of progesterone administration: group-1 (<25thpercentile), group-2 (25-50thpercentile), group-3 (50-75thpercentile) and group-4 (>75thpercentile) did not show statistically significant differences in terms of live birth rate, which were 51.6%, 55.1%, 54.9% and 56.4%, respectively. The groups also did not significantly differ in implantation and clinical pregnancy rates. To determine whether there is a threshold for E2 level to predict pregnancy or not, receiver operating characteristic (ROC) analysis was conducted. Based on ROC curves, the E2 level on the day of progesterone administration was not predictive for implantation (AUC: 0.490, p=0.98), clinical pregnancy (AUC: 0.507, p=0.80) and live birth (AUC: 0.514, p=0.60).

Limitations, reasons for caution: The study has the limitations inherent to its retrospective nature.

Wider implications of the findings: Our results suggest that serum E2 level measured on the day of progesterone initiation does not predict live birth rate in patients undergoing single frozen-thawed top quality blastocyst transfer in artificial endometrial preparation cycles. Thus, our data may provide guidance to physicians managing this group of patients.

Trial registration number: not applicable

P-415 Effect of levothyroxine treatment and prevalence of thyroid autoimmunity in a cohort of 1065 patients with recurrent pregnancy loss

Abstract withdrawn by the authors

P-416 Measuring serum progesterone level on the day of transfer can be an additional tool to maximize ongoing pregnancies in single euploid frozen blastocyst transfers.

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Study question: Is there a relation between serum progesterone(P) levels on day of embryo transfer(ET) and ongoing pregnancy rates(OPR) ?

Summary answer: OPR is found to be significantly diminished in patients where serum P level is less than 14,6 ng/ml in single euploid vitrified-warmed blastocyst ET.

What is known already: Since contemporary comprehensive chromosomal screening (CCS) applications necessitate embryo cryopreservation at the blastocyst stage, number of euploid FBT cycles are steadily increasing worldwide. it has been reported that transfer of euploid blastocysts can result in high clinical and ongoing pregnancies. On the other hand, in nearly 30-35% of these cycles, a successful pregnancy can not occur.The role of monitorization of P levels on day of FBT in artificial cycle is controversial and the common practice is not to monitor. Studies in which an optimum serum P threshold or wiindow on ET day is evaluated for better pregnancy outcome are scarce.

Study design, size, duration: This prospective cohort study includes patients who underwent single euploid FBT between March and August 2018. All patients received HRT with estradiol valerate (EV) and 50 mg intra muscular (IM) P. Patients with uterine anomalies, pathologies and endometrial thickness <7 mm were excluded. In all cycles, only embryos that were biopsied on day 5 were utilized for FBT. Next generation sequencing (NGS) was used for comprehensive chromosomal analysis. The primary outcome measure was OPR.

Participants/materials, setting, methods: One hundred and sixty-eight patients undergoing euploid single FBT were analysed. Following 10-13 days of EV use, vaginal ultrasound examination was performed to confirm endometrial thickness >7 mm and serum progesterone level<1 ng/ml. FBT was scheduled 117-120 hours after starting 50 mg IM P. Serum P level was analysed 1 hour before the 6th day of P administration. For each pregnant patient, OPR was monitored by ultrasound at the end of 16th weeks of pregnancy.

Main results and the role of chance: Overall, OPR was 58.9%(99/168). In logistic regression analysis, ongoing pregnancy was taken as the dependent variable and female age(years), BMI(kg/m2), duration of infertility(years), number of previous cycles, previous pregnancy history, endometrial thickness(mm), P and estradiol level at the starting day of progesterone P, P level on the day of FBT, blastocyst morphology were taken as independent variables. BMI(p=0,041) and P level on the day of FBT(p=0.013) were found to be significant predictors of OP. Mean serum P on the day of FBT was 33.2±23.3 ng/ml (Percentiles 25, 14.6; 50, 24.7; 75, 54.4). OPRs according to serum P quartiles were Q1:26.2(11/42); Q2:74.4%(32/43); Q3:60.5%(26/43); Q4:75%(30/40). The OPR of Q1 was significantly lower than Q2–Q4: 26.2% versus 70.6%; p :0.007 RR (95%CI):0.28(0.11-0.69). Serum P levels on the day of FBT were found to be correlated only with BMI (rho:-0.013 p=0.001) and P level at the starting day of P (rho:0.258 p=0.001). The ROC curve showed a significant predictive value of serum P levels on the day of FBT for OPR, with an AUC (95%CI)=0.69(0.60-0.77). The optimal serum P threshold in which sensitivity and specificity for OPR were both >50% was 20.9 ng/ml (70.7% sensitivity, 55.1% specificity).

Limitations, reasons for caution: Patient population included only women with appropriate endometrial thickness and good quality euploid blastocysts transfer. Validation of P levels on different population should be extrapolated. The results needs to be confirmed in larger studies.

Wider implications of the findings: The study suggests a minimum threshold of serum P values on the day of FBT in artificial cycles to

optimize pregnancy rates. Large variations in P levels should be documented and evaluate. Individualization of progesterone dosage and optimal route especially in obese patients should be evaluated in further studies.

Trial registration number: None

P-417 An examination of density and clustering of four major immune cells in women with recurrent miscarriage in precisely timed endometrial samples compared with fertile controls

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Study question: Is there any difference in the density and infiltration level of four major endometrial immune cell types between women with recurrent miscarriage and fertile controls?

Summary answer: There was a significant increase in the density of CD3+, CD68+ and CD56+ cells and clustering between CD68+ and CD56+ cells in recurrent miscarriage women.

What is known already: Serval studies showed an increased uNK cell density on day LH+7 of women with recurrent miscarriage (Lash *et al*., 2016)(Chen *et al*., 2017). A few studies examined other endometrial immune cells (T cell/Macrophage/B cell) but with inconstant results (Mei *et al*., 2010)(Yin *et al*., 2012), partly because the specimens were not precisely timed. To better understand the contribution of different immune cells to recurrent miscarriage, it is necessary to simultaneously examine major immune cells in the same specimen to determine the relative magnitude of the cell density changes (if any) and the degree of clustering between two immune cell types.

Study design, size, duration: This was a retrospective study of archived endometrial specimens carried out in a university teaching hospital in Hong Kong between Aug. 2017 and Sep. 2018. A total of 30 women with unexplained recurrent miscarriage (three consecutive pregnancy losses) and 30 fertile controls were included in this study.

Participants/materials, setting, methods: Endometrial biopsies were collected precisely 7 days after LH surge. A multi-colour immunofluorescent method was used to simultaneously measure the density of CD3 for T cells, CD 20 for B cells, CD56 for uNK cells and CD 68 for macrophages in the same endometrial biopsy. The degree of clustering of different pairs of endometrial immune cells was measured by the infiltration level, using the spatial distribution toolbox 'spatstat' in R (Carstens *et al*., 2016).

Main results and the role of chance: The median CD3+/CD20+/CD68+/CD56+ cell density in the fertile controls was 1.3%, 0.4%, 2.8% and 7.1%, respectively; and for women with recurrent miscarriage was 2.7%, 0.5%, 4.3% and 11.9%. In women with recurrent miscarriage, there was a significant increase density ($p<0.05$) of CD3+ T cells (107.6%), CD56+ uNK cells (40.3%) and CD68+ macrophages (34.8%), with no significant increase in CD20+ B cell density. In addition, there was a significant infiltration level between CD56+ uNK cells and CD68+ macrophages, compared with fertile controls.

Limitations, reasons for caution: The prognostic value of the measurements has not been examined in this study.

Wider implications of the findings: Recurrent miscarriage is associated with alteration of multiple immune cells density in the endometrium and the degree of clustering between the different immune cell types.

Trial registration number: This work was supported by the Health and Medical Research Fund in Hong Kong (No. 04152786) and 2018 Hong Kong Obstetrical and Gynaecological Trust Fund.

P-418 Comparison of live birth rate between fresh embryo transfer and vitrified-thawed embryo transfer

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Study question: Does vitrified-thawed embryo transfer (VET) result in higher live birth rate (LBR) than fresh embryo transfer (ET) in women without polycystic ovary syndrome (PCOS)?

Summary answer: VET resulted in higher LBR than fresh ET in women without PCOS.

What is known already: Theoretically, VET may result in higher LBR than fresh ET due to a more favorable intrauterine environment for embryo implantation by avoiding the supraphysiologic condition that occurs after ovarian stimulation, and results from most previous studies of limited sample size did observe a higher LBR after VET. However, two recently published randomized clinical trials (RCTs) showed the LBR did not differ significantly between fresh ET and VET in women without PCOS.

Study design, size, duration: This was a retrospective cohort study involving a total of 28,672 transfer cycles (3,389 fresh ETs and 25,283 VETs) of in vitro fertilization/intracytoplasmic sperm injection from 2006 to 2014.

Participants/materials, setting, methods: LBR was compared by patient and treatment characteristics, including female age, BMI, infertility duration, gravidity, parity, previous full-term births, tubal-factor infertility, PCOS, endometriosis, male-factor infertility, ovary stimulation protocol, endometrial thickness on ET day, number of embryos transferred, treatment year, and transfer type. Thereafter, we used generalized estimated equation regression models to calculate unadjusted and adjusted ORs for the association between LBR and the aforementioned characteristics in the whole population and women without PCOS, respectively.

Main results and the role of chance: For the whole population studied, the overall LBR was 34.4% (9865/28672). VET was associated with a significantly higher LBR than fresh ET (35.6% vs. 25.5%, OR 1.67, 95% confidence interval [CI] 1.54-1.81, P<0.001). After adjusting for confounding factors, VET still had a significantly higher LBR compared with fresh ET (adjusted OR 1.28, 95% CI 1.15-1.41, P<0.001). For women without PCOS, the overall LBR was 33.8% (9143/27042). VET was also associated with a significantly higher LBR than fresh ET both in the unadjusted (35.0% vs. 25.1%, OR 1.61, 95% CI 1.48-1.75, P<0.001) and adjusted (adjusted OR 1.27, 95% CI 1.15-1.41, P<0.001) analyses.

Limitations, reasons for caution: This was a retrospective study based on data from a single center. Moreover, progesterone level on the day of trigger was not available, which limited our further comparison of LBR between fresh ET and VET under different progesterone subgroups.

Wider implications of the findings: VET resulted in higher LBR than fresh ET both in the general population and ovulatory women, which supports the hypothesis that VET can result in more favorable intrauterine environment for implantation. Transfer of fresh versus frozen embryos in ovulatory women should be further assessed in RCTs of large sample size.

Trial registration number: Not applicable.

P-419 Autologous Intrauterine Platelet-Rich Plasma Instillation for recurrent implantation failure: A Pilot Study

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Study question: To evaluate the effects of intrauterine infusion of Autologous Platelet-Rich Plasma (PRP) on RIF regarding to implantation rates, clinical pregnancy rate and abortion rate.

Summary answer: The use of autologous PRP may increase clinical pregnancy and implantation rate in patients with recurrent implantation failure.

What is known already: Recurrent implantation failure (RIF) is defined as unsuccessful implantation after repeated transfers of at least 4 good quality embryos in a minimum of three fresh or frozen cycles in a woman under 40 years into a normal uterus. The definition of RIF is under constant scrutiny.

Study design, size, duration: 80 patients with the history of RIF from 01.01.2017 until 01.01.2018 were recruited in this Randomized control trial.

Participants/materials, setting, methods: PRP was administered at the day of oocyte puncture. Intrauterine instillation of autologous PRP was done in Forty patients as PRP group, and forty patients in control group.
Main results and the role of chance: The mean age for whole study group was 35.7±6.2 years (PRP 35.5±5.2, controls 35.4±4.7). 10/40 (25%) patients had a clinical pregnancy after embryo transfer in PRP group while 6/40 (15%) patients in control group. The implantation rate was 14.1% in PRP, 7.3% in control group.
Limitations, reasons for caution: Further research in the form of large scale randomized controlled trials is needed.
Wider implications of the findings: This study suggests that the use of autologous PRP holds promise in the treatment of women with Recurrent implantation failure.
Trial registration number: none

P-420 A comparison of expression of angiotensin II receptor type 1 in peri-implantation endometrium in women with RIF and fertile controls

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Study question: Does local renin angiotensin system play a role in peri-implantation endometrial vascularization and function?
Summary answer: The expression of angiotensin II receptor type I in women with recurrent implantation failure (RIF) was significantly lower than in fertile controls.
What is known already: Altered vascularization status in the endometrium around the time of embryo implantation may contribute to different implantation outcome. It is known that Angiotensin II promotes the growth and vascularization of the endometrial stroma. However, it is not known that if angiotensin pathway plays a role in implantation outcome.
Study design, size, duration: It was a prospective cohort study. A total of 33 women were recruited in the study, including 10 fertile women and 23 women withRIF. All endometrial biopsies were collected precisely on day LH+7.
Participants/materials, setting, methods: Endometrial biopsied tissue was collected and immunostained for Angiotensin II receptor type I(AT1)and Angiotensin (1-7) mas receptor Results was graded and calculated according to an H-score equation. The H scores were measured in luminal epithelium, glandular epithelium, and stroma separately.
Main results and the role of chance: The expression of AT1 was observed in all compartments in the peri-implantation endometrium, and a significantly lower expression in all compartments of the endometrium was observed in peri-implantation endometrium of women with RIF (luminal epithelium: 104±72 ; glandular epithelium: 97±66 ; stroma 76±50), compared with fertile control (luminal epithelium: 218±46 ; glandular epithelium: 191±50 ; stroma 151±54). Moreover, we found that the expression of Ang(1-7) mas receptor was confined in endometrial stromal compartment.
Limitations, reasons for caution: More components of renin angiotensin system components need to be confirmed (such as AT2, Angiotensin II, Renin, ACE), and a larger sample size need to be recruited to verify this novel finding in the future.
Wider implications of the findings: The renin angiotensin system may be involved in the endometrial vascularization. The role of renin angiotensin system in peri-implantation endometrium is worthy of further study.
Trial registration number: None

P-421 Endometrial receptivity analysis in recurrent implantation failure: A prospective study comparing benefits in own versus donor cycles.

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Study question: To determine if endometrial receptivity analysis (ERA) is beneficial for universal application in recurrent implantation failure (RIF) or in select groups.
Summary answer: ERA was found to be more beneficial in RIF patients recruited in donor cycles when compared to those using own gametes.
What is known already: The factors affecting implantation potential are multi-factorial encompassing many different growth factors, cytokines and a plausible shift in implantation window during fresh IVF cycles following hyper-stimulation. ERA serves as a study of measurable RNA and 238 genes and found to be beneficial in cases of RIF, considering 25% have a shift of this window and another 12% are non-receptive at any given time. The testing allows personalized embryo transfers at the receptive phase thereby enhancing success rates to almost 67%. But is it universally applicable in all cases especially in donor programs remains to be seen.
Study design, size, duration: A prospective study was conducted from Oct 2013 - Dec 2018 with the sample size of 391 women with recurrent implantation failure undergoing assisted reproduction. The mean age of women was 34.66 ± 5.69 years. Recurrent implantation failure was defined as more than two IVF failures in self and donor programs. ERA was performed on day 21 of a hormone replacement cycle. The reports were generated as receptive, pre or post receptive endometrium.
Participants/materials, setting, methods: The study participants were divided into two groups. Group A (n= 91) comprised of women using own gametes and Group C (n= 92) comprised of women using donor gametes. Both groups were compared to their respective controls B (n=107) and D (n=101). ERA was performed on groups A and C following standard hormone replacement therapy protocol. Clinical pregnancy rates, fetal miscarriages and live birth rates were compared between study and respective control (non-ERA) groups.
Main results and the role of chance: In group A (ERA, own cycles) 65.93% of women had receptive endometrium, 27.45 % had pre receptive and 6.59% of women had post receptive endometrium. In group C (ERA, donor cycles) 68.48% of women had receptive endometrium, 17.39 % had pre receptive and 14.13% of women had post receptive endometrium.

There were no statistically significant differences with respect to clinical pregnancy (p=0.128) and fetal miscarriage rates (p=0.133) between groups A (ERA) and B (non-ERA) in RIF patients using own gametes. However there were statistically significant differences in clinical pregnancy (p=0.013), live birth (p=0.032) and fetal miscarriage rates (p=0.04) between groups C (ERA) and D (non-ERA) in RIF patients using donor gametes. This signifies the benefits of performing ERA in recurrent implantation failure patients who have failed despite using donor gametes, where poor response or poor embryo quality had previously been more of a deterrent than the endometrium per say.
Limitations, reasons for caution: A larger sample size would add more value to the study.
Wider implications of the findings: The study groups were demographically comparable to their respective control groups there by having fewer limitations and adding significance to performance of ERA in donor cycles where recurrent failure implicates an endometrial factor in implantation.
Trial registration number: N/A

P-422 The role of endometrial and circulating miRNAs in the recurrent implantation failure (RIF)

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Study question: Has the expression of endometrial and circulating miRNAs in two different phase of menstrual cycle significantly altered between RIF and the control group?
Summary answer: The study results showed that four selected miRNAs were significantly dysregulated in between RIF and control groups samples.
What is known already: In the menstrual cycle, it has been identified that endometrium specific miRNAs have different expression levels in endometrial tissues and maternal serum. A previous study has been suggested that miRNAs are related with mediating endometrial responses to maternal hormones and regulating the modulation from the proliferative into the secretory phase. Some Research demonstrated that endometrial miRNAs are altered in patients with

RIF, and suggested that they may be important regulators of endometrial receptivity and thus implantation success. Accordingly, these RIF-associated miRNAs might possibly be employed for diagnosis and treatment of embryo implantation failures.

Study design, size, duration: In this study, there are two groups; RIF and normal fertility samples which are collected as two blood and two endometrial biopsies in two different phase of mestural cycle. In first step, total RNAs are isolated from endometrium tissue and sera exosomes, next step is quantitative analyses of targeted miRNAs (hsa-miR-31, hsa-miR-30b, hsa-miR-145 and hsa-miR-23b) in samples and the last step is bioinformatic analysis of quantitative RT-PCR results. Study duration lasted from 2016 until 2019.

Participants/materials, setting, methods: In total, twelve RIF samples (age range, 25-38) and eight normal fertility samples (age range, 29-40) which were obtained as two peripheral blood and two endometrial biopsies; the proliferative phase (cycle day 7-10) and the secretory phase (cycle day 20-24). In this study, all of endometrial and serum miRNA exprassions are measured by quantitative real-time PCR (qRT-PCR) technique which is a high sensitive method for measures quantitatively expression of targeted miRNA in biological sample.

Main results and the role of chance: The significant feature of this study is the analysis of miRNA expression level in two different type of samples in same cases with colected sample in two varied menustral phase. In total, study results are dysregulated miRNAs selected by expression analyses in two different sample in RIF versus control groups. The statistical analysis showed that four selected miRNAs (hsa-miR-145, hsa-miR-23b, hsa-miR-31and hsa-miR-30b) were significant ($P<0.05$) up-regulated in proliferative phase and down-regulated in secretory phase of endometrium samples. In serum sample only two miRNA (hsa-miR-145 and hsa-miR-23b) was significantly down-regulated in both of proliferative phase and secretory phase. Target analysis for dysregulated miRNAs in different target scan databases (Target Scan, Miranda and Pictar) identified important, validated and predicted genes for implantation process. Pathway analysis of targeted genes showed critical pathways with $P<0.05$ which are related to cell important cell functions such as proliferation, apoptosis, invasion, adhesion and differentiation.

Limitations, reasons for caution: In this study, one of the challenge was endometrial biopsy step; the obtaining sample from normal fertile women in two different phase of menstrual cycle was difficult. Another challenge was exosomal RNA isolation step; the low concentrations of circulating RNAs limits the detection with Nanodrop (~2 ng/μL).

Wider implications of the findings: The findings of this study showed that selected miRNAs can be effective in the biological progression of the RIF. Finally, the results can create a basis for developing a new theory for the potential treatment of RIF and can be used for non-invasive molecular biomarkers in early diagnosis of RIF.

Trial registration number: This PhD thesis was supported by Istanbul University Scientific Research Projects Department (grant no. 20535)

P-423 RCT of the impact of the online lifestyle coaching platform 'Smarter Pregnancy' on modifying peri-conceptional behaviours in women presenting with subfertility or recurrent miscarriages.

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Study question: Is the online lifestyle coaching platform 'Smarter Pregnancy' more effective at modifying peri-conceptional behaviours than standard advice offered by the UK National Health Service (NHS)?

Summary answer: This online lifestyle coaching platform is an effective alternative means of delivering lifestyle advice and modulating behaviours in women with reproductive failures.

What is known already: The impact of peri-conceptional lifestyle factors on fertility and pregnancy outcomes has been well described and clinical guidelines increasingly advise optimization of BMI, nutrition, smoking and other factors prior to attempting conception. The peri-conceptional intrauterine environment, which has been shown to be modulated by diet, is a determinant of early embryo development and programming. Modifiable lifestyle behaviours therefore have an impact on fertility, miscarriage risk, fetal development and on longer term health outcomes for future children. However, cost effective means of altering peri-conceptional lifestyle remain elusive. The use of an online personalized lifestyle coaching platform may address this challenge.

Study design, size, duration: Between June 2016 and August 2018, 400 women were recruited to a two centre randomised controlled trial comparing the impact of an online based, smartphone accessed personalised lifestyle coaching program to that of referral to an NHS provided information website.

Participants/materials, setting, methods: Women referred to two NHS clinics for treatment of subfertility or recurrent miscarriages who met the inclusion criteria were randomised to receive either the personalised online lifestyle coaching platform 'Smarter Pregnancy' or standard peri-conceptional advice provided by NHS websites. All participants were requested to complete a validated lifestyle questionnaire at baseline, 12 and 24 weeks. T-tests and chi-squared tests were used to compare differences in nutritional (vegetable and fruit) and folic-acid supplement intake between groups.

Main results and the role of chance: Of the 400 women recruited into the trial, 264 women were randomised (n=131 intervention group, n=133 control group). At baseline, for the intervention group, mean vegetable intake was 136.7g per day (standard deviation (s.d.) 83.0g) and mean fruit intake per day was 2.34 pieces per day (s.d.1.8 pieces/day). At baseline, for the control group, mean vegetable intake was 126.7g per day (s.d. 92.4g) and fruit intake was 1.96 pieces per day (s.d. 1.8 pieces/day). At 12 weeks, there was a significant difference in the mean fruit intake (intervention group 2.82 pieces per day (s.d.1.9 pieces/day) vs control group 2.02 pieces per day (s.d.1.7 pieces/day), p-value 0.001, 95% CI 0.33, 1.28), but no significant difference in the mean vegetable intake between the two groups was observed (intervention group 147.7g per day (s.d. 80.9g) vs control group 135.2g per day (s.d. 92.3g), p-value 0.28, 95% CI -10.2, 35.3) The folic acid intake was not significantly different between intervention and control groups at baseline, or 12 weeks after randomisation (At baseline: 91/124 for intervention group vs 96/130 for control group, p-value 0.58) and at 12 weeks: 79/108 for intervention group vs 91/116 for control group, p-value 0.44)

Limitations, reasons for caution: Although clinicians were blinded throughout, due to the nature of the study, it was not possible to blind women who were randomised. The women's diet and lifestyle were self reported through questionnaires and it was not possible to eliminate reporting biases.

Wider implications of the findings: This personalised online lifestyle coaching platform has the potential to be a useful adjunct to standard resources in modifying behaviours. It may represent an empowering and cost effective means of delivering peri-conceptional advice to women suffering from subfertility or recurrent miscarriages.

Trial registration number: ISRCTN 89523555

P-424 Rheumatic autoimmune diseases as a risk factor for primary ovarian failure - a case control study

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Study question: The objective of this study was to investigate the relation between premature ovarian failure (POF) and autoimmune diseases (AIDs).

Summary answer: We found a high prevalence of AIDs in patients with POF. Screening for AID should therefore be offered to every POF patient.

What is known already: Premature ovarian failure (POF) is defined as a hypergonadotropic amenorrhoea, persisting more than 4 months before the age of 40 years with elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels. This condition affects 0.3 - 1% of women

Study design, size, duration: This prospective study included patients with POF. Exclusion criteria were chromosomal abnormalities, chemo- or radiotherapy and ovarectomy. AMH was measured with electrochemiluminescence

(ECLIA; Roche diagnostics). The test measures concentrations between 0.01 – 23 ng/ml. Depending on age, normal values range from 0.77 – 9.49 ng/ml.
Participants/materials, setting, methods: From 2009 until 2017 52 consecutive women between the age of 18 to 40 years and POF were included and screened for AID at the department for rheumatology
Main results and the role of chance: In 40.4% (95% CI: 26.9-53.9%) of the patients at least one AID was detected. In more than half (57.1%) of the patients POF preceded the diagnosis of the AID. The average age for the first diagnosis for POF was 29.5 years (13-40 years). Most patients reported menopausal symptoms. All patients showed low AMH-values (Median: 0.05; 0.0-0.9 ng/ml). Without considering the quite common Hashimoto's disease, still 15.4% (95% CI: 7-28.1%) of the patients were diagnosed with a systemic AID like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), psoriasis, Crohn's disease and celiac disease (both biopsy-confirmed), presenting with typical clinical symptoms and antibodies.
Limitations, reasons for caution: The relatively small number of patients in our study limits its significance, but up to now this is the biggest prospective study to show the high prevalence of AID in patients with POF. All our patients received the same standard diagnostic approach.
Wider implications of the findings: Autoimmune diseases can be found in around 40% of patients with primary ovarian failure and are associated with a poorer infertility treatment-outcome. Screening for autoimmune diseases therefore should be offered to all POF patients
Trial registration number: Ethics committee number:196/2009BO2

P-425 Endometrium epithelial cells from recurrent implantation failure (RIF) patients inhibit HTR8/SVneo cell proliferation, invasion, and promote apoptosis via exosomal pathway.

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Study question: Could endometrial epithelial cell-derived exosomes of RIF patients impact embryonic development and implantation?
Summary answer: Exosomes derived from endometrial epithelial cell of RIF patients decreased embryo blastocyst formation rate, inhibited HTR8/SVneo cell proliferation, invasion, and induced apoptosis.
What is known already: Recurrent implantation failure is a clinical diagnosis which occurs in repeated good-embryo transfer without pregnancy. It is generally accepted that impaired endometrial receptivity and embryo-endometrium cross talk play significant roles in RIF. Exosomes, one of the extracellular vesicles, play important roles in mediating cell-cell communication. Recently, exosomes were identified within the uterine cavity of woman and they contributed to the implantation and embryonic development.
Study design, size, duration: Endometrial tissue samples in the late proliferative phase were obtained from women who undergone hysteroscope in two groups (RIF group: 14 women who underwent embryo transfer number at least 3 times without pregnancy; fertile group: 9 women who had a live birth or conceived in 2 years). Primary endometrial epithelial cells were isolated from samples and modulated by estrogen and progesterone to mimic the receptive-phase.
Participants/materials, setting, methods: Exosomes were isolated from the conditioned media by ultracentrifugation, and characterized by transmission electron microscopy and Western blot. Murine 2-cell embryos and HTR8/SVneo cells were treated by exosomes or PBS (control group). The blastocyst formation rate, hatching rate and blastomere count were counted. Exosomes labeled by 1,1-dioctadecyl-3,3,3'3'-tetramethylindotricarbocyanine-iodide fluorescent dye were tested endocytosis. HTR8/SVneo cell proliferation, apoptosis and invasion capacity were accessed by CCK8, immunofluorescence staining of caspase3 and transwell invasion assay.
Main results and the role of chance: The data showed that primary endometrial epithelial cells were able to secret exosomes with a biconcave morphological feature. Exosomes from RIF group significantly decreased the blastocyst formation rate (84.2%) compared to fertile group (97.1%) and control group (93.1%). Exosomes can be endocytosed by HTR8/SVneo cells. Compared to the control and fertile group, exosomes from RIF group inhibited HTR8/SVneo cell proliferation ($p<0.05$), invasion ($p<0.05$) and promoted apoptosis ($p<0.05$).
Limitations, reasons for caution: We used only in vitro methods to show the regulation of proliferation, apoptosis and invasion effects of exosomes. Thus, future studies should determine the mechanisms involved in this process.
Wider implications of the findings: This study found that endometrial epithelial cell derived exosomes participate in the regulation of embryonic development and implantation, especially in RIF patients. Inefficient maternal communication may be the pathology of RIF via exosome pathway.
Trial registration number: not applicable

P-426 Adjunction of paternal activated peripheral mononuclear cells (PBMCs) with autologous PBMCs to immune-modulate endometrium in cases of recurrent implantation failures (RIF).

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Study question: Does intrauterine administration of co-cultured maternal and paternal PBMC improve pregnancy and implantation rates in patients with RIF?
Summary answer: Intrauterine administration of at least 2 millions of co-cultured maternal and paternal PBMC is an effective approach to improve embryo implantation in patients with RIF.
What is known already: The physiopathology of mechanism of RIF is not easy to define. Recently, immunological factors such as infammatory reaction, lymphocyte T helper 1/T helper 2 ratio and cytokines production have been proposed as the cause of endometrial receptivity defect.

Some studies suggested the role of endometrium immuno-modulation with maternal activated PBMC in implantation success. However, the effect of intra uterine insemination of mixed paternal and maternal activated PBMC before embyo transfer in RIF cases has not been studied enough. Indeed, paternal PBMC facilitate immune reactions including blocking antibodies.
Study design, size, duration: Prospective study conducted between February 2018 and January 2019. Thirty six couples with RIF were included.The patients were categorized into two groups with regard to their treatment type, autologous PBMC: group A (n=17) and co-cultured maternal and paternal PBMC: group B (n=19). Subgroups were defined according to the number of PBMC inseminated : $<$ 2 millions (Group A1 (n=8) and group B1 (n=9); and $\geq$ 2 millions (Group A2 (n=9) and group B2 (n=10).
Participants/materials, setting, methods: Mononuclear cells were isolated from patient's peripheral blood by density gradient centrifugation using commercially available lymphocyte preparation and then cultured for 3 days and transferred into the endometrium cavity prior to embryo transfer. All patients were selected on the following inclusion criteria: failure to achieve a pregnancy following a minimum of three IVF/ICSI cycles in which more than 5 high-grade embryos were transferred, age $<$40 years old, primary infertility and absence of uterine pathology
Main results and the role of chance: The median age of our patients was 36 years. Vitamin D level ranged from 15,3 to 78 ng/ml. The couples studied had a mean of 5 previous failed IVF cycles, and had been trying to conceive for a mean of 7 years. All patients had normal karyotype.

The pregnancy rates were significantly higher when at least 2 millions of co-cultured maternal and paternal PBMC were inseminated, group B2, (60%) in comparison respectively to group A1, A2 and B1 ((37,5%; 33%; 11%); ($p<0,05$). The implantation rate was also significantly higher in group B2 (35,3%) in comparison respectively to group A1, A2 and B1 ((18,7%; 26,6%; 8,7%); ($p<0,05$).

Main baseline characteristics and main ovarian stimulation outcomes had no statistically significant differences between groups A and B or between subgroups, nor did the number of embryo transferred per cycle.
Limitations, reasons for caution: Sample size must be increased and further studies are required to assess if ART outcome could be improved using our immunomodulation procedures in case of RIF.

In our study, sperm abnormalities patients were included which could not avoid the effect of sperm DNA fragmentation on implantation failure.

Wider implications of the findings: Further studies are needed to define other factors that may improve implantation rates in RIF cases.
Trial registration number: Not applicable

P-427 Hormonal stimulation modulating the expression of genes responsible for endometrial receptivity: an in vitro study

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Study question: To determine, in endometrial stromal cells, the impact of decidualization on the expression of genes involved in endometrial receptivity. Does hormonal hyperstimulation affect this profile?
Summary answer: *In vitro* decidualization affects the expression profile of genes involved in endometrial receptivity. Hormonal treatment mimicking ovarian hyperstimulation protocols seems to affect this specific fingerprinting
What is known already: Despite great progresses achieved by assisted reproductive techniques, the pregnancy rate still remains low, with implantation process representing one of the most crucial step. In this regard, endometrial receptivity is an essential prerequisite for a successful embryo implantation; alterations in development and maturation of the endometrium can cause implantation failure and infertility. Moreover, several evidences suggest that protocols of controlled ovarian hyperstimulation currently applied in assisted reproductive techniques may affect endometrial physiology, with possible negative effects on the embryo implantation
Study design, size, duration: We investigated the expression of 19 selected genes involved in endometrial receptivity using primary endometrial stromal cells (HESCs) isolated from endometrial tissue biopsies. Endometrial specimens have been obtained from healthy women who underwent surgical interventions starting from March to October2018 and signed an informed consent. Gene expression analysis of 19 genes involved in endometrial receptivity was carried out in primary HESCs, before and after decidualization, associated or not with FSH, LH, hCG treatment
Participants/materials, setting, methods: HESCs isolated from endometrial tissues collected from fertile women during implantation window have been exposed for 4 days to estrogen, progesterone and cAMP to mimic the decidualization process or with estrogen alone as a control. They have been treated for 24h with FSH alone or in combination with LH or hCG, reproducing the hormonal hyperstimulation conditions. mRNA extracted was subjected to qRT-PCR and the expression profiles of selected genes were evaluated by using precustomised array
Main results and the role of chance: Our results highlighted that most of the analysed genes, even if expressed in both decidualized and non-decidualized HESCs, are significantly modulated by *in vitro* decidualization process. The expression of several genes resulted to be significantly increased by decidualization: between others, *WNT4* and *SPP1*, genes codifying for proteins having role in cell-cell interactions, as well as *LIFR*, the LIF receptor. By contrast, *in vitro* decidualization significantly decrease the expression of *BMP2* and *IGF1*, genes encoding for multifactorial growth factors. Moreover, we observed that *in vitro* gonadotropins treatment significantly affects the gene expression profile of *WNT4* and *IGF1,* whose mRNA levels were reduced in both decidualized and non-decidualized HESCs. Interestingly, the treatment with FSH alone induced in non-decidualized cells an increase in WNT4 mRNA levels, and the treatment with FSH in combination with LH in IGF1 mRNA level in decidualized cells. *BMP2*, *SPP1*and *LIF* display an opposite trend of expression after the hyperstimulation treatment Before and after the decidualization process. Indeed, all hormonal treatments up-regulate LIFR mRNA levels.
Limitations, reasons for caution: The present results were obtained using primary endometrial stromal cells and *in vitro* decidualization protocols. In addition, different parameters may influence the response to hormonal hyperstimulation protocols, hence, further studies are necessary to elucidate whether these mechanisms operate similarly *in vivo*.
Wider implications of the findings: This approach allowed us to identify key genes whose expression is significantly modulated in decidualized compared to non-decidualized HESCs, also in response to hormonal hyperstimulation. This study, if replicated in a larger population, might contribute in understanding impact of the ovarian hyperstimulation protocols used in ART cycles
Trial registration number: Not applicable

P-428 Are endometrial receptivity assay results correlated with obstetrical outcomes and body mass index in patients undergoing embryo transfer in embryo transfer cycle immediately following assay

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Study question: Are pregnancy and live birth rates in the cycle subsequent to endometrial receptivity assay (ERA) increased, and is body-mass index (BMI) correlated with abnormal ERA.
Summary answer: There is a high rate of pregnancy and live birth in cycle subsequent to ERA for patients with adjusted and unadjusted embryo transfer (ET) cycles.
What is known already: The ERA test helps assess a woman's window of implantation (WOI) and optimize the time of ET. Up to 20-30% of women may have a displaced WOI, and for those who have failed previous ET with and without euploid blastocysts, this may be a useful tool to identify an individual's WOI and optimize ET timing. It is also possible that excessive adiposity leads to higher peripheral estrogen conversion, and thereby a higher rate of out-of-phase receptivity in the overweight/obese population due to an altered hormonal milieu.
Study design, size, duration: This is a retrospective observational study. Demographics of 35 patients undergoing ERA over an 18-month period were recorded. These patients had ERA performed due to failed implantation following multiple ART cycles.
Participants/materials, setting, methods: This is a single institution studyof patients who had undergone fresh ET cycles with high grade embryos, and/or frozen cycles with either genetically tested euploid blastocysts or untested high grade embryos. Patient age at ERA, BMI, and number and type of previous ET cycles were documented. Modifications to the subsequent ET cycle were performed when "non-receptive" test results were received. The correlation between ERA results and BMI was performed by chi-square analysis.
Main results and the role of chance: 43 ERA tests were performed for 35 patients; 8 patients had repeat biopsies during mock frozen ET cycles after hormonal/timing alterations due to non-receptive results. 22 patients had receptive results. Two patients had early/late receptive results, and subsequent adjustment by 12 hours of ET as recommended. 11 patients had pre- or post-receptive results, and subsequent ET cycles were adjusted accordingly. 25/35 patients proceeded with a subsequent frozen ET cycle subsequent to ERA. 22 patients achieved pregnancy in their next ET after ERA (88%); all 10 patients whose ET cycles were adjusted due to ERA results achieved pregnancy in their next cycle (100%), as did 12/15 (80%) with receptive ERA and unadjusted cycles. 13/22 pregnancies resulted in live birth (59.1%). 59% pregnancies occurred with euploid SET, and 41% with untested embryos. 11/35 patients had a BMI >25 (overweight or obese). 3/11 of patients with BMI>25 had abnormal ERA results (27%), while 8/24 women with BMI 19-25 had abnormal ERA results (33%) (p=0.36). As our sample population is 35 patients, the role of chance and possible regression to the mean in terms of success rates factors into our results
Limitations, reasons for caution: Our results did not confirm that BMI is related to endometrial receptivity, however, further investigation with a larger sample size is warranted to determine significance of our findings in terms of obstetrical outcomes, as well as the population to whom ERA is beneficial.
Wider implications of the findings: The high success rate in unadjusted ET cycles warrants further exploration as to whether the reason for success is related to changes in hormonal stimulation, or to the proximity of ERA and the endometrial effects of the biopsy on subsequent ET.
Trial registration number: N/A

P-429 Rnasequencing of trophectoderm cells biopsied from human blastocysts cultured in a new three-dimensional in vitro model reveal major pathways predicting implantation

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Study question: Can we use a three-dimensional *in vitro* model to study early human embryo implantation and do trophectoderm cells express predictive markers for embryo implantation potential?
Summary answer: This three-dimensional *in vitro* model sustains growth until day 14 of human embryo development. RNAsequencing of trophectoderm cells revealed key pathways decisive for embryo implantation.
What is known already: Successful implantation requires a receptive endometrium and a competent embryo, but little is known about the mechanisms regulating this crosstalk. Therefore understanding the first events of implantation is crucial. Identifying an objective criterium to select the embryo with the best implantation potential has been one of the most challenging aspect of artificial reproductive treatment. A number of models have been set up but so far only limited advancements have been achieved because of obvious ethical and technical restrictions (Teklenburg et al. 2009). Nevertheless studies have highlighted the crucial role of the trophectoderm cells during implantation (Ahlström et al. 2011).
Study design, size, duration: To set up the *in vitro* model 70 healthy oocyte donors were recruited. Between January 2014 and December 2018 they donated an endometrial biopsy on the day of pick-up. Day 5 good quality human blastocysts donated to research were warmed between 2014 and 2018 and cultured. Trophectoderm biopsies taken on day 6 were divided in two parts for (1) RNA sequencing and (2) DNA sequencing using Next Generation Sequencing (NGS). Twenty seven embryos were sequenced.
Participants/materials, setting, methods: This three-dimensional model was composed of decidualized stromal cells (using cAMP and Progesterone) embedded in Matrigel and a layer of Ishikawa cells. Day 6 biopsied human embryos were put in co-culture and after 48 hours the embryos were allocated to a group: "implantation" or "no implantation". Prolactine (PRL) and human Chorionic Gonadotropin (hCG) were analyzed by ELISA. Trophectoderm biopsies were analyzed with NGS using Illumina Hiseq and library preparation was obtained with SMARTseq.
Main results and the role of chance: To validate stromal cell decidualization PRL was measured in spent medium of the model after 72 hours of culture. Day 6 embryos were co-cultered in the three-dimensional model until day 14 of embryo development. Attachment and implantation were evaluated using the reaction of the embryos when pipetting the medium up and down. An invasion rate of 60% (16/27 blastocysts invaded) was achieved after 48 hours of co-culture. The invasion rate was confirmed with the measurement of hCG.

Trophectoderm biopsies obtained before co-culture were further matched to the fate of the embryos and allocated to the "implantation" or "no implantation" groups. DNA sequencing outcome (euploidy vs aneuploidy) could not be linked to the invasion capacity of the embryos. Unlike DNA, RNA sequencing with NGS revealed a number of pathways with differential expression in both groups such as WNT and epithelial-to- mesenchymal transition. Finally the presence of a number of miRNA's was specifically found in the "implantation" group.
Limitations, reasons for caution: Data from an *in vitro* model may not be extrapolated to the *in vivo* situation. The model is composed of Ishikawa cells showing similarities to primary epithelial cells but different mechanisms cannot be excluded. The absence of other cells present *in vivo*, like natural killer cells, may have an effect.
Wider implications of the findings: These findings support the role of trophectoderm cells during implantation and the capacity of the TE biospy to predict the pregnancy outcome. The pathways will lead to specific markers that could be used in the clinic to choose the best embryo and as a result reduce de time to pregnancy.
Trial registration number: not applicable

POSTER VIEWING
MALE AND FEMALE FERTILITY PRESERVATION

P-430 Anti-Müllerian Hormone levels after ovarian protection with gonadotropin-releasing hormone agonist during chemotherapy in breast cancer patients

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Study question: Is there is a difference in the ovarian protective effect among different administration timing of gonadotropin-releasing hormone (GnRH) agonist during chemotherapy in breast cancer patients?
Summary answer: There was no difference in ovarian protective effect among different timing of administration of GnRH agonist.
What is known already: GnRH agonists requires several days to achieve pituitary suppression. The effect of ovarian protection when chemotherapy has been administered before pituitary suppression is unknown.
Study design, size, duration: Prospective cohort study. Between October 2009 and August 2014, we included 93 breast cancer patients under age 40 who had their anti-Müllerian hormone (AMH) evaluated at least once after chemotherapy.
Participants/materials, setting, methods: Breast cancer patients received GnRH agonist at least one day prior to- and during chemotherapy. Serum AMH was measured before and 12 months and 24 months after completion of chemotherapy. The AMH value was compared among the three groups, divided by the date difference between the GnRH agonist start day and the chemotherapy start day: 1-6 days, 7-13 days, and 14 days or more
Main results and the role of chance: The mean age of subjects was 32.3 $\pm$ 4.7 years. There was no difference in the initial AMH levels among the three groups (5.4 $\pm$ 3.2 ng/ml, 5.3 $\pm$ 6.2 ng/ml, and 6.4 $\pm$ 3.8 ng/ml in the 1-6 days group, 7-13 days group, and 14 days or more groups, respectively, P = 0.23). Among the 93 women initially included, AMH value was available in 57 women at 12 months. The mean AMH levels at 12 months were 1.8 $\pm$ 2.2 ng/ml, 2.0 $\pm$ 2.5 ng/ml, and 1.6 $\pm$ 1.3 ng/ml in the three groups, respectively, and there was no statistically significant difference among them (P = 0.87). The AMH value was available in 38 patients at 24 months, and also did not show significant difference across groups (1.6 $\pm$ 1.7ng/ml, 1.5 $\pm$ 2.0 ng/ml, and 1.3 $\pm$ 0.9 ng/ml, respectively, P = 0.91).
Limitations, reasons for caution: Limited number of patients were available at 12 months'- and 24 months' follow up.
Wider implications of the findings: The action of GnRH agonist on ovarian protection, when administered at least one day prior the chemotherapy, works equally regardless of the timing of administration.
Trial registration number: None

P-431 A six-year experience of social freezing and IVF after warming oocytes

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Study question: Which are the current outcomes of social egg freezing?

Summary answer: Of all the women that vitrified their oocytes in a six-year period, 5.7% have returned to used them, with a 19% live birth rate.

What is known already: For several decades now, the mean age at which women have their first child has increased across developed countries. This has lead to a steady increase in age related sterility, as well as a demand of assisted reproduction techniques. The main reason given by women for their delayed motherhood is the lack of a suitable partner, over other reasons like accomplishment of education or career goals. Social oocyte vitrification has been proposed as an option for women who decide to postpone maternity due to sociodemographic changes, however, large studies on the outcomes of this technique are still lacking.

Study design, size, duration: Retrospective cohort study of women who underwent a social oocyte vitrification treatment, between 2011 and 2017, in one private fertility clinic**.**

Participants/materials, setting, methods: 1,519 women sought information to freeze their oocytes. Of these, 1,006 started a stimulation cycle, and 860 had their oocytes vitrified. As 197 women underwent stimulation more than once, 1284 vitrification cycles were performed in total.

Main results and the role of chance: The mean age at the time of vitrification was 37.5±3.17, with a steady and encouraging decrease in age at treatment, from 40 years in 2011 to 36.6 in 2017. The mean number of cumulus-oocyte complex (COC) and metaphase II (MII) obtained were 10.4±8.07 and 7.9±6.32, respectively. There were 12% (n=164) cancelled cycles and 0.3% (n=35) cases without any MII at ovum pick-up. On average, each woman underwent 1.26 stimulation and vitrification cycles. During this period, 67 ET were scheduled (from 57 patients), of which 63 were finally performed (2 fertilization failure after ICSI, 1 no survival after warming and 1 no ET due to PGT-A results). The mean age at the time of ET was 41.4 years (for these women, mean age at vitrification was 38.5 years). Of the women that came back to use their oocytes, 55.6% performed IVF with a male partner, while the others opted for donor semen. Biochemical, clinical, ongoing pregnancy and live birth rates were 30.2%, 23.4%, 23.4% and 19%, respectively.

Limitations, reasons for caution: Only 5% of women who vitrified their oocytes used them during the time of the study and mean age for these women was higher than average for the program. Therefore, IVF results of these women may not be representative of the population of women who vitrified oocytes**.**

Wider implications of the findings: Although the age of women who have vitrified their oocytes has been decreasing in the last years, more educational efforts are needed to further decrease the age at vitrification, in order to improve the reproductive results associated to this technique.

Trial registration number: not applicable

P-432 Oocyte cryopreservation for fertility preservation in women with ovarian endometriosis

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Study question: Is the oocyte cryopreservation feasible fertility preservation (FP) option for women with ovarian endometriosis (endometrioma)?

Summary answer: In women with endometrioma, oocyte cryopreservation before ovarian cystectomy could be a feasible option for FP.

What is known already: Ovarian endometriosis by itself reduces the ovarian reserve by affecting ovarian physiology in the healthy ovarian tissue surrounding it. Ovarian cystectomy is a treatment option for endometrioma in most cases. However, ovarian cystectomy has been associated with a risk of diminished ovarian reserve. Women with endometrioma are at a higher risk of decreased ovarian reserves and are potential candidates for FP. However, there are few published papers on the topic of FP in endometriosis.

Study design, size, duration: This retrospective study was conducted at a tertiary university hospital. We performed controlled ovarian stimulation (COS) and oocyte cryopreservation in 26 women with endometrioma before a planned ovarian cystectomy between May 2016 and October 2018.

Participants/materials, setting, methods: Clinical characteristics and the COS results were analyzed in 26 women with endometrioma before a planned ovarian cystectomy. We compared COS outcomes of the first cycle in FP patients with endometrioma with the COS outcomes in infertile patients without endometrioma who underwent in vitro fertilization treatment (24 patients owing to male, tubal, or unexplained cause). We analyzed the number of oocytes cryopreserved in repeated COS cycles and evaluated the differences between the cycles.

Main results and the role of chance: The median [interquartile range] of endometrioma size was 5.3 [3.9, 7.1] cm. The median age and serum anti-Mullerian hormone (AMH) levels of the women were 30 [26, 38] years and 1.38 [1.00, 2.34] ng/mL, respectively. The number of oocytes retrieved and the number of oocytes cryopreserved were 5.0 [3.0, 8.0] and 4.0 [2.0, 7.0], respectively. Despite there being no difference in the AMH levels in patients with bilateral and unilateral endometriomas, the number of oocytes cryopreserved was lower in patients with bilateral endometrioma (5.5 [3.8, 8.3] vs. 3.0 [2.0, 6.0], p=0.044). Although the ages were similar, the AMH level was significantly lower in the FP patients with endometrioma compared to that in infertile patients without endometrioma (1.38 [1.00, 2.34] vs. 3.35 [2.14, 4.20] ng/mL, p<0.001) The number of oocytes retrieved was lower in the FP patients with endometrioma, but not statistically significant (4.0 [3.0, 8.0] vs. 7.0 [4.3, 11.0], p=0.069). Total 10 (38.5%) patients with endometrioma obtained more oocytes through more than one COS cycle. Retrieved oocyte number at the first, the second, and the third cycle were 3.0 [2.0, 6.0], 4.5 [2.0, 7.3], 3.0 [2.0, 7.5], respectively.

Limitations, reasons for caution: The retrospective study design and the small number of study subjects are limitations. We showed only the result of oocyte cryopreservation, and further studies are needed to evaluate the results after thawing.

Wider implications of the findings: Women with endometrioma should be counseled about oocyte cryopreservation for FP before surgery. The optimal number of oocytes for cryopreservation can be secured by repeated oocyte retrieval.

Trial registration number: not applicable

P-433 Is vitrification damaging oocytes? Results from 37.520 fresh and vitrified sibling donor oocytes

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Study question: Are reproductive outcomes of IVF cycles with vitrified donor oocytes comparable to IVF cycles with fresh donor oocytes?

Summary answer: IVF with vitrified donor oocytes provides similar reproductive outcomes to fresh oocytes when the same number of oocytes is available for fertilization after warming.

What is known already: Oocyte vitrification is used to preserve the reproductive potential of oocytes. Several RCTs, carried out by highly trained and experienced groups have shown that this technique provides comparable reproductive outcomes to the use of fresh oocytes. However, large registries analyses have consistently reported lower live birth rates after using vitrified oocytes. It is not clear whether this decrease may be due to an inherent damage that vitrification causes to the oocytes or to the lower efficiency of the technique, as a variable proportion of the allocated oocytes (around 10% even in the best laboratory conditions) do not survive warming.

Study design, size, duration: Retrospective cohort analysis of 1,844 cycles of oocyte donation, each woman providing sibling oocytes for at least one recipient of fresh oocytes (2,562 cycles) and one other of vitrified oocytes (2,472 cycles), representing a total of 37.520 MII sibling oocytes included in this study. All recipient cycles (n=5,034) were carried out between 2011 and 2017 (embryo transfer date).

Participants/materials, setting, methods: Differences in reproductive outcomes between groups were evaluated using Chi^2 tests and regression analysis adjusted for recipient's age, BMI, sperm origin (patient/donor), sperm state (fresh/frozen), day of ET, morphological score and number of transferred embryos. In addition, we performed a subanalysis of 1,336 IVF cycles where the number of inseminated oocytes was the same for the recipients of fresh and vitrified oocytes to test whether vitrification/warming efficiency impacts reproductive results.

Main results and the role of chance: Baseline characteristics of recipients and number of inseminated and fertilized MII were comparable between groups. Overall, vitrified oocytes provided lower reproductive outcomes than their sibling fresh oocytes: ongoing pregnancy (32.4% vs 37.9%; p<0.001) and live birth (31% vs 36.2%; p<0.001). When the number of inseminated oocytes was the same for fresh and vitrified donor cycles, reproductive outcomes in recipients became comparable: ongoing pregnancy (33.5% vs 34.1%; p=0.82) and live birth rate (32.1% vs 32%; p=0.97), indicating that the efficiency of the vitrification process, rather than an intrinsic oocyte damage due to vitrification, affects the reproductive outcomes of oocytes. The multivariable analysis confirmed these results; the OR (95%CI) of vitrified vs fresh oocytes was 0.869 (0.764, 0.990) for ongoing pregnancy and 0.882 (0.773, 1.006) for live birth at the main analysis; and 1.110 (0.865, 1.425) for ongoing pregnancy and 1.150 (0.892, 1.483) once controlling for oocyte loss due to warming. In summary, when the allocation of oocytes to recipients provides the same number of oocytes after warming than would be allocated fresh, reproductive results up to live birth are comparable.
Limitations, reasons for caution: An open vitrification system was used for all cases, and oocyte vitrification/warming was performed by experienced embryologists with consistently high survival rates; caution must be exerted when extrapolating our results to closed vitrification systems or to IVF units with survival rates <90%.
Wider implications of the findings: This is the biggest cohort study comparing reproductive outcomes of vitrified and fresh sibling donor oocytes so far. Our results indicate that vitrification *per se* does not affect oocyte developmental competence; we recommend implementing strict indicators of vitrification/warming efficiency in clinics, and developing vitrification/warming protocols to maximize oocyte survival.
Trial registration number: not applicable

P-435 Isolation and in vitro maturation of immature oocytes from a small piece of ovarian tissue in fertility preservation treatments.

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Study question: Can immature oocytes retrieved during the ex-vivo preparation of ovarian tissue prior to cryopreservation be matured in vitro, fertilized and cryopreserved for future fertility treatments?
Summary answer: Immature oocytes can be successfully retrieved from ovarian tissue, matured in vitro, fertilized and cryopreserved for future fertility treatments.
What is known already: Fertility preservation in women prior to gonadotoxic therapies can be achieved by the cryopreservation of ovarian cortical tissue. Immature oocytes recovered during the preparation can be matured in vitro and lead to live births, thereby providing an additional option for fertility preservation (Fasano et al., 2017; Prasath et al). Here, the feasibility of this approach in a setting with unilateral biopsy of a small piece of ovarian tissue and minimal tissue preparation prior to shipment to an external cryo-bank was tested.
Study design, size, duration: Feasibility study at the University Women's Hospital Heidelberg (Germany) initiated in December 2017. Until December 2018 n= 9 patients requiring chemotherapy were recruited and provided written informed consent, of which n= 2 decided not to undergo ovarian tissue extraction. Unilateral biopsy of approx. 50% of ovarian tissue was performed on n= 7 patients. The tissue was minimally prepared by separation of the cortex from the medulla before shipment to an external cryo-bank.
Participants/materials, setting, methods: An unilateral biopsy of approx. 50% of the ovary was performed on n= 7 female patients. Immature oocytes were retrieved during minimal preparation of ovarian tissue before transport for cryopreservation in an external cryo-bank. In vitro maturation was performed as described previously (Roesner et al., 2017). Intracytoplasmic sperm injection (ICSI) was performed either directly or after vitrification of Metaphase II (MII) oocytes (Kitazato, 91171, 91182). Pronuclear stage oocytes were cryopreserved by slow freezing (Vitrolife, 10166).
Main results and the role of chance: The patient's age was 20 to 33 years (25.6 ± 5.0 years (mean ± stdev); n= 7). The anti-Müllerian hormone level (AMH) ranged from 0.65 to 8.15 ng/ml (4.1 ± 3.1 ng/ml (mean ± stdev)). The number of immature oocytes recovered was n= 23 (n= 1 – 8 (min. – max.); 3.3 ± 2.2 (mean ± stdev)). The maturation rate after 24 hours was 34.8% (n= 8/23). MII-oocytes were either vitrified (n= 3, n= 3 patients) or directly used for ICSI (n= 5, n= 3 patients) with a fertilization rate of 60.0% (n= 3/5). Pronuclear stage oocytes were cryopreserved (n= 3). Vitrified MII-oocytes were warmed (n= 2, n= 2 patients) with a post-warming vitality rate of 50.0% (n= 1/2) and used for ICSI with a fertilization rate of 0% (n= 0/1, n= 1 patient).
Limitations, reasons for caution: A limitation of this study is the small sample size. No embryo culture and transfers were performed so far because of fertility preservation purposes in cancer patients, therefore no conclusion on the oocyte's developmental potential and clinical outcomes can be drawn. Fertilization of vitrified MII-oocytes still needs to be shown.
Wider implications of the findings: This study shows that immature oocytes can be successfully recovered from small unilateral pieces of ovarian tissue during minimal preparation. In agreement with data from Fasano et al. (2017) these oocytes can be matured in vitro and fertilized, thereby contributing to the fertility preservation treatment offered to patients.
Trial registration number: The study was approved by the Ethics Committee of the Medical Faculty Heidelberg (S-222/2017) and registered at the DRKS (registration number DRKS00013170).

P-436 Key recommendations for high quality female oncofertility care based on international clinical practice guidelines

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Study question: Which guideline-based key recommendations can be selected for high quality female oncofertility care?
Summary answer: A set of eleven key recommendations for high quality female oncofertility care was selected to enable future oncofertility care monitoring and improvement.
What is known already: Young women scheduled to undergo gonadotoxic therapy are at risk to become infertile. To ensure the ability for biological children, care activities to preserve fertility are necessary. However, this oncofertility care is still suboptimal. Evidence based key recommendations (KRs) for high quality female oncofertility care have to be selected to identify and monitor the specific needs of improvement.
Study design, size, duration: To select a set of KRs for female oncofertility care, the Delphi method was used. First, recommendations from (inter)national clinical practice guidelines were selected in four domains: risk communication, referral, counselling and decision-making. Thereafter, they were scored, per domain, on their importance for high quality oncofertility care in two Delphi rounds. Finally, the selected KRs were presented for approval in a third round.
Participants/materials, setting, methods: A multidisciplinary, national oncofertility expert panel, consisting of patients, referrers, and counsellors, participated in the three online questionnaires' rounds. Differences in perspectives between subgroups of the expert panel were analysed.
Main results and the role of chance: The expert panel (N=86) selected eleven KRs for high quality female oncofertility care. KRs in the domains risk communication and referral focused on information provision and offering referral to a reproductive specialist to female cancer patients. Regarding the counselling domain, KRs focused on all aspects of counselling including different methods, safety, and pros and cons. In the decision-making domain, KRs focused on shared decision-making and supporting the decision with written information. The final set of selected KRs was approved by 91% of the experts. Differences in perspectives were found between subgroups, particularly patients found recommendations regarding decision-making and information provision more important than referrers and counsellors.

Limitations, reasons for caution: Expert panel's opinions were obtained through online questionnaires, which could have led to the lack of subtleties provided by a face-to-face discussion. However, by using online questionnaires, participants from all over the country could take part and they could not be influenced by the (strong) opinions of other participants.
Wider implications of the findings: After transcribing these KRs into quality indicators, these are well suited as a measuring tool for identifying problems and improving the quality of female oncofertility care and quality of life in survivors in the Netherlands and internationally.
Trial registration number: Not applicable

P-437 In vitro activation (IVA) of follicles: effective in human frozen-thawed ovarian tissue but influenced by the type of incubation medium and its volume

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Study question: Does the type of culture medium and its volume affect the frozen-thawed ovarian tissue (follicles, stroma) during the in vitro activation of follicles?
Summary answer: In vitro activation of follicles is effective in the frozen-thawed ovarian tissue, but is affected by the type of culture medium and its volume.
What is known already: In vitro activation (IVA) of follicles in the ovarian tissue with PTEN inhibitor and phosphatidylinositol-3-kinase stimulator and re-transplantation is a promising method of assisted conception. During IVA procedure, the ovarian tissue is incubated in the medium with activators for 48 hours, which may impair the tissue (structure, vitality, and DNA fragmentation of follicles/oocytes) and success of subsequent transplantation. Therefore, the aim of this study was to elucidate whether the type of culture medium (simple, for IVF designed and tested Flushing Medium vs. complex DMEM-F12 medium) and its volume affect the quality of frozen-thawed ovarian fragments (follicles, stroma) treated with IVA.
Study design, size, duration: During two years, the ovarian tissue of one patient (female-to-male transgender surgery) was slowly frozen (25 cryotubes), thawed, sub-fragmented, and exposed to activators of follicles bpV(Hopic) and 740YP, diluted in two different culture media: complex DMEM-F12 and Flushing Medium of two volumes (1 and 5 ml). After thawing and 48-hour incubation in the medium with activators or without them, the ovarian fragments were compared in terms of histology, vitality, and DNA fragmentation of follicles/oocytes.
Participants/materials, setting, methods: The ovarian (cortex) fragments were frozen using the slow freezing with 1,2-propanediol and sucrose (Fabbri et al., 2010). After rapid thawing, they were treated with bpV(Hopic) and 740YP for 24 hours and with 740YP alone for another 24 hours in a complex DMEM-F12 (+$NaHCO_3$/glucose/L-glutamine/albumin) or Flushing medium (+HEPES; Origio). To evaluate the histology, vitality, and DNA fragmentation of tissue/follicles, the hematoxylin-eosin staining, vital stains (Trypan Blue, Neutral Red), and Roche DNA fragmentation kit were used.
Main results and the role of chance: Just after thawing, the histology of the ovarian tissue was quite comparable to the fresh tissue of the same patient. The IVA procedure significantly decreased the proportion of optimal – the best preserved follicles (oocyte nucleus/chromatin, follicular cells) and increased the proportion of vital but suboptimal (less preserved) follicles, and dead follicles in comparison to the tissue just after thawing regardless of the culture medium (7.5 %, 89.0 %, 3.5 % vs. 97.1 %, 2.9 %, 0 %; $P<0.01$, Chi-Square Test). In the ovarian fragments cultured in the DMEM-F12 and Flushing Medium there was a relatively high and comparable proportion of vital/alive follicles (98.0 % vs. 94.7 %) and more growing follicles after the IVA procedure, as revealed by histology and vital staining (Neutral Red, Trypan Blue). However, the incubation of ovarian fragments in the Flushing Medium resulted in a less-preserved stroma with a mass of pycnotic cells and a higher proportion of follicles/oocytes with DNA fragmentation. In the optimal DMEM-F12 medium the histology of ovarian tissue was favorable, with more optimal and less dead follicles, when cultured in a higher volume of medium (5 ml: 8.7 %, 0 % vs. 1 ml: 0 %, 3.9 %; $P < 0.05$).
Limitations, reasons for caution: To establish the optimal condition, different culture conditions were tested and analyses performed in the ovarian tissue of the same patient to avoid the bias due to heterogeneity of the ovarian tissue. In the next step, this procedure will be applied to the ovarian tissue of different patients.
Wider implications of the findings: In vitro activation of follicles is effective in the frozen-thawed ovarian tissue and is promising for oncological and infertile patients with cryopreserved ovarian tissue, when optimal. It allows the infertility treatment in women with severe ovarian infertility and residual follicles in their ovaries (e.g., premature ovarian failure, low ovarian reserve).
Trial registration number: Approved by the Slovenian Medical Ethical Committee: 0120-484/2017/4
Trial registration number not needed.

P-438 Systematic proposal of fertility preservation (FP) by oocyte freezing in patients with recurrent benign ovarian tumors (BOT)

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Study question: How about COH outcomes in terms of mature oocytes number and risks-benefits balance in case of oocyte freezing for fertility preservation in patients with recurrent BOT ?
Summary answer: Oocyte accumulation is recommended in young patients with BOT to have sufficient frozen oocytes and further chances of pregnancy.
What is known already: Benign ovarian cysts are frequent during women's life. They are diagnosed with pelvic pain or fortuitously during an ultrasonographic exam. In some case, cysts are recurrent and multiple surgeries are needed leading to a significant risk of ovarian damage by follicular depletion. To date, there is no cohort study dedicated to fertility preservation by oocyte freezing in this specific subgroup of patients. An effective strategy of fertility preservation has to be evaluated and propose for these young patients.
Study design, size, duration: This is a prospective observational study performed from 2014 to 2018 at the IVF centre of the Lille University Hospital. All women, age between 18-35 years, with recurrent ovarian cysts were proposed to an oocyte accumulation program (1 to 3 ovarian stimulation cycles) to preserve their fertility. The primary endpoint is to evaluate the efficiency in terms of metaphase II oocytes number. The secondary end-point is to assess safety and compliance to the procedure.
Participants/materials, setting, methods: 54 patients were included and 81 FP cycles were analysed. COH was performed by using antagonist protocols after baseline ovarian reserve assessment (AMH measurement and sonographic antral follicle count). Triggering was achieved by hCG when at least 3 follicles reached a 18mm diameter. Mature oocyte vitrification was realized 2 hours after retrieval in a closed device. (Rapidl Kit Vitrolife®). Statistical analysis was performed with the SAS Software V9.3 by the Biostatistics Unit of Lille's Hospital.
Main results and the role of chance: 54 patients, 50% with endometriomas (endometriosic group) and 50% with dermoid/mucinous cysts (non-endometriosic group) were included with a mean age of 26.7 ± 4.9 years. Mean AMH levels were 12.4 ±9.2 pmol/L (2;43). The mean number of total retrieved oocytes per cycle was 6.9 ± 4.6 (0;19). The mean number of Metaphase II oocytes per cycle was 4.6 ± 3.7 (0;16). We found a high proportion of patients (37%) exhibiting a poor ovarian response (i.e. less than 4 mature oocytes) at the first cycle. To date, 21 patients achieved 2 or 3 cycles. After accumulation, a mean number of 8.8 M2 vitrified oocytes per patient were obtained. 54% of patients had more than 8 meta II oocytes frozen after accumulation. AMH levels were strongly correlated to the Meta II oocyte number but did not appear to be predictive according to the ROC curve analysis (AUC=0.75, sensibility = 0,63; specificity=0.75).

No difference was found between endometriosic and non-endometriosic groups.

No serious adverse events occurred and the procedure was well tolerated by patients.

Limitations, reasons for caution: Our results are preliminary. Long-term follow-up is needed in order to assess the efficiency, clinical relevance and cost-effectiveness of the procedure through the analysis of reutilization and further pregnancy rates.

Wider implications of the findings: The mean number of vitrified mature oocytes after only one COH cycle in young patients with history of recurrent ovarian cysts and surgery is too low to further provide good chances of pregnancy. Hence, oocyte accumulation may be proposed as it appears to be well-tolerated and safe for patients.

Trial registration number: not applicable

P-439 Predictors of oocyte yield in social egg freezing

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Study question: Can oocyte yield in social egg freezers be predicted by clinical, biochemical and radiological markers?

Summary answer: Age and BMI, along with oestradiol level and number of follicles >12mm at trigger can help predict oocyte yield in Social Egg Freezing (SEF) cycles.

What is known already: SEF offers women the opportunity to preserve their reproductive potential prior to the inevitable decline in ovarian reserve. Extensive counselling is essential to ensure women are fully informed, including the possibility of unsuccessful treatment in the future with a finite number of stored eggs. To minimise this risk, the timing of vitrification and determination of how many oocytes to store is important. However, other than age, there is currently little evidence to determine other factors that predict the number of oocytes retrieved in SEF.

Study design, size, duration: This was a retrospective cohort study which included 315 SEF cycles in a single fertility clinic in the United Kingdom between 2008-2018.

Participants/materials, setting, methods: The number of oocytes retrieved from 315 SEF cycles was calculated. Clinical, biochemical and radiological data including age, body mass index (BMI), anti-Mullerian hormone (AMH), antral follicle count (AFC) and oestradiol level and total number of follicles >12mm at trigger were assessed. Numerical variables were summarised by the median and range. Multivariable Poisson regression was used to evaluate the impact of each variable, whilst adjusting for the other variables, on the number of oocytes retrieved.

Main results and the role of chance: The median (range) for the variables of interest were: total number of oocytes retrieved per cycle 7 (1-37); number of metaphase II oocytes 6 (1-23); age 38 (29-45) years; BMI 22 (17-34) kg/m2; baseline AMH 10.0 (0.7-98.3) pmol/l; AFC 11 (1-47); oestradiol level at trigger 6744 (557-23924) pmol/l; total number of follicles >12mm at trigger 9 (1-69). Multivariable Poisson regression demonstrated that an increase in age by one-year results in a statistically significant 4% decrease in yield after adjusting for all other variables (IRR 0.96; 95% CI 0.94-0.98; p< 0.001). BMI (IRR 1.02; 95% CI 1.00 -1.03; p=0.04), oestradiol level per 1000 pmol/l increase (IRR 1.05; 95% CI 1.04 -1.07; p<0.001) and the number of follicles >12mm at trigger (IRR 1.02; 95% CI 1.01-1.03; p<0.001) were all significant independent predictors of oocyte yield. There was no significant effect on oocyte yield of either AFC (IRR=1.00; 95% CI 0.99-1.02 p=0.43) or AMH (IRR 1.00; 95% CI 0.99 – 1.01; p=0.71).

Limitations, reasons for caution: The data from this study was retrospective and from a single centre.

Wider implications of the findings: Our data demonstrates that age, BMI, oestradiol level and number of follicles >12mm at trigger can all be used to help predict oocyte yield in SEF cycles. This information can be used to optimise counselling in SEF, help manage expectations and guide decisions over future treatment.

Trial registration number: Not applicable

P-440 Novel cryopreservation medium reduces post-thaw oxidative stress and improves viability/survival

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Study question: Does the new cryopreservation medium improve sperm parameters and provide better cryoprotection from injury and oxidative stress (OS) -induced damage compared with other sperm freezing media?

Summary answer: The novel sperm freezing medium preserves sperm vitality and confers protection from oxidative stress induced injury compared with established media for all subjects.

What is known already: Cryopreservation allows men to preserve their fertility. However, cryopreservation greatly reduces sperm parameters and increases DNA damage. The addition of cryopreservation-media maintains post-thaw sperm parameters including post-thaw motility. The use of glycerol, animal versus human protein, antioxidants, and other additives in cryomedia remains highly debatable. The use of egg yolk lacks clinical popularity due to its potential to elicit antibody production in the vaginal canal and its associated risk of prion disease. A novel media called Arctic sperm cryopreservation medium (ASCM) is free from animal protein and has a dual buffering capacity because of presence of HEPES and MOPS.

Study design, size, duration: Semen samples from 22 normozoospermic donors and 20 infertile men were examined for sperm concentration, motility, viability, oxidation-reduction potential (ORP), and sperm DNA fragmentation (SDF). ORP was measured using MiOXSYS system and SDF by TUNEL assay using bench flow cytometer. Identical freezing protocols were used using Arctic sperm cryopreservation medium ;ASCM and another competitive medium called Origio sperm freezing medium; OSFM) but with different dilution ratios (ASCM: 1:3 and OSFM: 1:1) as per manufacturer instructions.

Participants/materials, setting, methods: Samples were randomized for freezing with ASCM or OSFM. Pre-freeze and post-thaw sperm concentration, motility, ORP and SDF were measured. For cryopreservation, samples were cooled to 4°C for 10 minutes, placed in liquid nitrogen vapors for 60 minutes, and then submerged in liquid nitrogen for 24 hours. Samples were thawed and same pre-freeze parameters were measured post-thaw. Data was analyzed using independent t-test or Mann-Whitney Test and 95% confidence intervals (CI) were used.

Main results and the role of chance: The baseline pre-freeze and post-thaw semen parameters such as sperm concentration, motility, SDF and ORP were significantly better (P<0.0001) in all donors versus infertile patients. ASCM required less volume of the medium than OSFM [(0.53ml (95% CI: 0.39-0.93 vs. 1.50ml (95% CI: 1.10-2.04 p<0.0001)] and provided no significant differences in post-thaw motility in patients [(20.0 CI: 14.4-32.2 vs 18.0 CI: 13.0-20.6, p=0.64)]. The change in normalized ORP from pre-freeze (PF) (PF NORP) to post-thaw (PT) (PT NORP) was found to be significantly increased in OSFM compared to ASCM (2.05, CI 1.15 to 3.23 mV/10^6/mL, vs.1.01, CI 0.54 to 1.75 mV/10^6/mL, p=0.011). The overall decrease in viability was significantly higher in OSFM compared to ASCM (39.0(CI: 38.0-47), 26.5(CI: 22-28), P =0.0048). Overall, post-thaw SDF was comparable in both patients and donors for the 2 media (20.3 ± 12.2% vs 23.1 ± 8.7%, p=0.36).

Limitations, reasons for caution: Future controlled studies with larger sample size are needed to compare ASCM with the other established sperm freezing medium such as Test yolk buffer and Sperm freeze solution to validate the findings of this study.

Wider implications of the findings: The novel medium is a xeno-free medium with dual buffering capacity, economical and can be used for better viability preservation and protection from OS especially in abnormal patient samples.
Trial registration number: Not applicable

P-441 The effect of Letrozol or Tamoxifen co administraton in fertility preservation protocols for breast cancer patients

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Study question: Does co-administration of Letrozole or Tamoxifern during standard IVF protocols for fertility-preservation influence treatment outcomes?
Summary answer: Co-administration of Tamoxifen is associated with lower rates of top quality embryos, whereas co-administration of Letrozole is associated with higher rates of abnormal fertilizations.
What is known already: Ovarian hyperstimulation protocols for fertility-preservation in breast cancer patients, give rise to supra-physiological estradiol levels.

In order to reduce the exposure to high and potentially harmful estrogen levels, modifications to standard protocols have been made using: aromatase inhibitors (Letrozole, Femara) and selective estrogen receptor modulators, such as Tamoxifen. The alteration of the Estrogen/androgen balance in the follicle habitat may have an effect on its development and thus on the success of the fertility treatment. To date little is known about the effect of Letrozole and Tamoxifen administration on fertility outcomes.
Study design, size, duration: This is a retrospective cohort study. Data was retrieved from patients electronic records. The treatment outcomes of 118 breast cancer patients undergoing controlled ovarian hyperstimulation for fertility-preservation between the years 2009 -2017 were analyzed.
Participants/materials, setting, methods: All the patients received a standard IVF protocol for induction of ovulation.

The study groups received additionally Letrozole (n=36) or Tamoxifen (n=30). The control group (n=52) received standard protocol only. The main outcome measures were: number of oocyte retrieved, mature oocytes rate (MII oocyte), fertilization rate and top quality embryo rate. Secondary outcomes measures included: duration of stimulation, total amount of gonadotropin administered, peak estradiol (E2) levels and rate of abnormal fertilization (1PN+3PN).
Main results and the role of chance: The mean age of the patients in the three groups were similar (34.3±4.4, 34.5±4.8 and 34.5±4.5 years).

No differences were found in the numbers of oocyte retrieved, mature oocyte, and fertilization rate between the study groups and the controls.

The percentage of top quality embryos was lower for the Tamoxifen group compared to controls (25% vs 39.4%, p=0.034), but not for the Letrozole group (37.9% vs 39.4%, P=0.8083).

No differences were found in duration of stimulation and total amount of gonadotropin administered between the treatment groups and controls.

As expected Peak E2 levels were significantly lower in the Letrozole group compared to controls (mean 746.4 ±537 pg/ml vs 1745.6± 1068 pg/ml, P< 0.0001). No statistical difference was found between the tamoxifen group and controls (mean 1883.6 ± 1404.3 pg/ml vs 1745.6± 1068 pg/ml, P=0.997).

The rate of abnormal fertilization (1PN + 3PN fertilizations) was higher in both study groups when compared to controls, however only for the Letrozole group it achieved significance (7.8% vs 3.60%, P=0.0148). Stepwise logistic regression analysis revealed that Letrozole administration (OR 11.9; 95% CI 2.2-60.4, P= 0.0013) and Maximal E2 (OR 1.0007; 95% CI 1.0002-1.0013, P= 0.0017 per unit change) were significantly associated with abnormal fertilization.
Limitations, reasons for caution: Main limitations: 1.Retrospective study 2.Small study groups
Wider implications of the findings: Our data suggests that Letrozole and Tamoxifen may affect intra and extra cellular mechanism in the oocyte accounting for its ability to fertilize normally.
Trial registration number: not applicable

P-442 Fertility preservation (FP) by oocyte accumulation in patients with low and very low AMH levels: is it worth it?

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Study question: *What are the results of FP in patients with low AMH levels? How many controlled ovarian stimulation are needed to offer real chances of pregnancy?*
Summary answer: *It is worth preserving and accumulating oocytes in women with low and even very low AMH levels, regardless the cause of the diminished ovarian reserve.*
What is known already: *Patients with low AMH levels have, by definition, a smaller number of antral follicles and therefore fewer metaphase 2 (M2) oocytes in controlled ovarian stimulation. In 2016, Cobo et al. showed that a minimum of 8 M2 oocytes were required to give real chances of pregnancy. No studies have analyzed so far the results of FP in patients with low and very low AMH levels. No studies compared the outcomes of FP depending on the etiology of the diminished ovarian reserve (DOR).*
Study design, size, duration: This is a retrospective study with data collected prospectively from 2011 to 2018, in the IVF Center of Lille University Hospital (France). 123 women with low AMH levels (<10 pmol/L) and undergoing FP for oocytes accumulation were included, resulting in 180 cycles. All patients were treated with an antagonist protocol, high doses of recombinant FSH and a trigger by recombinant HCG. Data were analyzed using the SAS software 9.4 (SAS Institute Inc, Cary, NC, USA).
Participants/materials, setting, methods: The main outcomes were the number of M2 oocytes vitrified following the first cycle, and the cumulative number after a maximum of 3 cycles. Patients were divided into 4 groups: pre-cancer (n=55), post-cancer (n=16), premature ovarian insufficiency (POI) (n=19) and history of ovarian surgery (n=33). The secondary outcomes were the number of M2 oocytes according to the serum AMH level (low 5-10 pmol/L or very low ≤5 pmol/L) and to the etiology of the DOR.
Main results and the role of chance: The mean number of M2 oocytes obtained after the first cycle of FP was 3.03 ± 2.69. This number increased to 5.48 ± 3.72 after a second retrieval (n=41) and to 8.53 ± 4.52 after a third cycle (n=15). 28 women (23%) had at least 8 M2 oocytes vitrified (10 after the 1st cycle, 12 after the 2nd cycle and 6 after the 3rd cycle). Women with low AMH obtained a higher number of M2 oocytes vitrified per cycle than women with very low AMH (3.39 ± 2.60 vs. 2.43 ± 2.76, p=0.001 respectively). 11,8 % of the women with very low serum AMH levels (8/68) had at least 8 M2 vitrified. After controlling for confounders in logistic regression analysis, the mean number of M2 oocytes per cycle did not differ significantly regarding the indication (3.25 ± 3.03 in pre-cancer, 2.83 ± 3.38 in post-cancer, 2.78 ± 1.71 in POI and 3.04 ± 2.41 in women with ovarian surgery history, p=0.47). The maturation rate was similar between the four groups. Lastly, only AMH level and estradiol level on trigger day were correlated with the number of M2 oocytes obtained.
Limitations, reasons for caution: In addition to the retrospective nature of this study, the specific topic and the inclusion criteria resulted in a small population size, which subsequently decreased the power of our study.
Wider implications of the findings: A low serum AMH level should not be an argument against FP and oocyte accumulation in patients with DOR, regardless of the etiology. Long term follow-up is needed to assess the efficiency, clinical relevance and cost-effectiveness of the procedure through the analysis of the reutilization and further pregnancy rates.
Trial registration number: not applicable

P-443 Perceptions, outcomes and regret following social egg freezing in the UK

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Study question: Why do women undergo social egg freezing (SEF) in the UK, and what are their perceptions and attitudes towards SEF after undergoing the process?
Summary answer: Women mostly undergo SEF because they do not have a partner. The majority of women are satisfied after SEF with less than 10% experiencing regret.
What is known already: Social egg freezing (SEF) enhances reproductive autonomy by empowering women with the capacity to delay their childbearing years. However age-related obstetric complications, economic implications and the risk of unsuccessful future treatment make it a controversial option. Some women have no reasonable alternative, such as single women approaching the inevitable physiological decline in their ovarian reserve. Despite the upward trend in women electively cryopreserving their eggs, there is little data about the women's perceptions and experiences, having undergone the process.
Study design, size, duration: This was a cross sectional survey which included 75 women who underwent SEF in a single fertility clinic in the UK between 2010-2018.
Participants/materials, setting, methods: An online questionnaire, utilising a 5-point Likert scale, was created to assess reproductive plans, motivations for SEF, perceptions to their fertility and SEF, along with attitudes to the treatment they received, specifically including regret.
Main results and the role of chance: 71 women responded. At the time of SEF, the majority (93.3%; n=65) were worried about their declining fertility. The predominant motivation was not having a partner with more than three quarters (n=55; 77.5%) saying it influenced their decision. Conversely, just 9.9% and 2.8% attributed career or education to impact their decision. Most intended to store their eggs for 3-4 years (n=25; 35%). However, 11.3% of women planned to store their eggs for >10 years, which is longer than current legal limits in the UK.The majority (81.7%; n=58) understood there was a chance of future treatment failure and that a livebirth could not be guaranteed. Whilst 49 women (69%) were aware that becoming pregnant at advanced maternal age was associated with increased pregnancy risk, 31% (n=22) were not. 19.4% (n=12) have successfully had a baby since SEF and two (2.8%) are currently pregnant. The majority (n=10; 71.4%) were naturally conceived, while the remainder (n=4; 28.6%) conceived using their stored eggs. Overall 90.1% (n=64) had no regrets over electively freezing their eggs. Of those who felt regret, the vast majority described it as 'a little' (n=6; 85.7%). None of the women who underwent SEF and subsequently became pregnant naturally experienced regret.
Limitations, reasons for caution: The data from this study was retrospective and from a single centre.
Wider implications of the findings: Despite significant physical, psychological and financial burden, with no guarantee of a livebirth, only a small minority of women experience regret after SEF. The suboptimal perceptions of associated risks demonstrated herein highlights the need for individualised extensive counselling to ensure women are fully informed including awareness of storage limits.
Trial registration number: Not applicable

P-444 THE 9 YEARS-EXPERIENCE OF FERTILITY PRESERVATION FOR BREAST CANCER PATIENTS AT ADVANCED FERTILITY PRESERVATION CENTER IN JAPAN.

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Study question: How effective we could provide fertility preservation (FP) for breast cancer patients at fertility preservation center with advanced role in Japan?
Summary answer: We have provided effective and safe FP care for 424 of breast cancer patients through 9 years, based on closed collaboration with multidisciplinary medical professions.
What is known already: Improvement in early cancer detection and multidisciplinary treatment have enabled survival rates of cancer patients to increase, loss of fertility known as the effects of cancer treatment has been focused. Breast cancer is most popular disease for FP. Embryo or oocyte cryopreservation after controlled ovarian stimulation with aromatase inhibitor is one of FP procedure for breast cancer patients who have Estrogen receptor positive (Luminal type) cancer. Also, there are no evidence that fertility preservation was associated with an increased risk of recurrence. And the long-term safety of pregnancy in breast cancer survivors is demonstrated including those with Luminal type cancer.
Study design, size, duration: Data were retrospectively obtained from the clinical records of 424 breast cancer patients who were referred to the Fertility Preservation Unit at the Center for Reproductive Medicine of St. Marianna University Hospital from 2010 to December 2018.
Participants/materials, setting, methods: The breast cancer patients who hope to preserve own fertility were received the oocyte, embryo, or ovarian cryopreservation. We investigated the clinical records of patients and figured out their backgrounds, kinds of FP option, outcome of FP, reproductive outcome using embryo or oocyte or ovarian tissues, and subsequent outcome in terms of cancer recurrence.
Main results and the role of chance: The total numbers of patients who visited for FP was 569, and 424 of them had breast cancer. Result in provision of information, 177 of 424 didn't receive FP treatment (only consultation). Also, 26 of 424 were not applicable cases for FP due to severity of primary disease. To develop safety and efficacy of FP treatment, we have established the closed collaboration with breast surgeons since 2014 (Breast-Obgyn: BROG conference). The members of BROG conference consists of the breast surgeons and the reproductive specialists, and nurse staffs and psychologists. At the BROG conference, we can determine the eligibility for FP treatment, and consecutive time course of FP to cancer treatment with multidisciplinary specialists. After establishment of BROG conference, the FP treatment details were 43 cases of oocyte cryopreservation, and 64 cases of embryo cryopreservation, and 31 cases of ovarian tissue cryopreservation (total 134, 4 were combined treatment). After FP treatment, 11 of 138 finished cancer treatment and restarted fertility treatment. Six of 11 received embryo transfer which used embryo which were preserved before cancer treatment, and 4 of them got pregnant. And one of 11 got pregnant naturally. In addition, 3 patients had a recurrence and 2 of them died.
Limitations, reasons for caution: To determine the efficacy and safety of our fertility preservation treatment, we need to follow up for long period after fertility preservation including perinatal outcome. Also, psychological care for patients who gave up FP is important to achieve high quality FP care.
Wider implications of the findings: Present investigation outcome demonstrated the efficacy and safety of FP for breast cancer patients, based on closed collaboration with multidisciplinary medical professions. Such collaboration may improve the quality of FP.
Trial registration number: This study received Institutional Review Board approval from St. Marianna University of Medicine. (approval No[S1] . 2973, 3464)

P-445 How rapid freezing affects active mitochondria, DNA integrity and seminal parameters of human sperm?

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Study question: What is the impact of rapid freezing with cryoprotectant on mitochondrial activity, DNA damage, as well as on motility, vitality, morphology and sperm concentration, and how the parameters are correlated?
Summary answer: Cryopreservation has been found to lead to a significant decrease of the spermatic quality.
What is known already: Infertility affects approximately 15% of all couples trying to conceive. Mitochondria are an important source of energy for spermatozoa necessary for both sperm motility and fertilization. Thus, plasma and mitochondrial membranes must remain intact to allow cell competence. The DNA damage has also been related to male infertility and reduced fertilization rates. Although several studies report a spermatic quality decrease with cryopreservation, the impact of freezing on sperm human cells is still controversial. Therefore, it is important to evaluate the impact of current freezing protocols on several spermatic characteristics.
Study design, size, duration: This study was performed on 26 anonymous semen samples provided by 26 adult men (22-46 years old), who attended the Fertility Support Centre consultations, at the Hospital Center of Trás-os-Montes and Alto Douro, between January and May 2018. The semen samples were cryopreserved with cryoprotectant by a rapid freezing protocol, and the percentage of sperm with active mitochondria, the DNA damage and the spermatic parameters obtained before and after 1 month of cryopreservation were compared.
Participants/materials, setting, methods: The percentage of sperm with active mitochondria (MitoTrackerTM Red FM dye), the DNA damage (assessed by Alkaline Comet assay and TUNEL assay) and the semen analysis (according to WHO, 2010) were assessed on fresh samples and after cryopreservation.

All samples were cryopreserved on cryovials, with Sperm Freezing Medium, on liquid nitrogen vapors and then plunged into liquid nitrogen.

The statistical analysis was performed (IBM SPSS Statistics 25 software) and p-values $<0,05$ were considered statistically significant.
Main results and the role of chance: The percentage of spermatozoa with active mitochondria decreased by 68% with cryopreservation (from 66% to 21%, p=0,001). The DNA damage increased from 88 arbitrary units (AU) to 172 AU ($p<0,001$) and the percentage of spermatozoa with fragmented DNA was similar before and after freezing (from 6% to 10%, $p>0,05$). The spermatic parameters were significantly worse ($p<0,05$) after cryopreservation. The motility was the most affected parameter, decreasing from 70% to 24%, and in particularly the progressive motility that decreased by 78% (from 58% to 13%). Vitality decreased from 77% to 27%, morphologically normal spermatozoa decreased from 4% to 2% and sperm concentration decreased from $81x10^6$ SPZ/mL to $42x10^6$ SPZ/mL. A strong positive correlation between mitochondrial activity and motility/vitality was also observed ($p<0,01$).

To our knowledge, few studies have compared and evaluated the impact of a current freezing protocol on mitochondrial activity, DNA damage, motility, vitality, morphology and sperm concentration simultaneously.

Limitations, reasons for caution:

- The small number of samples.
- This study was conducted among patients from fertility support consultations, thus it doesn't represent a broadest population.

Wider implications of the findings: The results are consistent with the literature and since cryopreservation is currently used for assisted reproduction, it´s urgent to enhance the efficiency of freezing systems, testing other protocols with different cooling/thawing rates.
Trial registration number: Not applicable.

P-446 LH in vitro treatment promotes DNA repair of mouse apoptotic follicles damaged by alkylating agents.

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Study question: Does luteinizing hormone (LH) incubation protect mouse follicles against chemotherapy-induced apoptosis by promoting DNA repair during exposure to alkylating agents?
Summary answer: LH promoted the homologous recombination mechanism allowing DNA repair to avoid follicle apoptosis in adult mouse ovaries cultured in vitro with alkylating drugs.
What is known already: High-dose chemotherapy treatment with alkylating agents in female patients may impose deleterious effects on the ovary. The follicular pool can be destroyed by the induction of DNA damage leading follicles to atresia, if cells are not able to repair it. In oocytes, the homologous recombination via ataxia telangiectasia-mutated (ATM) pathway is a main mechanism for DNA damage response.

Previous studies suggested that LH incubation preserves the ovarian reserve against follicular depletion induced by chemotherapy in mouse models. We aim to elucidate if LH in vitro treatment protects follicles or promotes DNA repair mechanisms after ovarian exposure to alkylating agents.
Study design, size, duration: Experimental in vitro study with 32 ovarian fragments obtained from eight 10 week-old CD1 mice. Ovarian fragments were randomly allocated to four experimental conditions (n=8/group): Control, Chemotherapy (ChT), ChT+LH-1IU and ChT+LH-5IU. Samples were conditioned with basal media for 24 hours before treatment. Tissue was pre-incubated with LH and one hour after, the ChT-treated groups were supplemented with alkylating drugs. Samples were recovered at 12 and 24 hours after treatments.
Participants/materials, setting, methods: Ovarian fragments were in vitro cultured with α-MEM basal media (37°C-5%CO_2) After 24 hours medium was renewed, and treatments added. LH supplementation was performed 1h before treatment by adding 1 or 5IU of LH. Then, controls and ChT treated groups were supplemented with Vehicle or 1.2µM of busulfan +12µM of 4-Hidroperoxiclyclophosphamide, respectively. Fragments were collected at 12 and 24h and analysed for follicle counts, TUNEL, apoptosis and DNA repair markers by western blot.
Main results and the role of chance: LH incubation, especially at 5IU, reduced the number of TUNEL-positive-oocyte follicles (C: 74.2±17.2%; ChT: 90.4±6.7%; ChT+LH-1IU: 86.7±23.1%; ChT+LH-5IU: 63.1±16.5%, p=0.03) and the percentage of follicles with >20% of TUNEL-positive granulosa cells (C:68.1±40.8%; ChT:96.9±50.3%; ChT+LH-1IU:68.6±30.8%; ChT+LH-5IU:37.6±21.1%, p=0.04) already damaged after 24h of exposure to alkylating drugs. These decreases appeared during the first 12h of incubation although not statistically significant. Furthermore, both LH dosages reduced the percentage of apoptotic cells by follicle at all examined timepoints (12h exposure; C:56.3±6.3%; ChT:71.1±33.5%; ChT+LH-1IU:26.8±22.2%; ChT+LH-5IU:45.6±15.9% and 24h exposure C: 25.8±21.1%; ChT: 70.0±11.6%; ChT+LH-1IU: 61.9±29.2%; ChT+LH-5IU: 27.7±17.0%). The expression levels of the antiapoptotic protein Bcl2 were also quantified in all samples. LH incubation increased the levels of Bcl2 at 12h (C:0.2; ChT: 0.5; ChT+LH-1IU: 0.8; ChT+LH-5IU: 2.1) and 24h (C:1.1; ChT: 0.6; ChT+LH-1IU: 1.3; ChT+LH-5IU: 0.9).

In order to evaluate if the LH protective effect was mediated by DNA damage repair systems, ATM pathway proteins were examined. We found that both LH treatments increased levels of ATM (C:0.3; ChT: 1.1; ChT+LH-1IU: 1.9; ChT+LH-5IU: 2.1), BRCA1 (C:1.0; ChT: 1.3; ChT+LH-1IU: 2.2; ChT+LH-5IU: 1.5) and Rad51 (C:0.7; ChT: 1.3; ChT+LH-1IU: 2.0; ChT+LH-5IU: 1.3) from the first 12h of incubation.
Limitations, reasons for caution: This is an in vitro study performed with mouse ovaries that should be confirmed in a preclinical study with a larger population of human samples prior to be proposed as a suitable clinical alternative to preserve fertility in cancer women.
Wider implications of the findings: LH administration promotes follicle recovery by inducing DNA repair via the expression of proteins involved in the ATM pathway. To elucidate mechanisms and promote repairing might be crucial,

as otherwise damaged follicles underwent atresia leading to follicular depletion and to ovarian failure.
Trial registration number: Not applicable

P-447 Fertility preservation for prepubertal boys: evaluation of controlled slow freezing of testicular prepubertal tissues

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Study question: Does Controlled Slow Freezing (CSF) of prepubertal testicular tissues enable the survival of Spermatogonial Stem Cells (SSCs) and maintain the integrity of the testicular niche?
Summary answer: CSF of prepubertal testicular tissues allows the survival of both SSCs and Sertoli cells and maintains the integrity of the testicular niche.
What is known already: Late onset effects of pediatric cancer treatments may include infertility. Fertility preservation options for prepubertal boys are very limited. Testicular tissues containing SSCs and testicular niche cells can be cryopreserved for potential future use. Cryopreservation using CSF may be an acceptable option for cryopreserving prepubertal testicular tissue.
Study design, size, duration: After approval by our institution review board, testicular tissue cryopreservation is offered as an experimental procedure to young prepubertal boys prior to gonadotoxic treatment. Informed consent is obtained from their parents. Following testicular biopsies, testicular tissues are cryopreserved by CSF. Some of the tissues are thawed and analyzed by H&E staining and immunostaining for MAGE-A4 - a marker of SSCs, and vimentin –a mesenchymal marker expressed by Sertoli cells.
Participants/materials, setting, methods: Five cancer-affected prepubertal boys (aged 6 to 14) before and after gonadotoxic treatment underwent the procedure. Following testicular biopsies, one testicular fragment was immediately fixed in 4% PFA and served as a control. The remaining fragments were cryopreserved by CSF. After thawing, fixing and embedding in paraffin, sections from various parts of the testis were immunostained for MAGE-A4 and vimentin. Testicular tubules were counted and evaluated for the presence of these markers.
Main results and the role of chance: Histopathological analysis of the tissues by H&E staining did not reveal any significant differences between control and thawed post-CSF testicular sections. Specifically, there were no significant differences in the number of tubules per section, tissue architecture, and structural integrity between the samples. Immunostaining for the vimentin, showed similar positive staining of the Sertoli cells in all tubules. No significant difference was observed in MAGE-A4 stained SSCs, between control and cryopreserved samples. The immunostaining demonstrated a clear difference between the testicular tissues of children who had been previously treated with chemotherapy: the overall number of SSCs in the sample was significantly lower. Interestingly, prior chemotherapy treatment did not affect the survival of the Sertoli cells. These results demonstrated that CSF of testicular tissue pieces from prepubertal boys preserved both the SSCs and the testicular niche. Moreover, testicular tissues exhibited a profound damage to the SSC caused by chemotherapy in the prepubertal boys, re-emphasizing the importance of fertility preservation prior to gonadotoxic treatment.
Limitations, reasons for caution: Due to low numbers of suitable donors, this study thus far includes few samples. As the reported study is ongoing, more samples will be collected and analyzed. More markers for the different components of the testicular tissue are needed. Differentiation between different chemotherapeutic regimen and doses should also be examined.
Wider implications of the findings: This study demonstrates that CSF presents a suitable option for cryopreserving prepubertal testicular tissues. It highlights the significance of cryopreservation of the testicular tissues prior to gonadotoxic treatments. CSF is a feasible and affordable cryopreservation method. It may, therefore, advance the possibility of future fertility restoration for cancer-affected prepubertal boys.
Trial registration number: not applicable

P-448 Glycogen synthase kinase 3β inhibitor (iGSK3β) decreases cisplatin-induced oocyte death in vitro inhibiting TAp63 activation

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Study question: Are pharmacological kinase inhibitors able to protect the ovary against cisplatin-induced damage and by which pathways?
Summary answer: GSK3b inhibitors significantly protect oocytes from cell death induced by cisplatin *in vitro* through TAp63a inhibition
What is known already: Advances in cancer therapies have increased survival rates, but there is a risk of premature ovarian insufficiency (POI) for female patients. Several recent studies have demonstrated a key role of kinases in oocyte death induced by cisplatin (Cs). Furthermore, Cs resistance in some cell types is attributable to reduced activity of certain kinases. In the ovary, it is known that inhibition of kinases CK1 and CHK2 protects primary oocytes from both Cs and g-irradiation, by blocking the tetramerization/activation of TAp63a, a pivot protein involved in programmed cell death of oocytes
Study design, size, duration: A GlaxoSmithKline library containing 341 new inhibitors against 14 different kinase pathways was tested for their protective action in vitro against Cs-induced damage on ovarian fragments obtained from postnatal day 4 (P4) mice (GFP/c-Kit and CD1 strains). Analyses were performed after T0h and T24h compound exposure. Whole ovaries from P8 CD1 mice were also treated *in vitro*, with analysis time points: T0h, T16h, T24h, T5days. iGSK3bwas tested against g-irradiation on P4 GFP/c-Kit ovarian fragments.
Participants/materials, setting, methods: Ovarian fragments were pretreated (T0) increasing concentration of inhibitors (0,003mM-3mM), before Cs(10mM) or g-irradiation(0.2Gy)treatment, and cultured for 24h. For each condition, GFP/c-kit positive and trypan blue negative healthy follicles were scored at T0 and T24h.

P8 whole ovaries cultured with Cs+iGSK3bwere analysed at different time points for apoptotic and DNA damage markers (cleaved-caspase3/Bax/g-H2AX) by SDS-page and immunofluorescence analysis. Native-page and in vitro assay were usedto verify the inhibition of TAp63aactivation.
Main results and the role of chance: At least two inhibitors from 14 different pathways were tested. Inhibitors of GSK3b were able to preserve follicles against Cs-induced damage in both type of analysis (survival rate 55% vs 25%; $P<0.05$). We used a commercial inhibitor of GSK3b (CHIR99021) to study the molecular pathways involved in this protection. WB analysis on whole P8 ovaries treated with inhibitor and Cs showed that iGSK3bdecreased protein expression levels of BAX and gamma-H2AX. Histological and immunofluorescent analysis of ovaries treated with iGSK3b and Cs vs only Cs showed a higher percentage of primordial follicles [AR1] (24h, Cs: 42.6±4.5 vs Cs+iGSK3b: 73.3±5.7; 5days, Cs: 15.6±4.1 vs Cs+iGSK3b: 66.5±7.3; $P<0.05$) and a decrease in cleaved-caspase3 (24h, Ctrl: 0.7[AR2] ; Cs+iGSK3b: 1.0; Cs: 2.6) In addition, we demonstrated by SDS- and native page, that iGSK3b inhibited the phosphorylation and consequent tetramerization/activation of TAp63a induced by Cs. I*n vitro* analysis showed that GSK3b was not able to phosphorylate TAp63a directly.

Survival analysis on ovarian fragments also showed that iGSK3b protected oocytes from g-irradiation (0.2Gy = 17±7; iGSK3b+0.2 Gy =49.5±13; $P<0.05$).

Limitations, reasons for caution: This study has been performed using short term in vitro assays in mice, thus the results need to be confirmed in vivo and at least in vitro in human and against other chemotherapy drugs. The molecular mechanisms underlying GSK3 protection against Cs is not known.

Wider implications of the findings: These results confirm the value of this culture system for rapid and simple drug screening effects on ovarian follicles. The use of GSK pathway inhibitors may provide a novel approach to protect female fertility against chemotherapy.

Trial registration number: Not applicable

P-449 Effect of sphingosine-1-phosphate on activation of dormant follicles in ovarian tissue from mouse and human

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Study question: Can sphingosine-1-phosphate enhance the number of activated follicles in ovarian tissue?

Summary answer: Sphingosine-1-phosphate upregulates the expression of genes downstream of the Hippo pathway, increases the number of activated follicles and promotes the survival of follicles

What is known already: The development from primordial follicle to a mature oocyte is a complicated and not fully understood process. Multiple signalling pathways are involved in maintaining dormancy of follicles. Activation of non-growing follicles in vitro is a novel approach to treat infertile women that cannot generate sufficient number of mature oocytes. The compound sphingosine-1-phosphate has been shown to promote follicle growth, in addition to having an anti-apoptotic effect on follicles. The aim of the study was to evaluate sphingosine-1-phosphate as a growth promoting agent in both murine and human ovarian tissue.

Study design, size, duration: The material consisted of donated human ovarian tissue and isolated preantral follicles from women undergoing ovarian tissue cryopreservation for fertility preservation. In addition ovaries and follicles isolated from juvenile mice (2-10 days old) were used. Culture of both human and murine ovarian tissue were done with or without a following xenotransplantation into immunodeficient mice. Isolated murine and human follicles were cultured.

Participants/materials, setting, methods: Human ovarian cortex (six patients) were cultured with sphingosine-1-phosphate for 24 hours, prior to xenotransplantation for six weeks. Follicle growth was evaluated by histology with counting and classification. Ovaries from juvenile mice were cultured with sphingosine-1-phosphate for 1-4 days. The ovaries where then used for analysis of gene expression, histology or xenotransplantation. Isolated human and murine follicles were cultured with sphingosine-1-phosphate and then evaluated for expression of genes in the Hippo signalling pathway.

Main results and the role of chance: A significantly higher expression of the downstream effector gene in the Hippo pathway, CCN2, was found in both murine and human follicles. Thereby, showing that sphingosine-1-phosphate induces a disruption of the Hippo signaling pathway and potential growth activation. For both sphingosine-1-phosphate treated mouse ovaries and grafted human ovarian cortex pieces, the treatment with sphingosine-1-phosphate increased transition of primordial follicles to the primary stage, but it did not seem to induce further growth compared to control. The results suggest that there is an increased shift from the primordial stage to the primary stage after sphingosine-1-phosphate treatment in both mouse ovaries and human ovarian cortex, but further growth was not evident. Interestingly, the human ovarian cortex study indicates an increase in follicle survival after sphingosine-1-phosphate treatment, compared to control.

Limitations, reasons for caution: Further studies are needed to describe the impact of sphingosine-1-phosphate on the further follicle growth and maturation. Additionally, the impact on the specific signalling pathways should be described in further details.

Wider implications of the findings: As the in vitro activation method needs to be optimized for increased success rate, new compounds that promote follicle growth, in addition to exerting further positive effects on follicles, are needed. The results indicate that sphingosine-1-phosphate may promote follicle growth and increase the survival rate of ovarian follicles.

Trial registration number: Not applicable

P-450 Why the differences in the oocyte survival rate in a donor vitrified program?

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Study question: What´s the cause of success in a vitrified donor program? The know-how of the embryologists, the number of oocytes retrieved or the donor herself?

Summary answer: The main factor influencing the survival rates in a thawing cycle is the donor's characteristics; not the embriologyst or the number of mature oocytes.

What is known already: Some studies have found differences on the survival rate depending on the vitrification experience of the operary. Furthermore, differences have also been found regarding the origin of the oocytes. Survival rates in donor oocytes were better than in non-donor oocytes.

To our knowledge, there are no studies which assess if, within the pool of donors which normally have good ovarian response, there is a subgroup which systematically have lower survival rates after oocyte warming.

Study design, size, duration: Retrospective study over donors who underwent at least five vitrification cycles and their corresponding thawing. Data compiled from 2012 to 2018 involving 39 cycles from seven donors. Survival rate was analyzed as dependent variable. The embryologist who vitrified and thawed the oocytes and, considering as a different dichotomic variable, if he performed both were independent variables. Personal experience was considered as the number of thawing cycles performed together with the oocytes thawed per cycle.

Participants/materials, setting, methods: All oocytes were vitrified and thawed using Kitazato media following their protocols strictly. Eight different embryologists with varied laboratory and thawing experience were analyzed. Each thawing cycle had a mean of 6,92 mature oocytes (95%IC:5,76-8,12) SPSS V20.0 was used for the statistical analysis ANOVA, the average comparison by t-student analysis as well as the Pearson correlation coefficient. Whilst, MANOVA was calculated using STATA v15.1

Main results and the role of chance: The survival rate per cycle was between 12,5 and 100%, with a mean of 81,56% (95%IC:73,71-89,41%) and median of 100%.

On ANOVA no statistical differences were observed concerning the embryologists who vitrified (p:0,452) or thawed the oocytes (p:0,439), neither differences were found on the means by T-Student testing to analyze if there is an effect if the embryologist was the same or not on both processes (p:0.947). Pearson testing didn't show any significant correlation between survival rates and the number of mature oocytes (p:0,568) nor with the embryologist's experience (p:0,408).

The use of one-way ANOVA to compare survival rates between the different donors revealed significant differences, being the survival rate means on their 5-6 cycles from 62,65 to 100% (F:4,46 p:0,002).

By a Multiple-way ANOVA we determined that the correlation is independent from previous confounders. MANOVA model with inclusion of these six variables was significant (p:0,0193) and therefore the donor is the only factor which showed influence on survival rates (p:0,0006) regardless of other five confounding factors.

Limitations, reasons for caution: The main limitation of our study is the small number of participants. The results observed in this study should be further confirmed with a larger sample number.

Wider implications of the findings: Standardization of vitrification-thawing protocols reduces the impact of the experience from each embryologist on the survival rates and variability of the results. The intrinsic characteristics of each donor may have a major effect over the survival rate. However, the causes remain unknown.

Trial registration number: Not applicable

P-451 A survey of French Gynecologist knowledge and attitudes toward fertility preservation (FP) in young patients with endometrial cancer (EC) or endometrial atypical hyperplasia (AH)

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Study question: What are current knowledge and attitudes toward fertility preservation in patients with AH or EC among gynaecologists in France?

Summary answer: Score of knowledge of FP in EC/AH was low; most physicians were not comfortable with conservative treatment for FP in EC/AH.

What is known already: The safety of FP in AH or grade 1 EC has been reported in various studies. Although, no clear guidelines have been established. Patients are often diagnosed with endometrial malignant or premalignant disease during infertility explorations. Remission is obtained using antigonadotropic treatment in approximately 80% of the cases, more frequently in AH than in EC. However, the extent to which this conservative management of AH or EC for FP is being implemented in practice is unclear. Despite a legal obligation and technical progress, there is a lack of information about FP given to French patients treated for cancer.

Study design, size, duration: Based on previous studies, one specific questionnaire was developed with one common and two specific parts: one for Gynecological Surgeon (GS) and one for specialists in Reproductive Medicine (SRM). Questionnaires were sent via email or given directly during congresses from April 2017 to April 2018. GS were members of the French Society of Gynecologic and Pelvic Surgery (N=434) and SRM belonged to Reproductive Medicine Society or French Study group on Fertility (N ~ 500).

Participants/materials, setting, methods: 140 physicians answered the survey, 87 GS and 53 SRM. Demographic, medical training and practice information were collected. FP knowledge in EC/AH was evaluated with a "score of knowledge" coding results of a four-point Likert scale. To evaluate attitudes toward FP, a five-point Likert scale was used coding a "score of attitudes". GS practice behaviors evaluation was FP information provided to patients. SRM were asked about their management of fertility after FP.

Main results and the role of chance: Knowledge score was low, 59.3 % of participants having a medium/low score. It was significantly higher for GS than SRM and for physicians working in a centre accredited for FP, with a significant EC/AH activity or in university hospitals. The two better known treatments were oral progestins and hysteroscopic resection. Among the 97 participants confronting EC/AH, 52.6% found "difficult" to take care of these patients and 61.8% regretted the lack of official recommendations.

Most physicians felt the upper age limit for FP was 42 or under; the SRM being stricter: 71.6% said '38' or '40' versus 52.9% for GS.

Most physicians seemed not very supportive and/or comfortable with FP in EC/AH, 57.2% having an 'attitude toward FP score' below 11/20. Physicians with a shorter time of practice (<10y), working in a centre performing FP or in a university hospital were more supportive. A significant positive correlation was found between knowledge and attitude scores.

GS "usually" or "always" give advice to patients about FP before EC/AH treatment. 56.6% of SRM wait for spontaneous pregnancy, maximum 3/6 months. If a fertility treatment is planned, 56.6% chose IVF to reduce time to pregnancy. 15.1% chose a mild stimulation to avoid hyperestrogenism.

Limitations, reasons for caution: Small sample size with only 20 % and 10% of response respectively in both populations (GS and SRM), may be inducing a bias. In addition, we asked only members of academic societies maybe more aware of FP options and management in EC/AH.

Wider implications of the findings: Despite reassuring results in the literature, French gynecologists are not very comfortable with FP by conservative management of EC/AH, probably due to a lack of confident knowledge. Specific guidelines are needed to help physicians to manage these young patients with EC/AH and their fertility.

Trial registration number: NA

P-452 Freezing for future fertility: does the Australian public support oocyte cryopreservation?

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Study question: What are the attitudes of the public towards medical and planned oocyte cryopreservation (OC)?

Summary answer: There is widespread support for medical OC but less support for the uptake and funding of OC to mitigate age-related infertility.

What is known already: Improvements in OC technology and outcomes have resulted in a significant increase in the uptake of OC; growing by almost 500% in the UK, and by almost 600% in Australia/New Zealand over the last 7 years. However, technical progress has outpaced policy development and ethical analysis, and there is a lack of clear or consistent policy guiding access to and funding for OC. Surveys on public attitudes from Europe and the US suggest using OC to manage the risk of age-related infertility (planned OC) is viewed more negatively than medical OC, however little is known about public attitudes in Australia.

Study design, size, duration: An online, cross-sectional survey was conducted in Australia between April 2018 and May 2018, open to members of the general public. Participants were asked to respond to multiple choice, 5-point Likert scale, and open-ended questions. The aim of the survey was to investigate the public's view about access to and funding for OC and whether policies should distinguish between medical and planned OC.

Participants/materials, setting, methods: This was a nationwide study that invited men and women aged between 18 and 60 years to give their views on both medical and planned OC. A total of 1,127 individuals initiated the survey: 89% female, 10% male and 1% identified as transgender or other. The data were analysed with descriptive statistics and multiple regression analyses.

Main results and the role of chance: This study surveyed the general public through a number of online fora and therefore an accurate response rate cannot be given. The median age of the participants was 29 years, 82% were <40 years old and 18% were ≥ 40 years old. There was almost unanimous support (agree/strongly agree) for the use of medical OC when treatments threaten fertility (*e.g.* chemotherapy) or for when medical illnesses threaten fertility (97.5% and 97.3%, respectively). Among the reasons for accessing planned OC, lack of suitable partner was the most supported (76%), followed by financial inability to raise a child (72.9%) and delaying childbearing for career/educational advancement (65.6%). Respondents ≥40 years was the only demographic group less supportive of planned OC for all reasons; *no partner* (OR: 0.41, 95% CI: 0.25 – 0.70, p<0.005), *financial* (OR: 0.34, 95% CI: 0.22 – 0.52, p<0.005) and *career* (OR: 0.31, 95% CI: 0.21 – 0.47, p<0.005). Although 82% of participants identified the costs of OC as a concern, <50% of participants agreed that medical OC should be publicly funded, and only 7% of participants supported public funding for planned OC, with higher support for self-funded (32%) or coverage through private health insurances (24.3%).

Limitations, reasons for caution: Given that people interested in, or experienced with, infertility are more likely to respond positively to new ARTs it is possible that results contain a selection bias in favour of OC. Furthermore, access to the survey was limited to the online format, therefore the findings may not apply more broadly.

Wider implications of the findings: This study contributes Australian findings to the limited data on public attitudes to OC and informs a critical review of the current fertility preservation policies in Australia. Our findings reiterate the perceived distinction between medical and planned OC, which raises difficult questions for policy and equity of access.

Trial registration number: not applicable

P-453 The role of Akt and mTOR pathways in follicle activation after grafting of human ovarian tissue

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Study question: What is the role of the Akt/FOXO1 and mTOR signaling pathways in follicle activation after ovarian tissue transplantation and burnout of the ovarian reserve?

Summary answer: Increased Akt phosphorylation and FOXO1 nuclear extrusion were observed in primordial follicles 3 days post-grafting. No change in mTOR was detected in primordial follicles post-transplantation.

What is known already: Ovarian tissue transplantation leads to massive loss of primordial follicles, together with an increase in the growing follicle population. This suggests that a double mechanisms is responsible for this event: follicle death and follicle activation. Follicle activation is irreversible and, once activated, follicles that are not selected to develop further will undergo atresia. Consequently, early exhaustion of the follicle pool and a shortened lifespan of ovarian grafts will occur. The Akt/FOXO1 and mTOR pathways have been extensively studied in animals and their role in follicle activation widely elucidated, nevertheless, no conclusive proof exists in the activation of human primordial follicles.

Study design, size, duration: Frozen-thawed ovarian tissue from six different patients was divided into 4 fragments, each measuring approximately 5×8×1.5 mm3: one to be used as non-grafted control and three to be grafted to SCID mice. Three grafting durations were evaluated: 3, 7 and 21 days.

Participants/materials, setting, methods: The ovarian tissue was collected from patients who underwent surgery for non-ovarian pathologies (age 25-35 years). The ovarian tissue and grafts after recovery were processed for hematoxylin and eosin (H&E) staining and immunohistochemistry. Follicle classification, growth by Ki67 analysis and and atresia by H&E and TUNEL analysis were analyzed, as well as, Akt and mTOR phosphorylation, and FOXO1 cytoplasmic localization.

Main results and the role of chance: At H&E staining, a significant decrease in primordial follicles and significant increase in growing follicles were detected after transplantation and more than 50% of follicles were atretic after 3 days, with a further 50% after 7 days of grafting. These results were confirmed by Ki-67 and TUNEL analysis, respectively. Moreover, extensive stromal cell death was also shown by TUNEL analysis. Akt phosphorylation significantly increased in primordial follicles after 3 days of grafting. No change in mTOR was observed in primordial follicles post-transplantation, but a significant upturn was recorded in growing follicles compared to primordial follicles, regardless of grafting time. We also found a higher percentage of primordial follicles with FOXO1 in the cytoplasm after 3 days of transplantation than in non-grafted controls.

Limitations, reasons for caution: Our study addresses Akt and mTOR phosphorylation and FOXO1 cellular localization, but animal studies also report the existence of other pathways involved in follicle activation.

Wider implications of the findings: We confirmed that primordial follicle activation is an early event post-grafting, and significant follicle death also contributes to burnout, eventually resulting in early depletion of the ovarian reserve. We evaluated for the first time that the Akt/FOXO1 and mTOR pathways in human ovarian tissue and documented their role in burnout.

Trial registration number: Not applicable

P-454 The evaluation of the role of patient navigators in female oncofertility care in a tertiary medical centre

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Study question: What are patients' and healthcare professionals' experiences with patient navigators in female oncofertility care and their recommendations for improvement?

Summary answer: Patients and healthcare professionals were satisfied about the supportive role of patient navigators. They added value as they became 'familiar faces' and provided personal care.

What is known already: Cancer treatment can cause infertility in young female cancer patients. Therefore, patients have to make a decision whether they want to undergo treatment to preserve their fertility in the stressful period of being diagnosed with cancer. Patients indicate that it is important to receive personal care, emotional support and information about the available options. However, many of these items remain unaddressed in current healthcare practice. To meet these needs and to provide additional support, two fertility nurses were assigned as patient navigators at the Radboud university medical centre fertility department.

Study design, size, duration: To explore patients' and healthcare professionals' experiences and recommendations for improvement, semi-structured in-depth interviews were conducted within nine patients and six professionals between July and September 2016.

Participants/materials, setting, methods: Participants were recruited from a tertiary academic centre, Radboudumc, which serves as a referral clinic for female fertility preservation care. The structure of the in-depth interviews was based on two separate topic lists. Interviews were independently coded and analysed by two authors using a method based on the concepts of grounded theory.

Main results and the role of chance: Patients were satisfied about the supportive role of the patient navigator in their fertility preservation process. They added value as they became 'familiar faces' who were easy to approach, aware of the process, and provided personal care and information in the process. Patient navigators reduced professionals' workload by taking over tasks and by being flexible about seeing patients outside regular consultation hours. To improve their role, both groups suggested that the patient navigator should always be present in fertility preservation counselling. Furthermore, attention should be paid to their availability to improve continuity of care.

Limitations, reasons for caution: Bias could have occurred, because most interviewed patients were diagnosed with breast cancer. Patients with other cancer types may have different experiences with patient navigators. However, the representation of breast cancer patients can be explained by the relatively high incidence of breast cancer in women of reproductive age.

Wider implications of the findings: Recommendations for improvement can be used to improve patient navigators' role in the future to ultimately improve female oncofertility care. Furthermore, the presented overview could be assessed by other centres when considering implementing patient navigators.

Trial registration number: Not applicable

P-455 Adipose tissue-derived stem cells boost vascularization in grafted human ovarian tissue by differentiation into endothelial cell lineages, yielding improved follicle survival rates

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Study question: What are the mechanisms by which adipose tissue-derived stem cells (ASCs) enhance vascularization and follicle survival in xenografted human ovarian tissue using a two-step transplantation approach?

Summary answer: Increased oxygenation, vascularization, proangiogenic growth factor secretion and follicle survival rates were observed in grafted human ovarian tissue supplemented with ASCs.

What is known already: Ovarian tissue cryopreservation and transplantation has gained ground as a valid fertility preservation and restoration approach thanks to its established effectiveness. However, there are still issues arising from the avascular nature of the grafting procedure, as up to 80% of follicles may be lost due to hypoxia. Shortening the hypoxic/ischemic post-grafting period is therefore paramount. Our team has recently developed a new transplantation technique using ASCs to improve follicle survival rates. ASCs have multilineage differentiation potential and proangiogenic properties, and have been shown to enhance vascularization in xenografted human ovarian tissue.

Study design, size, duration: Ten severe combined immunodeficient (SCID) mice were grafted with frozen-thawed human ovarian tissue from 5 patients. ASCs were labeled by lentiviral transfection for expression of enhanced green fluorescent protein (eGFP) to enable cell tracking. They were then transferred to peritoneum inside a fibrin implant 14 days prior to ovarian tissue grafting (n=5 mice). In the control group (n=5 mice), ovarian tissue was transplanted using the standard one-step approach. Ovarian grafts were collected on day 7.

Participants/materials, setting, methods: Prospective experimental study conducted in an academic gynecology research laboratory. Human ovarian tissue xenografts were analyzed by histology, immunohistochemistry,

immunofluorescence and real-time reverse transcription polymerase chain reaction (RT-qPCR), to assess proangiogenic factor secretion by ASCs (anti-human vascular endothelial growth factor [VEGF]). Immunofluorescence was also applied to investigate ASC differentiation into endothelial cell lineages and graft vascularization (eGFP, CD34 and CD31 for labeled ASCs and human endothelial cell lineages respectively).

Main results and the role of chance: The eGFP antigen revealed ASCs surrounding and infiltrating ovarian tissue grafts. Significantly higher vessel density was observed in the ASC group (p=0.0182) on day 7. Among human vessels, 12.15 ± 3.16% exhibited co-expression of both eGFP and CD34 antigens, confirming ASC differentiation into human endothelial cell lineages. RT-qPCR showed significantly higher expression of VEGF in the ASC group (p=0.0182), as did immunohistochemistry targeting anti-human VEGF (p=0.033). VEGF and eGFP immunofluorescence also displayed higher growth factor expression in areas surrounding ASCs, demonstrating co-expression.

Limitations, reasons for caution: Long-term studies should be carried out to investigate ASC safety and efficacy, with a view to future clinical application in patients undergoing ovarian tissue transplantation.

Wider implications of the findings: This is a promising step towards potentially improving ovarian tissue quality and lifespan, and extending ASC use in regenerative medicine. The present study provides further insights into the mechanism of action underlying the beneficial effects of ASCs in grafted ovarian tissue.

Trial registration number: no

P-456 The chronology of sperm sample storage and use in patients attending a national fertility preservation centre

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Study question: Are there patterns of storage and use of sperm samples from patients about to undergo oncology treatment?

Summary answer: 6% of patients died before using their samples and 2% returned for treatment which gave a 14% pregnancy rate and a 7% live birth rate.

What is known already: It is thought that there is a low return rate on stored sperm. The number of oncology patients that are referred and attend for sperm storage is much higher than the number that return for fertility treatment. Currently there are 585 oncology patients that have sperm samples stored at this clinic.

Study design, size, duration: Retrospective study looking back at 5 years of data.

Participants/materials, setting, methods: All oncology storage patients were included in this study. Methods included reviewing sperm storage and fertility treatment data.

Main results and the role of chance: On average this clinic stores for 47 oncology patients a year. Currently there are 585 oncology patients that have sperm samples in storage. Since 2014, 14 patients have returned to use their stored samples in fertility treatment meaning only approximately 2% of oncology patients that have stored prior to their treatment have returned to use their samples. Two patients also returned for a second cycle using stored samples. The median age of patients at storage date is 31 and at thaw date is 40. The interval between freeze date and thaw rages between 1 and 23 years. Of the 14 patients that received treatment there were two positive hCG tests however only 1 resulted in a live birth. It was also noticed that 13 patients have exported samples to other clinics, so it would be assumed that their intentions were to be treated elsewhere.

Limitations, reasons for caution: Data collected for treatment was from the last 5 years however the life of the storage data is much longer. Includes newly stored patients potentially still undergoing treatment and some are still too young to be considering starting a family. Outcome data for exported samples was not included.

Wider implications of the findings: Improved management of oncology patients that have stored within the service is required. This may assist with managing patient's expectations when they store as it suggests that there is a possibility that a large number of patients are still able to conceive naturally after their treatment.

Trial registration number: not applicable

P-457 The developmental potential of the in vitro matured oocytes recovered from excised ovaries in oncology patients: a pilot study

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Study question: Do the oocytes recovered ex vivo and matured in vitro have the potential for fertilization and further successful development?

Summary answer: Ex vivo retrieved oocytes from oncology patients have lower potential for fertilization and development, however, in rare cases good quality blastocysts can be obtained.

What is known already: Immature oocytes could be identified during most cases of ovary preparation for cryopreservation and can serve as an additional option for fertility preservation. However, only several cases of fertilization and embryo development from such oocytes have been reported, and most of them are presented as case reports. The published in vitro maturation (IVM) rates of ex vivo retrieved oocytes range from 3 to 100. Only three pregnancies after transfer of frozen-thawed day 2-3 embryos obtained from such oocytes were reported. Two of them resulted in life births.

Study design, size, duration: This study included 14 cancer patients aged 16-42 who underwent oophorectomy in the tertiary referral hospital from July 2018 to January 2019 for fertility preservation. In 12 patients ovarian tissue was frozen along with IVM, and in 2 patients due to nosology only IVM was performed. The main outcome measures were: maturation rate, fertilization rate, cleavage rate and blastocyst formation rate.

Participants/materials, setting, methods: The cumulus oocyte complexes (COCs) were retrieved from visible follicles by aspiration and from the remained fluid after ovarian tissue dissection. COCs were cultured in the IVM medium (Sage, CooperSurgical) supplemented with 0,75 IU/ml HP-hMG (Menopur, Ferring) for 48-72 hours. All matured oocytes were vitrified or fertilized with the husband's or donor's sperm. All normally fertilized zygotes were cultured until day 7.

Main results and the role of chance: In total 212 COCs from 13 patients were retrieved. The mean number of oocytes obtained per patient was 15 (from 1 to 77 COCs per patient). Oocytes were not retrieved from one patient. 60 oocytes (28,3%) matured in 48 hours, 28 oocytes (13,2%) - in 72 hours. The total maturation rate was 41,5% (88/212), and 31,6% of oocytes degenerated (67/212). After denudation 3 oocytes had 1 or 2 visible pronuclei, and 6 were found on the cleavage stage. Remaining 48 oocytes (22,6%) arrested at the germinal vesicle or MI stage. 32 mature oocytes were vitrified according to the patients' will. 55 mature oocytes were fertilized by ICSI. 18 hours after fertilization 11 zygotes (20%) had 2PN, 11 (20%) had 1 or 3PN, the remaining oocytes had no signs of fertilization or cleaved early. The cleavage rate was 100% (11/11), and the blastocyst formation rate was 27% (3/11). All 3 blastocysts were obtained from 1 patient and had 5BB, 5BB, and 3CC score accordingly to Gardner criteria).

Limitations, reasons for caution: Larger study is needed in order to determine whether the oocytes potential is compromised by the patient's nosology, the method of oocyte retrieval or the maturation protocol. None of the vitrified blastocysts have been thawed and their viability remains unknown.

Wider implications of the findings: This pilot study showed that immature oocytes can be harvested from excised ovaries and matured in vitro. However, such oocytes have low potential for fertilization and blastocyst formation. New modified protocol of IVM should be developed and strict criteria for patients who can benefit from such procedure should be set.

Trial registration number: n/a

P-458 Interactions between PI3K/Akt and Hippo signaling pathways during in vitro spontaneous and chemotherapy-induced follicular activation in mouse ovaries

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Study question: Do PI3K/Akt/mTOR and Hippo signaling pathways interact during spontaneous and chemotherapy-induced follicular activation in vitro?
Summary answer: PI3K/Akt/mTOR and Hippo signaling are involved in follicular activation. The inhibition of mTORC1 also affected the Hippo pathway, suggesting an interaction between the two pathways.
What is known already: The development of a complete in vitro culture system supporting follicular growth is an attractive option for restoring fertility using cryopreserved ovarian tissue in cancer patients. However, one of the remaining challenges is the massive follicular activation (FA) that occurs in vitro. FA is also part of the mechanism involved in chemotherapy-induced follicular depletion. Previous studies showed that PI3K/Akt/mTOR is a crucial pathway involved in FA. Moreover, disruption of the Hippo pathway after ovarian sectioning also induces FA. Here, we addressed the involvement of both PI3K/Akt/mTOR and Hippo signaling pathways in in vitro spontaneous and induced FA.
Study design, size, duration: Postnatal-day 3 (PND3) mouse ovaries were used as a model because they contain mainly primordial follicles that represent the follicular reserve. Whole ovaries were cultured in control medium (Dspontaneous activation) or exposed to 4-hydroperoxycyclophosphamide (4HC, 3µM or 20µM for 4h or 24h- chemotherapy-induced FA) with or without mTORC1 inhibitor (Everolimus 100nM- EVE). Ovaries were analyzed after 4 or 48 hours of culture in the different conditions. Each experiment was performed at least three times.
Participants/materials, setting, methods: The involvement of PI3K/Akt/mTOR and Hippo pathways during spontaneous and chemotherapy-induced FA was assessed after 0, 4 and 48 hours of culture by qPCR (gene expression) and Western Blot (protein analyses). Histological analyses were performed after 48h of culture. FA and apoptosis were evaluated by follicular counting and TUNEL staining.
Main results and the role of chance: Although PI3K/Akt/mTOR pathway gene expression was not modified during in vitro spontaneous activation, the protein levels of P-Akt were decreased compared to fresh ovaries as the medium did not contain any growth factors. In contrast, the expression levels of CCN2 and c-myc, genes regulated by the Hippo pathway, were increased after 4 hours of culture, despite the absence of ovarian fragmentation before culture. The proportion of growing follicles increased after 48h of culture in all conditions, irrespective of chemotherapy exposure. Nevertheless, a dose-dependent increase in apoptosis and morphological abnormalities was observed after 24 hours of 4HC exposure. Chemotherapy exposure induced an increase in PI3K pathway phosphorylated protein levels, such as P-Akt and P-rps6, at 4 and 48 hours of culture. The Hippo pathway was also affected by chemotherapy at both concentrations, 3µM and 20µM, with an increase in CNN2 expression after 48h of culture. As expected, the inhibition of mTORC1 by EVE significantly decreased P-rps6 levels but not P-Akt levels. Surprisingly, EVE also prevented the increase in CCN2 expression after 4HC exposure at 48 hours of culture. Therefore, EVE was able to reduce both spontaneous and chemotherapy-induced FA.
Limitations, reasons for caution: This study was limited to the evaluation of the two major signaling pathways. The PND3 model was chosen to evaluate a homogeneous population of early stage follicles but did not take into account the follicular interactions between growing and resting follicles occurring in adult ovaries.
Wider implications of the findings: Spontaneous and chemotherapy-induced follicular activation in vitro involves both the PI3K/Akt/mTOR and Hippo pathways. mTORC1 inhibition acts on both pathways to reduce follicular activation, suggesting cross-talk between the PI3K/Akt/mTOR and Hippo pathways. This represents an interesting approach to regulating follicular activation in vitro and reducing the chemotherapy-induced "burn out effect".
Trial registration number: not applicable

P-459 Inhibithion of Sirt1 protects ovarian primordial follicles from the gonadotoxic action of cyclophosphamide

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Study question: Does inhibition of SIRT1 activity protect ovarian primordial follicles (PF) from gonadotoxic action of cyclophosphamide (CPM), one of the most used anticancer drugs?
Summary answer: SIRT1 inhibition *in vitro* prevents PF activation induced by the active CPM metabolite phosphoramide mustard (PM) without compromising follicle viability and ability to resume growth.
What is known already: CPM gonadotoxicity is related to follicle apoptotic damage and disturbance of PF activation. Use of fertoprotective strategies has helped in understanding these mechanisms. Physiologically, SIRT1, a NAD^+-dependent deacetylase, is essential for follicle homoeostasis. A possible role for SIRT1 during the PF activation has been suggested based on increased nuclear expression of SIRT1 in primordial oocytes during transition to primary follicle and decrease of NADH/NAD^+ nuclear ratio. Moreover, increased SIRT1 expression is part of the early adaptive response of mouse ovaries to *in vivo* administration of CPM provoking 60% loss of PFs (Tatone et al., 2018).
Study design, size, duration: Cortical strips of bovine ovarian tissue 1×1×0.5mm were cultured in gas-permeable dishes (PD) for 6 days. Experiment 1: aimed to evaluate possible protective effects of the SIRT1 inhibitor Ex527 (control, treated with PM or with Ex527 alone, or in combination). Experiment 2: aimed to understand whether protected PFs were able to resume growth (control day 3 and 6, PM+Ex527 day 3, PM+Ex527 3 days + medium alone for the next 3 days).
Participants/materials, setting, methods: Bovine ovaries were minced through a tissue chopper and cultured in PD in alpha-MEM plus supplements at 38.8 °C, 5% CO_2 and 95% humidity in air. Strips were incubated in medium alone, with PM 10µM or with EX527 10µM alone or in combination. Follicle quality, stage and viability of fresh and cultured strips were analyzed through histology and after live/dead assay through confocal microscopy.
Main results and the role of chance: Experiment 1 (956 follicles): At day 0, most follicles were at the primordial stage (primordial, 57.7; primary, 26.9; secondary, 15.4%). At day 6, culture with PM promoted a higher follicle activation and growth compared to control (primordial, 18.9 vs 26.2; secondary, 40.4 vs 26.8%; $p<0.01$). EX527 prevents follicle activation and growth compared to control (primordial, 47.3 vs 18.9; secondary, 12.5 vs 40.4%; $P<0.01$). EX527+PM prevents follicle activation and growth compared to PM alone (primordial, 53.2 vs 18.9; secondary, 11.5 vs 40.4%; $P<0.01$). Follicle viability was not affected by treatments except for Ex527 alone (control 71.5 vs Ex527 38.7%; $P<0.01$). Experiment 2 (990 follicles): At day 3, Ex527+PM prevents PF activation and growth compared to control (primordial, 79.6 vs 61.8; secondary, 5.4 vs 9.6%). Follicles in samples exposed to Ex527+PM for 3 days followed by culture in medium alone for the next 3 days are able to resume activation and growth reaching values similar to control (primordial, 58.1 vs 55.4; secondary, 12.4 vs 11.3%; NS). Follicle viability was not affected by treatments. Overall, PM accelerates follicle activation and growth and its effect can be prevented by Ex527 without compromising follicle viability and their subsequent ability to resume growth.
Limitations, reasons for caution: This is an in vitro study in an animal model.
Wider implications of the findings: Understanding the mechanisms underlying PF activation induced by the gonadotoxic action of CPM may contribute to build up strategies aimed to protect the ovarian reserve to preserve the fertility potential of female oncological patients.
Trial registration number: not applicable

P-460 Dynamic culture of human ovarian cortical tissue enhances follicle health, growth, and steroids secretion

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Study question: Does dynamic in vitro culture of human ovarian cortical tissue improve follicle growth and health?
Summary answer: Dynamicin vitro culture of human ovarian tissue in perfusion bioreactors (PB) does improve follicle health, progression to the secondary stage and steroids secretion.
What is known already: In vitro culture of human ovarian cortical strips in gas-permeable dishes (PD) enhances follicular health and secondary follicle growth over conventional dishes by improving oxygen availability in situ. However, the static culture systems adopted so far lack physiological biomechanical cues and generate stagnant media layers around the tissue with a consequent deprivation of nutrients and accumulation of catabolites. Dynamic culture in continuous perfusion bioreactors enables disruption of solutes concentration gradients and application of physiological fluid mechanic stresses on tissue and may improve ovarian cortical tissue culture.
Study design, size, duration: Biopsies of human ovarian tissue (HOB) were cut into 1×1×0.5mm strips and cultured in PD and in PB (ten strips each) for 6 days. At the end of culture, the following endpoints were assessed: 1) follicle health and stage; 2) follicle viability; 3) estradiol (E2) and progesterone (P4) levels in spent media. This study was approved by the ethical committee.
Participants/materials, setting, methods: HOBs were obtained from five consenting patients (age 24.8±6.4)during laparoscopic surgery for benign gynecologic conditions, minced into strips using a tissue chopper and cultured for 6 days in PD and PB. Fresh and cultured strips were fixed and stained for histological analysis of follicle health and stage, labelled with live-dead far red and Hoechst 33342 for viability assessment by confocal microscopy, and day 6 spent media were analysed for E2 and P4 levels.
Main results and the role of chance: Overall, 1696 follicles were analyzed. At day 0 most follicles were primordial (primordial, 75.6%; primary, 21.1%; secondary, 3.3%), with high viability (78.4%) and good quality (grade 1, 41%; grade 2, 31.6%; grade 3, 27.2%). At day 6, PB culture was superior to PD culture in terms of follicle progression (primordial, 31.5% vs 17.3%; primary, 51.3% vs 54.5%; secondary, 17.2% vs 28.2%; $p<0.01$) and health (grade 1, 27.1% vs 33.9%; grade 2, 33% vs 38.2%; grade 3, 39.9% vs 27.9%; $p<0.01$). Viability was not significantly different between samples. E2 secreted by the tissue in spent media at day 6 was higher in PB (2.5pg in PD vs PB, 4.2pg in PB), whereas P4 remarkably increased in PD (16.8ng in PD vs 1.1ng in PB). Overall, culture in PB provided the best conditions yielding a higher proportion of healthy secondary follicles, and an increased ratio between estradiol and progesterone levels (E2/P4: 0.0002 in PD; 0.004 in PB).
Limitations, reasons for caution: This is a preliminary study and should be validated on a larger cohort of patients.
Wider implications of the findings: Culture in a dynamic system permits the disruption of stagnant media layers and the application of shear stress, and improves ovarian cortical tissue culture. Use of a dynamic system yielding enhanced number and health of secondary follicles could represent a valuable tool for multistep in vitro folliculogenesis.
Trial registration number: none

P-461 Cumulus growth pattern of in-vitro matured cumulus-oocyte-complexes (COC) of transgender persons obtained during cryopreservation of ovarian tissue correlates with the maturation status.

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Study question: Is the morphology growth pattern of COCs from female-to-male (FtM) transgender persons during *in-vitro* maturation (IVM) an indicator for oocyte meiotic competence?
Summary answer: The growth pattern of the cumulus cells is a significant predictor for maturity of ex-vivo *in-vitro* matured oocytes in FtM transgenders.
What is known already: It has been shown that during ovarian tissue processing and cortex freezing, COCs can be found in the remaining medulla suspension. These COCs can be in-vitro matured for 44-48 hours until they reach metaphase II stage. This has been described for fertility preservation programs for oncology patients (Kristensen, *et al.* 2011, Wilken-Jensen, *et al.* 2013) and by our group for transgender persons (De Roo et al. 2017). During the IVM culture, the cumulus cell morphology pattern changes and can be a useful tool for predicting the maturation status of the oocyte (Smitz J. et al., 2004).
Study design, size, duration: At the moment of cryopreservation of ovarian tissue (OT), the COC's were collected and cultured for 44-48h in a commercial IVM medium (Origio). Cumulus mass (CM); cumulus expansion (CE) and contact between CC and the oocyte (CO) were scored according to Smitz J.(2004). The size of the expansion was measured and calculated on day0 (collection date) and day2 (after 44-48h IVM) after which denudation took place and the maturation status of the oocyte was assessed.
Participants/materials, setting, methods: Total of 1934 COCs were collected at time of transition surgery from 91 FtMs (23.3±5.9y). All participants were on testosterone treatment prior to OT preservation. Combinations of CM,CE and CO were established and mixed logistic regression was applied with patient as random factor, the combination categories as fixed predictor and vitrification as outcome variable. Separate models were applied for day0 and day2. Model based estimated proportions of vitrification per category are reported with their 95%CI.
Main results and the role of chance: On day 0 the highest estimated proportion of matured MII oocytes were for the COCs which were fully enclosed, with moderate expansion of 10 or more layers of cumulus cells. The estimated proportion of matured MII oocytes was 43.3% (95% CI: 33.6% to 53.6%). The lowest estimated proportion of matured MII oocytes on day 0 were COCs that were fully enclosed, with tight dense cells of 10 or more layers of cumulus cells. The estimated proportion of matured MII oocytes was 12.9% (95% CI: 10.7% to 15.6%).

On day 2 the highest estimated proportion of matured MII oocytes were those COCs that lost their tight dense connection between oocyte and cumulus cell layers, also called naked oocytes after 44-48h IVM. The estimated proportion of matured MII for those COCs was 42.5% (95% CI: 36.1% to 49.3%). The lowest estimated proportion of matured MII oocytes on day 2 were COCs that were fully enclosed, with tight dense cells of 3 to 10 layers of cumulus cells. The estimated proportion of matured MII oocytes was 4.3% (95% CI: 0.6% to 25.3%).
Limitations, reasons for caution: This study is based on material donated from FtM transgender persons which were under testosterone treatment at the time of OT cryopreservation and COC collection. We couldn't compare this data with literature nor with data from oncology patients in our center, because we do not have the same large dataset.
Wider implications of the findings: The morphological growth pattern during maturation can help us to study the maturation competence of these COCs that are obtained during the ovarian tissue cryopreservation under testosterone treatment. This cohort of COCs makes it possible to design a prognostic model for clinical use in fertility preservation programs for transgender persons.
Trial registration number: This research is conducted with the approval of the local ethics committee
(2015/0124 – B670201523543).

P-462 Prospective assessment of clinical and oocyte morphological quality in oncological fertility preservation in comparison with matched-age social fertility preservation patients.

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Study question: Are clinical/oocyte quality parameters in oncological fertility preservation (FP) patients, and in breast cancer subgroup (Letrozol effect), altered in comparison to social FP patients?

Summary answer: Oncological FP have more morphological oocyte alterations and lower AFC compared to social FP. Letrozol subgroup (Breast cancer) does not differ from other cancer types.

What is known already: Advances in oncological treatments have increased life expectancy but the risks for reproductive potential is uncertain. Fertility preservation for oncological patients in reproductive age represents hope to them reach maternity in the future. Oocyte vitrification, an effective technique for FP, is a establish option for these patients. Follicular environment – follicular fluid, cumulus complex - is responsible to support oocyte development. When follicular environment is unbalanced by inflammation, such as in PCOS, oocyte quality is decreased. In oncological patients, inflammation reaches a systemic level, but inflammatory status in follicular environment and the oocyte morphological alteration have not been explored yet.

Study design, size, duration: Prospective, non-interventional study; oncological and social fertility preservation patients were monitored between 2014 and 2018 at Huntington Medicina Reprodutiva clinic, Brazil. Patients under treatment for social FP (n=105 patients/105 cycles) and oncological FP (n=77 patients/86 cycles; in which n=54 patients/59 cycles were diagnostic with breast cancer) were included in this study. In oncological patients, reproductive-associated tumors represented 78% of cycles (69% breast, 7% uterine, 7% ovary tumors).

Participants/materials, setting, methods: Social and oncological FP patients under treatment with antagonist cycle and agonist trigger were included. Breast cancer patients also used Letrozol. Clinical parameters such as type of tumor, antral follicular count, number of follicles ≥15mm and total gonadotrophins dose were analyzed. Laboratory parameters such as number of oocytes retrieved, number of mature oocytes (MII) and oocytes alteration (shape, cytoplasm, extra-cytoplasmic) were compared between groups. Student's T-test and Fisher's exacted test were used as appropriate.

Main results and the role of chance: Social FP mean age was 31.22±4.98 and oncological FP 32.37±4.10 years old (p=0.24). Breast cancer patients were older (33.06±3.60) when compared with other tumor types (29.64±5.90), p=0.0042. Antral follicular count mean in social FP was higher than in oncological FP (14.9±6.76 vs 12.41±7.83, p=0.0005), but no difference was observed between breast cancer vs other tumors (12.23±7.83 vs 12.425±8.05, p=0.70). Number of follicles ≥15mm during ovary stimulation and total gonadotrophins used were not different between social and oncological FP (13.78±7.63 vs 12.78±8.46, p=0.20; 3042.86±776.70 vs 2996.22±877.72, p=0.94), neither between breast cancer vs other tumors (12.5±8.9 vs 12.08±9.29, p=0.37; 3029.82±819.86 vs 3064.42±930.25, p=0.96). Number of oocytes retrieved, number of MII and %MII were not different between social and oncological FP (17.5±7.69 vs 17±11.07, p=0.19; 12.08±5.58 vs 12.4±9.32, p=0.37; 70.27% vs 72.37%, p=0.31), neither between breast cancer and other cancer types (17.58±10.64 vs 15.74±12.07, p=0.32; 12.85±9.15 vs 11.26±9.63, p=0.16; 72.30%±0.18 vs 72.53%±0.21, p=0.91). The number of cycles with oocyte alterations were higher in oncological than social FP (77% vs 55%, p= 0.0072), but were not different between breast cancer and other tumors (78% vs 70%, p=0.54). Frequency of oocyte alterations by type – shape, cytoplasmic and extra-cytoplasmic – was not different between groups.

Limitations, reasons for caution: Low number of patients of both social and oncological FP have requested the use of preserved oocytes, what difficult the evaluation on procedure efficacy and the implication of oocyte alterations in reproductive outcome. Oncological patients were not tested for inflammatory markers.

Wider implications of the findings: Although clinical parameters did not differ, lower AFC and increased oocyte morphological alterations in oncological FP highlight the need of studies regarding the alterations on follicular environment in response to cancer pathophysiology. Implication of these oocyte alterations should be investigated/punctuated for the potential use of these cells in the future.

Trial registration number: Not applicable.

P-463 Fertility preservation in breast cancer patients: clinical experience and outcomes

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Study question: What are the short and long-term outcomes of fertility preservation in breast cancer patients- do they respond differently to ovarian stimulation?

Summary answer: Patients show a high return rate with good clinical pregnancy rates. Response to ovarian stimulation is similar to matched non-cancer infertile controls.

What is known already: Breast cancer can often affect young women of childbearing age. Whilst treatments such as chemotherapy and long-term endocrine medication are proving to be effective interventions, they can have a significant adverse impact on fertility. Whilst more women are choosing to have oocyte or embryo cryopreservation- there remains little data on short and long-term outcomes. Moreover, data that compares response to IVF in these patients as compared with matched controls are limited.

Study design, size, duration: We retrospectively analysed a matched, cohort of 152 breast cancer patients who proceeded with oocyte or embryo cryopreservation at the Hammersmith Hospital IVF unit between 2002 and 2018. The primary aims were controlled ovarian hyperstimulation outcomes, including oocyte retrieval and maturity number and percentage, total dose and duration of gonadotrophin stimulation, number and percentage of zygotes created, peak serum oestradiol and incidence of complications.

Participants/materials, setting, methods: Fertility preservation patients were matched for age, suppression protocol, gonadotrophin starting dose and antral follicle count. Total dose and duration of gonadotrophin stimulation, oocyte number, zygote number and peak serum oestradiol were compared. Mann-Whitney U test and independent t-tests were performed to demonstrate statistical difference between breast cancer patients and controls. We also report long-term return results of patients with breast cancer who had IVF and compare cycle outcomes of this cohort to matched controls.

Main results and the role of chance: No statistical significance was seen in any of the cycle outcomes compared between breast cancer and non-cancer infertile patients. Furthermore, letrozole had no impact on immediate cycle outcomes. 21 patients returned for 30 cycles of frozen embryo transfer (FET). 51 successfully thawed embryos were transferred. This resulted in 16 clinical pregnancies (31.4% per embryo transferred); 2 were ectopic (3.9%), 8 resulted in miscarriage (15.7%) and 6 pregnancies were carried to term and produced healthy babies (11.8%). A further 11 patients achieved 13 spontaneous pregnancies; 10 of which were carried to term, one miscarriage and two terminations due to continued tamoxifen use.

Limitations, reasons for caution: Although we work in a busy fertility preservation unit the overall number of patients remains small, and outcomes should remain viewed with some caution. The significant number of patients conceiving naturally is an important outcome and should be considered by patients and doctors during decision making.

Wider implications of the findings: Long-term follow-up shows a high return rate with good clinical pregnancy rates. Similarities in response between cohort and control groups suggest comparable efficacy of IVF in breast cancer patients. Further large studies are required to make meaningful comparisons of FET outcomes in cancer patients to non-cancer patients.

Trial registration number: N/A

P-464 Combining fertility preservation procedures to spread the eggs across different baskets: a retrospective single-centre cohort study

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Study question: What is the reproductive potential of combining ovarian stimulation, in vitro maturation (IVM) and ovarian tissue cryopreservation in female patients seeking fertility preservation (FP)?
Summary answer: In selected patients, combining different FP procedures is a feasible approach. Reproductive outcomes following FP in patients who return to attempt pregnancy are promising.
What is known already: Fertility preservation is increasingly performed in infertility clinics but an algorithm to select the most suitable FP procedure according to patient characteristics and available timeframe is currently lacking. Vitrification of mature oocytes and ovarian tissue cryopreservation (OTC) are most commonly performed, although in some clinical scenarios a combination of procedures, including IVM, to spread the sources of gametes may be considered with the goal of enhancing options for the future.
Study design, size, duration: Retrospective, observational study in a university-based, tertiary infertility center involving all female patients who underwent urgent medical FP between January 2012 and December 2018. Descriptive analysis of various FP procedures, either stand-alone or combined, was performed, and reproductive outcomes of patients who attempted pregnancy in the follow-up period were recorded.
Participants/materials, setting, methods: 207 patients (between 0 and 42 years of age) were referred to our centre for medical or oncological fertility preservation. Patient-tailored strategies and procedures were selected after multidisciplinary discussions. When deemed feasible, fertility preservation procedures were combined to cryopreserve different types of reproductive tissue and to enhance future reproductive potential. The main outcome measure was the number of mature oocytes. Live birth rates (LBR) were analysed in patients who returned for reproductive treatment.
Main results and the role of chance: Among patients seeking FP, 95/207 (46%) had breast cancer, 43/207 (21%) had haematological, and 31/207 (15%) had gynaecological tumours. Mean age was 27 +/- 8.3 years.

Eighty-four (40.8%) patients underwent controlled ovarian stimulation (COS), yielding 10.8 +/- 7.1 metaphase II (MII) oocytes. Eleven (5.34 %) patients had multiple COS cycles. Transvaginal oocyte retrieval for IVM was performed in 17 (8.3%) patients, yielding 8.9 +/- 10.5 MII oocytes. Thirty-one (15.0%) patients underwent OTC combined with ex-vivo IVM, yielding 4.0 +/-4.3 MII oocytes in addition to ovarian fragments. Sixteen (7.8%) patients had OTC combined with ex-vivo IVM and transvaginal retrieval of oocytes from the contralateral ovary for IVM, resulting in 12.6 +/-9.3 MII oocytes. In 13 (6.3%) patients, OTC with ex-vivo IVM was followed by stimulation of the contralateral ovary, yielding 11.3 +/-6.6 MII oocytes in total.

So far, 28/207 (14%) patients have returned. Of those, nine (32.1%) patients had preserved ovarian function conducive to ART treatment with fresh oocytes, resulting in five (55.6%) live births (LB) and two ongoing pregnancies. The remaining 19 (67.9%) patients had their cryopreserved potential thawed. Of those, eight (42.1%) patients had a LB, of whom three after ex-vivo IVM. To date, 5/207 patients achieved a spontaneous pregnancy.
Limitations, reasons for caution: The FP program in our centre is based on a patient-tailored approach rather than based on an efficiency-driven algorithm. The data presented are descriptive, which precludes firm conclusions.
Wider implications of the findings: Combining different FP procedures has the potential to enhance the reproductive competence of patients undergoing gonadotoxic treatment, but our data need confirmation in larger follow-up studies. Live birth rates are promising in patients who return after FP to undergo ART, whether or not they present with ovarian insufficiency.
Trial registration number: Not applicable

P-465 Knowledge, attitudes, and practices regarding fertility preservation among oncologists and healthcare providers in China

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Study question: What is the knowledge level, attitude, and practice towards fertility preservation in cancer patients among Chinese oncologists and healthcare providers?
Summary answer: Chinese oncologists and healthcare providers exhibited positive attitude towards fertility preservation in cancer patients, although the knowledge of the techniques and institutes specializing was insufficient.
What is known already: As cancer treatment can potentially affect patient fertility, fertility preservation prior to tumor treatment is an effective method for preserving patients' fertility. However, the knowledge, attitude, and clinical practice regarding to this topic among Chinese healthcare providers remains unclear. Awareness of guideline and multi-disciplinary discussion including oncology, reproductive medicine and other healthcare providers are important to ensure they provide patients with effective and timely information on fertility preservation.
Study design, size, duration: In this cross-sectional study conducted at community oncology practicer in China, we anonymously surveyed 1,532 oncologists and healthcare providers from September 2017 to February 2018.
Participants/materials, setting, methods: The study used a cross-sectional design to evaluate the knowledge, attitudes, and practices of FP among oncologists and clinical healthcare providers in China. This survey consisted of 20 questions. Oncologists and healthcare providers who worked in the fields of clinical oncology, internal medicine, surgery, gynecology, and pediatrics in hospitals in China were surveyed using this questionnaire.
Main results and the role of chance: Among the 1,532 healthcare providers (mean [standard deviation] age, 34.3 [7.2] years; 38.4% male and 61.6% female), 1,372 (89.56%) believed it was necessary to recommend fertility preservation to cancer patients. With regard to male and female fertility preservation, 1,017 (66.38%) and 989 (64.56%) respondents were aware of the topic, and 217 (14.16%) and 213 (13.90%) were aware of correct institutes, respectively. Age, department, area, hospital grade, professional qualifications, and educational background ($P<0.01$) of the healthcare providers affected the practice of recommending fertility preservation to their patients. "I think the patient is not suitable for childbirth" (28.39%), "I am unaware of fertility preservation" (28.13%), and "lack of information regarding the institutes/departments providing fertility preservation" (26.37%) were the 3 main reasons for non-recommendation of fertility preservation for cancer patients among healthcare providers.
Limitations, reasons for caution: The present study has certain limitations such as the unbalanced sample size. The sample size in some provinces was relatively small, hence the findings might not represent the views of all clinicians. Moreover, we were unable to examine the specific conditions under which healthcare providers recommend FP to their patients.
Wider implications of the findings: It is necessary to include fertility preservation in physician education to increase knowledge and awareness of this topic among oncologists and other healthcare providers in China.
Trial registration number: N/A

P-466 Differential mouse-oocyte radiosensitivity as a function of the follicle stage

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Study question: What is the quantitative and qualitative impact of X and γ irradiations on oogenesis in the mouse?

Summary answer: Irradiations trigger oocyte death in a dose dependent manner in primordial follicles. Strikingly, there is little impact on oocytes survival in growing follicles.

What is known already: During the last decades, the use of irradiations for medical purposes increased tremendously. It is therefore of great importance to determine the consequences of these expositions on female fertility. Human data were so far collected from women who were irradiated years ago under highly variable conditions. Moreover, such approaches did not allow the study of the quality of the surviving oocytes and were performed with high doses of radiations. We know that low-dose irradiation alters the integrity of human somatic cells. These low doses are used for medical imaging pointing the importance to study their effect on female fertility.

Study design, size, duration: This prospective experimental study conducted from 2018 to 2019 aimed to quantify the number of oocytes and DNA damages induced by various types (X and γ) and doses of irradiations in a murine model.

Participants/materials, setting, methods: Irradiations were performed using GSRD Ce137 and X LINAC irradiators on NMRI prepubertal mice at 8 days post partum (dpp). Non irradiated and irradiated mice (0.02 Gy, 0.1 Gy, 0.5 Gy, 2 Gy) were sacrificed at 10 dpp to determine oocytes survival after ovarian histological analysis of each group (n=3). DNA damages were quantified at 4 hours post irradiations using γH2AX labeling.

Main results and the role of chance: We observed a difference of radiosensitivity between primordial and growing follicles. At the dose of 0.1 Gy the number of oocytes from primordial follicles was significantly decreased by half (average of 1150 per ovary) and tended to disappear at 0.5Gy compared to non irradiated mice. Oocytes from growing follicles were radioresistant as their average number was similar to control (350 oocytes per ovary). These results were similar between X and γ irradiations sources. Quantification of γH2AX labeling revealed an important increased of DNA damages with an average of 12 nuclear foci per oocyte at 4 hours post irradiation time compared to control ($p<0.0001$). This increase was not significantly different between primordial and growing follicles ($p=0.78$) suggesting similar initial DNA damages.

Limitations, reasons for caution: The murine model allows experimental flexibility to perform histological and immunolocalization approaches but our preliminary results will require further consolidation with human follicles.

Wider implications of the findings: The data of this work should allow us to improve female fertility preservation, estimate the risks of giving birth with irradiated oocytes and later develop new technics of radioprotection of human oocytes.

Trial registration number: Not applicable.

P-467 Fertility preservation and oocyte quality in young cancer women: a prospective analytical study.

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Study question: Does cancer disease influence on the oocyte quality and ovarian stimulation outcomes in female oncology patients?

Summary answer: The number of dysmorphic oocytes is similar between two groups, even if a particular abnormality was observed in the study group.

What is known already: Fertility preservation (FP) has become an important component in cancer management. Oocyte vitrification is no longer experimental and this option is elective for FP in young women. In assisted reproduction vitrified oocyte give results nearly similar to those of fresh oocyte. While it is known that cancer treatments can impair fertility in women, it is not yet clear whether oncological diseases have an impact on ovarian reserve and oocyte quality influencing ovarian stimulation outcomes, mainly metaphase II (MII)-total oocyte ratio and dysmorphic oocyte ratio after retrieval in cancer patients.

Study design, size, duration: This is a prospective analytical study performed in the IVF laboratory at the Sandro Pertini Hospital in Rome between 2016 and 2018. The aim of this study is to determine the effect of cancer diseases on number, above all on quality of oocyte in oncology patients and to define if there is a typical pattern of dysmorphic oocyte in cancer women compared to women age-matched who undergone ICSI treatment for tubal or male factor infertility.

Participants/materials, setting, methods: A total of 48 patients recently diagnosed with cancer underwent ovarian stimulation for oocyte cryopreservation is the study group (Group A). A total of 50 patients under 38 age underwent ICSI treatment for tubal or male infertility is the control group (Group B). Baseline characteristics are age, body mass index (BMI) and antimulleran hormone (AMH) value. The primary outcome was number and quality of retrievd oocyte from ovarian pick-up (OPU).

Main results and the role of chance: Baseline characteristic are comparable. Despite similar ovarian stimulation protocol, similar recombinant FSH total dose (Group A: 2258,33 U.I.; Group B: 1604,72 U.I.), similar mean stimulation duration (Group A: 11,31 days; Group B: 11,45 days), similar oestradiol peak at triggering (Group A: 1127,12; Group B: 932,02) patients from Group A had lower number of MII ($P<<0.01$)and higher number of immature oocytes than Group B ($P<<0.01$) . There are no differences in the ratio of dysmorphic oocyte though in Group A dysmorphic analyzed oocyte have a particular abnormality: a perivitelline space (SPV) with granularity.

Limitations, reasons for caution: Limitations concern the paucity of groups and the fact that we haven't a follow up to evaluate the competence of vitrified MII oocytes for oncology patients. Also it was not possible to stratify the study group according to the cancer diagnosis to discriminate ovarian tumors one.

Wider implications of the findings: There are few studies about fertility preservation that investigate oocyte quality and not only quantitative parameters of ovarian stimulation. This allows a more exhaustive counseling to oncology patient and an optimized treatment for FP.

Trial registration number: Not applicable

P-468 AMH, AFC and FSH long term follow up before and after chemotherapy in patients undergoing temporary ovarian suppression with GnRH analogs

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Study question: Is there a difference in long-term fertility outcomes after chemotherapy among patients suffering from hematologic, breast or ovarian cancer undergoing temporary ovarian suppression with GnRHa?

Summary answer: There is a clinical difference in AMH, FSH, AFC decrease rate and recovery time among the three groups.

What is known already: The increase in cancer survival rates changed survivors attitude about reproductive options and desires.

Premature ovarian failure (POI) is a well-recognized consequence of chemotherapy, leading to infertility and menopausal symptoms. Temporary ovarian suppression with GnRH agonist during chemotherapy is a strategy to reduce the gonadotoxic impact and incidence of POI. Chemotherapy induces apoptosis of the granulosa cells in the growing follicles and the subsequent increase of FSH stimulate the proliferation of granulosa cells increasing their sensitivity to chemotherapy. GnRHa co-treatment decreases FSH levels suppressing ovarian function and reducing the gonadotoxic effect of chemotherapy.

Study design, size, duration: Prospective study including 201 oncological patients with a pending gonadotoxic treatments, aged 18 to 45 (mean age 29,2±4,3), enrolled from 1997 to 2018 at the Bologna University IVF and infertility Center.99 had hematologic neoplasms, 64 had breast cancer, 36 had pelvic-related (ovarian and cervical) cancer, 2 had colorectal cancer.

Participants/materials, setting, methods: All the patients started GnRH agonist treatment before and continued it during chemotherapy. AFC, FSH and AMH basal levels were previously measured. A complete follow up of AFC, FSH and AMH levels during chemotherapy and 6 and 12 months after the end of therapies was recorded. Menses resumption and spontaneous pregnancy after treatment were also investigated. T-student test was used to analyze the data.

Main results and the role of chance: The overall results of the study reported a significant decrease in AMH and AFC when comparing basal and during-chemotherapy levels, followed by a significant increase at 6 and at 12 months after the end of antineoplastic therapy. Similarly, FSH increased significantly during chemotherapy and then significantly diminished 6 months after the end of treatments. 52 (59,4%) patients experienced a menstrual resumption.

Considering the 99 patients suffering hematologic neoplasm, 28/44(63%) resumed regular menses and 3/28(10.7%) had a spontaneous pregnancy. Out of the 66 breast cancer patients, 24 (36%) resumed menstrual cycles and 1 (1.5%) had a spontaneous pregnancy. Of the 36 patients with ovarian cancer 12/19 (62%) had menses resumption and 3(25%) of them got pregnant spontaneously.

The results of the study, in agreement with the literature, confirm that GnRH agonist during chemotherapy should be suggested in all cancer patients that desire to preserve their fertility. GnRH should be started one week before the beginning of therapies and prolonged until the end of chemotherapic cycles.

Limitations, reasons for caution: The main weakness of the research is that a small number of patients who attended the center completed the whole follow up. Furthermore the monocentric nature of the study could create a bias in the interpretation of the data.

Wider implications of the findings: In all cancer patients with a scheduled chemotherapic treatment and the desire to preserve their ovarian function, GnRH agonist should be always recommended, alone or in combination to other fertility preservation techniques.

Trial registration number: /

P-469 Feasibility of follicle isolation, culture and oocyte maturation from ovaries recently gonadotropin-stimulated for IVF. A proof of concept study using an experimental mouse model

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Study question: Is it feasible to isolate and culture secondary follicles up to oocyte maturation from ovaries recently stimulated with gonadotropins for in vitro fertilization (IVF)?

Summary answer: Secondary follicle isolation, culture and oocyte maturation were feasible, percentage of follicles fulfilling IVM conditions and ratio of MII oocytes were similar to non-stimulated control.

What is known already: Mature oocyte cryopreservation is an established option to preserve fertility of cancer patients, while in certain cases, only a few or no mature oocytes can be collected after controlled ovarian hyperstimulation (COH). We designed a study to investigate if ovaries recently treated with COH could provide secondary follicles that could grow and mature in culture, as an additional source of mature oocytes for fertility preservation.

Study design, size, duration: Controlled experimental study. Ovaries from five female mice C57Bl/6J 3-4-weeks old were obtained after completion of hormonal stimulation and egg retrieval as in COH for IVF and five non-stimulated mice served as controls. Secondary follicles were isolated and cultured for 12 days and matured in vitro according to the method of R.Cortvrindt (1996).

Participants/materials, setting, methods: Pregnant mare serum and human chorionic gonadotrophin (hCG) were administered by intraperitoneal (i.p.) injection to the mice and the ovaries were collected 13.5 hours after hCG injection. Secondary follicles were isolated and cultured. Follicle growth was recorded daily. On day 12, follicles that reached at least 500 mm of diameter were matured by adding hCG and epidermal growth factor (EGF) to the culture medium. Oocyte maturation status was evaluated after 16-20 h.

Main results and the role of chance: Comparable numbers of isolated secondary follicles per ovary were obtained from non-stimulated (n=9.9±4.20) and stimulated ovaries (n=9.7±2.03). During the 12-day culture, follicles from both groups showed similar growth curves. In the non-stimulated group 86.1% of 79 cultured follicles reached day 12 whereas only 54.4% of 68 cultured follicles grew till day 12 in the stimulated group ($p<0.001$). Despite this it was possible to obtain similar percentages of follicles fulfilling the conditions of in vitro maturation (IVM) (59.5% in the stimulated group vs 66.2% in the non-stimulated group, p=0.68) and similar ratios of metaphase II-stage (MII) oocytes in both groups, 51.1% in the non-stimulated group and 36.4% in the stimulated group, p=0.38).

Limitations, reasons for caution: This study was performed in animal model in which secondary follicle isolation and culture have been established and are less challenging compared to large mammals or human. The further confirmation of our findings in ovaries of larger mammals is suitable.

Wider implications of the findings: Our results show that isolation of secondary follicles from IVF-stimulated ovarian tissue is feasible and that mature oocytes can be obtained in culture. Additionally, similar percentages of follicles fulfilling IVM conditions and similar ratios of MII oocytes can be achieved comparing to non-stimulated ovaries.

Trial registration number: not applicable

P-470 Primordial Follicle Viability Following Culture in to the Decellularized Ovarian Scaffold in vitro

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Study question: What is the effect of decellularized scaffold on viability of cultured mice ovarian follicles?

Summary answer: Mice ovarian follicles can be viable and seed into the acellular scaffold.

What is known already: Decellularization is a novel technique in regenerative medicine. Recently decellularized ovarian cortex introduced as a scaffold in the field of human fertility preservation. Studies demonstrated that follicle growth and the survival rate are depend on type, structure, and rigidity of the scaffold, so scientific groups attempt to find ideal scaffold to support follicle development. Acellular ovarian cortex due to its high resemblance to the origin niche of the follicles, can ensure their growth and development.

Study design, size, duration: In the present study bovine ovarian cortical fragments (2 × 2mm) were prepared and divided in to two groups (SDS and SDS-Triton-Ammonium). 120 primordial follicles from two 14 days old NMRI mice were isolated and put into the SDS-Triton-Ammonium decellularized scaffold (n= 30/ 4 scaffold) then cultured for 4 days at 5% CO_2 and 37 °C.

Participants/materials, setting, methods: SDS group was decellularized with 0.1% SDS for 24 hours and SDS-Triton-Ammonium group was decellularized with 0.5% SDS for 2 hours and 1% Triton-0.1% ammonium for 22 hours.

H&E and DAPI staining **were** applied to prove decellularization. Orcein and Masson's trichrome staining was carried out to evaluate the presence of elastin and collagen fibers respectively. For evaluating scaffold's cytocompatibility, MTT test was done. Follicles viability and seeding were tested by H&E staining and SEM analysis.

Main results and the role of chance: According to the H&E staining results, the bovine ovarian cortex was decellularized in both groups. No residual nuclei were observed by DAPI staining.

Elastic and collagen fibers were kept after the decellularization process in both groups.

OD values of eluted formazan of MTT test showed that the SDS-Triton-Ammonium decellularized scaffolds were cytocompatible than SDS group.

The results of the in vitro culture showed that after 4 days 111/120 follicles were viable (92.2%) and seeded into the scaffold.

Limitations, reasons for caution: The study was performed in vitro just for 4 days so long term culture and extra analysis such as maturation of oocyte and grafting in ovariectomized mice are needed.

Wider implications of the findings: We showed for the first time follicles can survive and seed in to the acellular matrix and decellularization of ovarian cortex with SDS-Triton-Ammonium has satisfactory results.

Trial registration number: -

P-471 return rate and utilization rate of oocytes and embryos after fertility preservation

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Study question: What is the return rate and utilization rate of cryopreserved oocytes and embryos after fertility preservation (FP) for patients with malignant disease?

Summary answer: Of patients that underwent FP prior to cancer treatment 52.7% returned with questions concerning their fertility. Cryopreserved material was used in 23.7% of all patients.

What is known already: Cancer therapy can lead to loss of fertility due to primary ovarian insufficiency (POI). FP prior to cancer treatment can give additional chances to conceive after the gonadotoxic treatment, especially in case of POI. Because infertility is known to have a negative effect on quality of life, referral to a fertility specialist for FP has become more routinely imbedded in cancer therapy. Although the number of patients that go through FP prior to cancer treatment has increased, not much data have been published about the use of this cryopreserved material.

Study design, size, duration: A single center retrospective study of patients who were counseled for FP between 2002 and 2012. In this timeframe 178 patients were counseled and 93 patients went through FP. Follow up was collected up to 2017.

Participants/materials, setting, methods: All patients counseled for FP in means of vitrification of oocytes or cryopreservation of embryos were included. Retrospective data were collected from medical records.

Main results and the role of chance: Between 2002 and 2012 a total of 178 patients with malignant disease were counseled in our clinic for oocyte vitrification and embryo cryopreservation. 93 patients (52,2%) underwent at least one cycle of FP prior to the cancer treatment. Of those 49 (52.7%) returned for information about their fertility up to 2017. 22 of the returned patients (23.7% of all the FP patients) used their cryopreserved material, which resulted in an overall pregnancy rate of 45.5% and birth rate of 36.4%.

Of the patients that underwent FP 47.3% did not return, 5.4% had passed away and in 7.6% frozen embryos were discarded because their relationship had ended. Information about the other patients is missing.

Not in all patients that did return after FP ovarian reserve was compromised. Those with normal ovarian reserve were advised to pursue natural conception and 34.7% did conceive naturally. This explains the relatively low utilization rate (23.7%) of the cryopreserved material.

Limitations, reasons for caution: The study size is relatively low. Follow up data of patients that did not return is incomplete. Time to follow up is at least 5 years, but patients might not be ready to try to conceive yet and utilization rate might be underestimated.

Wider implications of the findings: FP prior to a gonadotoxic treatment is increasing. This study shows that the utilization rate of the stored material is relatively low. Discussion is needed to determine the indications of FP and whether it is performed too often.

Trial registration number: Not applicable

P-472 Oocyte vitrification negatively affects the Ca2+-releasing pattern and oocyte activation potential of mouse oocytes

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Study question: Can vitrified-thawed instead of fresh mouse oocytes be used to determine the oocyte activation potential of human spermatozoa?

Summary answer: Vitrified-thawed mouse oocytes display reduced Ca2+-releasing potential, necessitating adapted classification criteria in order to be used for assessing the activation potential of human spermatozoa.

What is known already: Mouse oocyte Ca2+ analysis (MOCA) is a diagnostic test whereby a patient's sperm is injected into mouse oocytes to investigate its ability to induce Ca2+ oscillations and assess the oocyte activation capacity. To facilitate large-scale diagnostic testing and decrease dependency of live mouse availability, the creation of a mouse oocyte bank with vitrified oocytes would be beneficial. However, vitrification-thawing might negatively impact oocyte activation by itself (i.e. the Ca2+-releasing machinery), or compromise embryonic development.

Study design, size, duration: Mouse oocytes were randomized into fresh or vitrified-thawed groups (each ≥ 15 oocytes) for further experimental investigations. Oocytes were injected with spermatozoa or parthenogenetically activated and checked for Ca2+ pattern analysis and developmental competence. Each experiment was repeated 3 times. We analysed the mean amplitude, frequency, and full width at half maximum (FWHM) of the Ca2+ peaks, as well as the area under the curve (AUC). For development, fertilization and blastocyst rates were observed.

Participants/materials, setting, methods: First, fresh and vitrified-thawed mouse oocytes (B6D2F1, 7-12 weeks old) were injected with frozen-thawed control human sperm. Secondly, mouse oocytes were exposed to SrCl2, or to a single or twice repeated (30 min time interval) ionomycin exposure, and monitored for their fertilization potential and Ca2+ patterns. Finally, oocytes were exposed to thapsigargin to evaluate the intracellular Ca2+ store content. Intracellular Ca^{2+} levels were measured by fluorescence microscopy using leakage-resistant FURA-2 AM as a Ca2+ indicator.

Main results and the role of chance: MOCA-test evaluation of sperm-injected vitrified-thawed oocytes (n=58) displayed a significantly lower amplitude ($p<0.001$) and AUC ($p<0.05$) of the induced Ca2+ pattern compared to fresh oocytes (n=44). The amplitude and FWHM of the Ca2+ peaks, as well as the AUC, were significantly lower and frequency significantly higher in vitrified-thawed SrCl2-exposed oocytes (n=60) compared to fresh oocytes (n=69) ($p<0.001$). The first ionomycin exposure resulted in a significantly lower amplitude, FWHM, and AUC in vitrified-thawed oocytes (n=48) compared to fresh oocytes (n=60) ($p<0.001$). The second ionomycin exposure also resulted in a lower amplitude ($p<0.001$), FWHM ($p=0.11$), and AUC ($p<0.001$) in vitrified-thawed oocytes. Fertilization rates after SrCl2 activation were similar between both groups (fresh 95.4%;n=87 and vitrified-thawed 94.4%;n=89; $p=0.76$). However, activation rates after a single or double ionomycin exposure were significantly lower in vitrified-thawed oocytes (43.2%;n=111 and 78.4%;n=51) compared to fresh oocytes (73.0%;n=100 and 100.0%;n=50) ($p<0.001$). By contrast, blastocyst rates did not differ between both groups. Thapsigargin exposure of vitrified-thawed oocytes (n=72) demonstrated oscillatory Ca2+ changes which were not observed in fresh oocytes which displayed a single transient Ca2+ increase (n=60) ($p<0.001$). The first peak showed a significantly lower amplitude and AUC in vitrified-thawed oocytes ($p<0.001$), indicating decreased Ca2+ store content after vitrification-thawing.

Limitations, reasons for caution: Oocyte vitrification negatively affects the Ca2+-releasing potential. Therefore, we cannot extrapolate results obtained in vitrified-thawed oocytes to fresh oocytes. Adapted, new classification criteria need to be established to correctly interpret MOCA results. Also, more research in human is recommended for gaining information about the Ca2+-signalling machinery in vitrified-thawed human oocytes.

Wider implications of the findings: When creating new classification criteria, we can perform large-scale diagnostic testing and decrease dependency of live mouse availability by using vitrified-thawed oocytes. Furthermore, one has to take in mind the reduced Ca2+-releasing potential of vitrified-thawed oocytes, especially when performing assisted oocyte activation on human vitrified-thawed oocytes in the IVF clinic.

Trial registration number: not applicable

P-473 Letrozole and fertility preservation in women with breast cancer

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Study question: The question is to investigate whether the use of letrozole is effective in fertility preservation for patients presenting with breast cancer ?

Summary answer: The use of letrozole in PF is effective because it does not reduce the average number of oocytes or embryos collected.

What is known already: Ovarian stimulation with exogenous gonadotropins leads to a significant rise in circulating estrogen levels. This could aggravate these particular patients' condition and even cause the spread of the disease. The use of anti aromatase agents such as letrozole could prevent this elevation

and protect the patients while remaining effective in terms controlled ovarian stimulation , as well as oocyte retrieval and overall outcome.

Study design, size, duration: We conducted a prospective comparative study from January 2015 to October 2018 and which involved 143 cycles for fertility preservation. We have compared the number of mature oocytes obtained between patients with breast cancer (Groupe 1) and patients with other types of cancer (group 2).

Participants/materials, setting, methods: Patients were referred by their treating physician. A FP consultation is provided by a gynecologist and a biologist. The evaluation of the ovarian reserve is done by Antral Follicle Count (AFC) ultrasound and AMH dosage.

The stimulation is conducted by antagonist protocol with random start and GnRH agonist triggering. For patients with breast cancer, the adjunction of letrozole 5mg/day was started the first day of the ovarian stimulation and continued 7 days after oocyte pick up.

Main results and the role of chance: 171 patients were referred to our FP visits, of which 143 patients had fertility preservation (oocyte/embryo vitrification) and 75 amongst them had breast cancer.

The average age of patients (year) in group 1 was 30.3 +/- 3.7 and 26.9 +/- 6.7 and the difference was not significant (p=0.73). The ovarian reserve was not statistically different : AFC (12.3 +/- 6.2 vs 13.9 +/- 6.4; p = 0.7) and AMH (2.43 +/- 2.3 ng/ml vs 2.8 +/- 2.45 ng/ml, p = 0.5).

The duration of the ovarian stimulation was not different : 10.2 +/- 2.3 days vs 11.8 +/- 3.1 days; p = 0.5).

The estradiol level on the day of ovulation triggering was 479 +/- 323 pg/ml in the breast cancer group versus 1701 +/- 682 pg/ml in the other group with a significant difference (p = 0.02) The number of CCOs obtained in the breast cancer group was 10.76 +/- 8.39 compared with 9.11 +/- 6.81 in the group 2 and the difference was not significant (p=1.83)

The mean number of mature metaphase II oocytes collected in the breast cancer group was 7.38 +/- 6.11 oocytes versus 6.09 +/- 4.72 oocytes in the group 2 and the difference was not statistically significant (p=1.33)

Limitations, reasons for caution: Amongst the limitations, some patients did not undergo FP upon fertility, because they could not afford the gonadotropins and were not included in the program unfortunately.

Wider implications of the findings: Letrozole would provide much ease and safety when stimulating women with breast cancer, as it a very frequent malignancy.

Trial registration number: 0

POSTER VIEWING
NURSING AND MIDWIFERY

P-474 Discontinuation before even starting fertility treatment: prevalence and prediction

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Study question: How common is discontinuation prior to starting fertility treatment and can this be predicted by women's or their partner's medical chart factors or infertility-specific distress?

Summary answer: One in five couples discontinued the fertility clinic trajectory before even starting treatment, most commonly between diagnostic tests, but no predictors were identified.

What is known already: 'Discontinuation' is the decision of a couple to opt out from the fertility clinic trajectory without being advised to stop by the clinic, without financial reasons and without having fulfilled their wish for a pregnancy or child. Most studies on discontinuation and infertility-specific distress focus on patients in treatment. Surprisingly, two studies reported rates of discontinuation prior to treatment ranging between 8-13% and identified women's higher age, lower education level and symptoms of depression as risk factors. To help couples achieve parenthood, more research into discontinuation and infertility-specific distress prior to starting fertility treatment is needed.

Study design, size, duration: This longitudinal study asked 104 couples first consulting a fertility clinic between March 2017 and July 2019 to fill out a questionnaire. Medical charts of couples were followed up for six months to assess 'discontinuation prior to treatment'. More specifically, we followed up how many couples did not start treatment, within six months after first consulting the clinic without reporting a pregnancy or having been advised by the clinic to delay their diagnosis or treatment.

Participants/materials, setting, methods: Heterosexual couples consulting a fertility clinic for the first time were eligible. Discontinuation, pregnancy, clinic censoring, and potential predictors (i.e. age and duration of infertility) were extracted from medical charts. Infertility-specific distress was assessed with the reliable Infertility-Distress-Scale questionnaire (IDS; range: 8-40; the higher, the more distress; Cronbach's α = 0.80). T-tests examined associations between discontinuation and the infertility-specific distress and age of both partners and couple's duration of infertility.

Main results and the role of chance: A total of 65 couples gave their consent and filled out the IDS-questionnaire (response rate=63%). Eleven couples were excluded due to spontaneous pregnancy (n=7) or due to clinic censoring to delay diagnosis and/or treatment (n=4; i.e. mainly because of obesity). Of our final 54 participating couples, ten discontinued the fertility work-up (19%). Most of them (n=7/10) discontinued in between diagnostic tests rather than not starting treatment after having received a fertility diagnosis (n=3/10). Participating women and men were on average, respectively, 31.1 (+/- 4.2) and 33.6 (+/- 4.9) years old. The couples had an average duration of infertility of no less than 18.5 (+/- 9.9) months. On the day of their first fertility clinic visit, the IDS showed considerable stress in both partners. Women's level of infertility-specific distress (mean=23.9; +/- 4.7) was significantly higher than their partner's (mean=21.0; +/- 4.9; paired difference=2.9 +/- 4.9; p<0.001). However, infertility-specific distress did not significantly predict discontinuation regardless of gender (women p=0.771; men p=0.595). Also women's (p=0.658) and men's age (p=0.501) were not predictive. Interestingly, a positive trend was observed between discontinuation and couple's duration of infertility (p=0.087); this can be re-examined in our final sample.

Limitations, reasons for caution: Our current sample size only allowed detecting medium effect sizes for potential numerical predictors (ES d=0.55; power=0.80, alpha=0.05; t-test). As recruitment is ongoing, we will report on a larger sample size and be able to detect smaller effect sizes for numerical predictors and include potential categorical predictors (e.g. education).

Wider implications of the findings: Before even starting treatment, women and men have considerable distress and a considerable proportion of couples discontinues fertility treatment. As research showed that many discontinuers still want children and regret discontinuation, clinics need to devote attention to the prevention of discontinuation and do so from patient's first clinic visit on.

Trial registration number: not applicable

P-475 An overview of the nursing management and pathway of patients undergoing Intravenous Immunoglobulin infusion therapy (IVIg)

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Study question: Intravenous immunoglobulin therapy is being prescribed as an additional treatment option to patients undergoing fertility treatment. Is there a need to implement a specialist nursing service to manage these patients?

Summary answer: Fertility nurses are well placed to manage the care of patients receiving Immunoglobulin therapy as part of their treatment.

What is known already: Intravenous Immunoglobulin therapy is a non evidence based treatment that is offered to some patients undergoing fertility treatment when they meet the criteria for the therapy. IVIg is concentrated and highly purified human immunoglobulins (antibodies) primarily IgG (immunoglobulin G). IVIg is prepared from pooled human blood donors. The immunoglobulin

therapy can be associated with a number of side effects including pain at infusion site, muscle pain, fever and low blood pressure. Chest tightness and blood clotting has also been associated with the therapy. Patients undergoing IVIg need to be monitored and observed both closely and cautiously throughout the infusion.

Study design, size, duration: Fertility nurses who are competent in the administration and information provision on the use of IVIg, manage the pathway and oversee the patients undergoing IVIg. The head of Nursing, clinical lead and theatre lead initiated the programme and devised a patient information and consent leaflet, clinical protocol and post infusion instructions in order to ensure that the patient is managed both safely and effectively pre, during and post infusion. The programme began in 2017.

Participants/materials, setting, methods: The programme consists of A clinical lead, head of nursing, senior fertility nurse and a theatre nurse. Evidence and research supporting the use of IVIg was reviewed and discussed at a preliminary meeting with the team. The protocols were devised and a log initiated to record the patients that meet the criteria of IVIg. Each member of the group were responsible for initiating the protocol and pathway.

Main results and the role of chance:

The HFEA, the Royal College of Obstetricians and Gynaecologists (RCOG), Science Advisory Committee and the American Society of Reproductive Medicine (ASRM) all agree that there is no strong evidence at the moment to justify immune testing and treatment in the context of fertility failure and recurrent miscarriages. However, if a patient is appropriately counselled on the use and given the opportunity to make an informed decision, safe protocols and pathways for the use of IVIg can be developed and managed by informed and competent staff in the delivery of the programme. Fertility nurses who are trained in the administration and use of the medication are able to deliver effective nursing care and manage the pathway for those receiving intravenous immunoglobulin infusions. It is crucial for these patients to be managed safely and to be well informed of all potential outcomes or adverse reactions and the fertility nurse is well placed to do this in accordance with the NMC Code 2018 - preserving safety, practising effectively, prioritising people and promoting professionalism and trust.

Limitations, reasons for caution: This project serves as a reminder that anyone providing care should not undertake a procedure unless competent to do so, whilst considering how they can best ensure competence and confidence to carry out activities that expands their scope of practice.

Wider implications of the findings: IVIg is non evidence based and there is division in the opinion of professionals that Immunoglobulin's are effective in the outcome of patients undergoing fertility treatment, IVIg is derived from a blood product so extreme caution should be taken when administering the infusion and nurses should receive further training.

Trial registration number: not applicable

P-476 Effectiveness of a spousal support program in improving the quality of life of infertile men in Japan: a pilot study

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Study question: What is the effect of a spousal support program on improvement of the quality of life (QOL) of male patients with infertility of various causes?

Summary answer: The spousal support program had a positive effect in terms of increasing the scores of the Relational and Emotional subscales of QOL.

What is known already: Infertile men experience infertility-specific anxiety, and socially isolated infertile men are more vulnerable to severe anxiety (Fisher & Hammarberg, 2011). Counseling and an educational program reportedly reduce stress in female patients but not in male patients undergoing infertility treatment (Boivin, 2003; Asazawa, 2015). The major predictors of the QOL of men undergoing infertility treatment are lack of spousal support, prolonged infertility period, and male factor (Asazawa, 2018). Therefore, a spousal support program for men with infertility from various causes and a short infertility period is needed.

Study design, size, duration: This a non-randomized controlled study involving a quasi-experimental one-group pretest posttest design to evaluate the preintervention and postintervention QOL and Distress scores. The program which aimed to obtain spousal support was conducted on a purposive sample of 38 couples of infertility patients in Tokyo, Japan, from April to August 2018. The sample size calculation involved a significance level of alpha = 0.05, power = 0.80, effect size to γ = 0.80, dropout rate = 35%.

Participants/materials, setting, methods: A single spousal support program provided information and used a participatory–interactive approach to enhance understanding and cooperation between couples undergoing fertility treatment. Interventions were performed for the couples, and surveys were conducted only in men. QOL and Distress were measured using self-administered valid and reliable questionnaires. The paired t-test was used to analyze preintervention and postintervention data.

Main results and the role of chance: During the study, 38 questionnaires were distributed to eligible men. Data from 31 questionnaires were usable for analysis, with a response rate of 81.6%. The mean age (± SD) was 38.6 (± 7.0) years. The causes of infertility were a low sperm concentration (i.e., oligospermia) of 61.3% and a poor sperm motility (i.e., asthenospermia) of 38.7%. There were no significant differences between the pretest-posttest scores in the three scales of QOL, Distress, and Spousal Support based on the paired t-test. The paired t-test was conducted for the QOL subscales. There were significant differences between the pretest-posttest scores of the Relational and Emotional subscales of QOL. The t-test showed that the posttest Emotional score (66.9 ± 16.9) was significantly higher than the pretest Emotional score (58.5 ± 13.5; $t(30) = 2.2$, $p < 0.05$). The posttest Relational score (71.2 ± 21.6) was significantly higher than pretest Relational score (60.8 ± 13.7; $t(30) = 2.3$, $p < 0.05$). In the high age group, there were significant differences between the pretest-posttest scores of the QOL ($p < 0.05$) and Distress ($p < 0.01$) scales based on the Wilcoxon signed-rank test. The majority of the participants (74.2%) were satisfied with the program.

Limitations, reasons for caution: This study involved a single group pretest-posttest design. The internal validity of the intervention effect was weaker. As this study had a small sample size and data were collected only from a single clinic, there is limited generalizability and potential bias.

Wider implications of the findings: The spousal support program was well received and had significantly improved part of the QOL of men with infertility from various causes. Therefore, a larger study using a pretest-posttest randomized controlled trial is recommended.

Trial registration number: noting.

P-477 Worlds Apart or Two Sides of the Same Coin? Attitudes, Perceptions and Motives of Potential Oocyte and Sperm Donors in Austria

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Study question: This study analyses the willingness to donate oocyte or sperm and focuses on differences between potential female and male donors in attitudes, perceptions and motives.
Summary answer: Significant differences between the donor groups "doubtful", "potential" and "non-donors" as well as significant sex differences with respect to attitudes, perceptions and motives are evident.
What is known already: Oocyte and sperm donors have been explored previously, mostly focusing on post-donation scenarios (Hershberger, 2004; Purewal and van den Akker, 2009; Sydsjö et al., 2012; Van den Broeck et al., 2013; Bracewell-Milnes et al., 2016, Thijssen et al., 2017, Provoost et al., 2018). Main issues of these empirical studies are characteristics, experiences, motivations, attitudes, disclosure, health risks, and the willingness to donate. Up to our knowledge we present the first pre-donation study of potential oocyte and sperm donors focusing on sex differences against the background of the literature-based variables attitudes, perceptions and motives.
Study design, size, duration: An electronic anonymous survey was conducted between April and June 2017. In total 556 students completed the questionnaire online. To allow the comparison between men and women regarding their attitudes, perceptions and motives as well as regarding their willingness to donate gametes we designed two separate questionnaires. Both generally consist of the same 44 items whereby the questionnaire for female respondents exclusively refers to oocyte donation and the male version exclusively focuses on sperm donation.
Participants/materials, setting, methods: Students from three Austrian universities received an email-invitation with a study description and a link directly leading to the online survey. Instructions on how to fill out the questionnaire were given online after participants had signed the informed consent form. Chi-square tests, two-way analyses of variance, and subsequent one-way analyses of variance with post-hoc tests (Scheffé) were used to analyse differences between the three donor groups and sex differences in the variables of interest.
Main results and the role of chance: Based on an item, concerning participants' willingness to donate oocytes/sperm at some point in the future, the study sample was subdivided in three groups: potential donors (n=133; 24%; women: 48%, man: 52%), doubtful donors (n=207; 37%; women: 76%, man: 24%) and non-donors (n=215; 39%; women: 68%, man: 32%). Results show that, compared with male potential donors (39%), the group of female potential donors (17%) is significantly smaller, while the groups of female non- (41%) and doubtful donors (42%) are significantly larger than the groups of male non- (28%) and doubtful donors (32%).

We can also provide evidence for sex differences and/or differences between doubtful and potential donors in motives for gamete donation. Doubtful donors differ significantly from potential donors in the role that altruism, passing on one's genes, and enhancing one's self-worth play with respect to the willingness to donate. Altruism is more important to doubtful than to potential donors, while passing on one's genes and enhancing one's self-worth is more important to potential than to doubtful donors. Beyond that altruism and the motive of passing on one's genes seem more relevant to men than to women. For the self-enhancement motive, we find no significant sex differences.
Limitations, reasons for caution: The study focuses on students of three Austrian universities, a non-representative study sample. Hence, we cannot generalize our results to all university students nor to the general population who would qualify as oocyte and sperm donors. Moreover, social desirability may have had an influence.
Wider implications of the findings: The study at hand presents relevant findings on the general perception of sperm and oocyte donation and especially focuses on the potential donors' perspective. Gaining a better understanding of potential donors in the existing donation framework helps to evaluate the given regimes in light of designing improved ones.
Trial registration number: not applicable

P-478 Women's reports of barriers to and facilitators of oral medication adherence during controlled ovarian hyperstimulation: A mixed methods study

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Study question: What are women's perceived barriers to and facilitators of oral medication adherence during controlled ovarian hyperstimulation cycles?
Summary answer: This study has identified multifaceted barriers and facilitators that could power new interventions tailored to optimize adherence behaviors in future trials and clinical practice.
What is known already: Adherence to lifestyle modification recommendations remain problematic for women undergoing fertility treatment raising concerns about the extent to which women adhere to prescribed medication regimens. There is a scarcity of research assessing mediation adherence during fertility treatment clinical trials although limited data have shown suboptimal oral medication adherence rates of 19%-74%. Yet, researchers have not established a systematic process to investigate medication-taking behaviors while women undergo controlled ovarian hyperstimulation to determine if interventions are needed to optimize adherence. Exploring barriers to and facilitators of medication adherence during fertility treatment cycles is a novel approach to understand women's perspectives on adherence.
Study design, size, duration: A prospective, cross-sectional, non-interventional, single-visit, mixed methods study was conducted among a sample of 30 women who were actively taking either letrozole or clomiphene citrate for ovarian hyperstimulation in conjunction with undergoing intrauterine insemination cycles. The study duration was 4 months.
Participants/materials, setting, methods: Women recruited were patients of a reproductive endocrinology and infertility center located in the Midwestern region of the United States of America. Medication adherence barriers were measured using a 20-item adherence barrier questionnaire. Medication adherence facilitators were assessed with structured interviews. The interviews were audiotaped, transcribed, and data coded using a thematic approach. The relationship between barriers, facilitators, and demographic variables were tested. Analysis included Pearson $\square^2$ or Fisher exact test.
Main results and the role of chance: Reported barriers included recently feeling sad, down, or blue (53%), taking medication more than once per day (40%), worrying if the medication would affect sexual health (17%), and forgetting to take medication (10%). Women also reported not having the medication with them when it was time to take it (47%), taking medication more or less than prescribed (27%), and having skipped or stopped taking a medication because it made them feel bad (20%). Race was significantly associated with having attitudes and beliefs related barriers ($p < .05$) and physical related barriers ($p < .05$). Facilitators included using physical aides as reminders (60%), establishing a daily routine (50%), social support (43%), motivation (27%), hoping the medication works (20%), relationship with healthcare provider (17%) , and remembering to take medication (10%). Level of infertility insurance coverage was significantly associated with having social support related facilitators ($p < .05$). Race ($p < .01$) and ethnicity ($p < .01$) were significantly associated with having knowledge related facilitators. Number of prior failed treatment cycles were significantly associated with having attitudes and beliefs related ($p < .05$) and motivation related facilitators ($p < .05$). Highest level of education was significantly associated with having cognitive related facilitators ($p < .01$).
Limitations, reasons for caution: This study was conducted at a single site fertility center and the sample size was small. The study participants were married (100%) primarily white (83%), college educated (87%), had a household annual income of at least $100,00 (57%), and lived in a suburban community (83%) which limited generalizability.
Wider implications of the findings: The dynamic interplay between perceived barriers and facilitators and women's medication-taking patterns could influence whether or not medication regimens are followed correctly. These study findings provide important implications for researchers to explore the relationship between medication adherence and reproductive outcomes in future clinical trials involving fertility medications.
Trial registration number: Not applicable

POSTER VIEWING

PSYCHOLOGY AND COUNSELLING

P-479 Identification of research priorities in infertility and assisted reproduction: a multicenter study in partnership with patients

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Study question: Which are the main research interests among assisted reproduction (ART) patients?

Summary answer: Patients identified as research priority the areas of: ART side-effects and health, coping resources, diet and exercise, success rates, oocytes quantity/quality, and genetics of infertility.

What is known already: The involvement of patients and caregivers in setting research agendas in medicine has gathered significant momentum in the last decade. Patient involvement in setting research priorities offers several benefits: improved patient awareness and knowledge of their condition; greater understanding of the medical professionals of the impact of the condition on patients' quality of life; reduce costs associated with redundant research activities. This is may be also applicable to research in infertility and ART, where patients' interests have never been explored before.

Study design, size, duration: Cross-sectional study; an anonymous online survey was sent up to three times to patients from 8 fertility centers in 5 countries between January-November 2018. The study was based in the James Lind Alliance priority setting partnership model that comprises four phases: 1) exploration: identification of patients groups; 2) consultation and prioritization: analysis of the research agenda; 3) integration: analysis of collected data and identification of priorities; 4) follow-up: analysis of patients' views' contribution into recommendations.

Participants/materials, setting, methods: Overall, 2112 patients were contacted, and 945 surveys were answered (RR: 44.7%). Female (845, 89.4%) and male (100, 10.6%) patients were included. In the first part of the survey, patients were asked about their experience living with infertility. In the second part they were asked to identify research questions in this are relevant to them. Answers were categorized in topics and ranked by frequency. A shortlist of the Top-10 research questions is presented.

Main results and the role of chance: Mean age of patients was 37.8 (SD 1.74). Most of the patients did not have children at the time of the survey (523, 59%), while 51 (5.7%) were pregnant. Sixty (6.3%) patients did not start treatment, 579 (61.3%) performed a treatment with their own gametes and 304 (32.2%) recurred to gametes donation.

Patients were mainly interested in the effectiveness of ART and side effects of drugs, protection of fertility, prevention of infertility, and psychological aspects. The top-10 research priorities identified were: 1) What are the side-effects of ART treatments? (41.6%); 2) What are the most effective methods to cope with infertility from the psychological point of view? (37.2%); 3) What effects could the diet have on fertility? (25.9%); 4) What are ART success rates per clinical profile? (25.9%); 5) Are there healthy habits and lifestyle that could prevent infertility? (20.0%); 6) What are the long term risks associated to ART? (18.5%); 7) Are alternative therapies as acupuncture, yoga and meditation effective to treat/prevent infertility? (18.5%); 8) What is the impact of exercise on fertility? (15.4%); 9) How does oocytes quantity and quality affect fertility? (9.5%); 10) What are the genetic patterns or hereditary conditions causing/related to infertility? (9.5%).

Limitations, reasons for caution: Although all respondents had attended a fertility center, not all had started treatment at the time of response, while a few were pregnant; their priorities for research might have been informed by their infertility journey. Participants came from private fertility centers; areas of interest may be different in public settings.

Wider implications of the findings: Researchers and clinicians should keep in mind that, in addition to improvement of treatments' success rates and side-effects, patients greatly value research on causes, prevention and emotional aspects of infertility. As their views might differ from those of medical professionals, patients' voices should be incorporated in setting infertility research priorities.

Trial registration number: not applicable

P-480 Experience of and coping with recurrent miscarriages: Risk for depression, anxiety and restricted social support

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Study question: Aim of the study was to identify the psychological risk and protective factors in couples with recurrent miscarriages and to derive the required professional support.

Summary answer: Already having a child increases anxiety and decreases depression in both partners. A high number of miscarriages correlate with low social support.

What is known already: Recurrent miscarriages are defined by the WHO as at least three consecutive losses of pregnancy before the 20th week of gestation. Anxiety, mourning and depression occur more frequently in women with recurrent than singular miscarriages. In one study a clinical relevant depression was found in 17% of the women and anxiety in 21%. A stable social network facilitates successful coping processes. Explicit statements about the emotional experience of the male partners are missing yet.

Study design, size, duration: A quantitative and qualitative questionnaire study was devised, approved by the Ethics Committee of the Heidelberg Medical Faculty. The ongoing study started in September 2018 and includes N=52 participants (25 couples, 2 women) so far. All women and their partners consulting the clinic for recurrent miscarriages at Heidelberg University Women's Hospital were invited to participate in the study.

Participants/materials, setting, methods: Participants completed a questionnaire with items regarding risk factors towards anxiety, depression and restricted social support (measured by the Screen-IVF), experience of miscarriages, coping (modified questionnaire on coping behaviour), impact on partnership, personal and social factors (COMPI Fertility Problem Stress Scales), attitudes towards treatment, need for support and sociodemographic variables. Data were analysed using chi square test, independent t-test analysis and ANOVA.

Main results and the role of chance: Questionnaires were handed out to 62 patients, whereof 52 completed the full assessment (response rate: 83.9%). In total, N=27 women (mean age: 35.1 years) and N=25 men (mean age: 36.2 years) have participated in the study so far. Of all subjects, 72.6% hold a high school or university degree. 40.4% of all participants already had one child in the current relationship. In the ScreenIVF, 84% of all participants showed at least one psychological risk factor. Overall, 66.7% revealed a risk for anxiety, 34.6% a risk for depression and 23.5% a risk for restricted social support. The risk for depression differs significantly between men and women with 48.1% for women and 20% for their partners (p=0.033). Having at least one child together decreases the depression risk in both women (p=0.043) and men (p=0.014), but increases the risk for anxiety in the whole group (p=0.033). A high number of miscarriages correlates with perceived restricted social support in the whole group (p=0.009).

Limitations, reasons for caution: Despite the high response rate, the generalizability of our findings is limited due to the small sample size. Furthermore, the participants in this sample showed an above-average high educational background.

Wider implications of the findings: Regarding the needs for emotional support, the study shows two results. Psychological counselling of couples with recurrent miscarriages and previous successful pregnancy should focus on anxiety rather than on depression. Couples with a high number of miscarriages should be encouraged to make demands on their social network.

Trial registration number: not applicable

P-481 Even in a population of patients satisfied of their medical care, ART burden remains an important issue to be addressed

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Study question: What is the perception and impact on quality of life among infertile French women and men having undergone assisted reproduction technologies (ART)?
Summary answer: Despite an overall high level of satisfaction concerning the treatment received, we observed the burden of infertility and treatments on most of the aspects investigated.
What is known already: Impact of infertility and ART on quality of life is well established. However, data focused on both women and men, their feeling, their main questions and preoccupations as well as the consequences of ART on their daily life are still lacking.
Study design, size, duration: We performed a cross-sectional study, from October 7, 2018 to October 28, 2018 in France. First, we screened 2,003 people aged 18 years or older, from a large panel representative of national population. From these, 1 045 patients (355 men; 690 women) had already undergone ART and accepted to answer to the online survey.
Participants/materials, setting, methods: The questionnaire included 56 questions on several domains: global feelings, treatment burden, rapport with medical staff, expectations… Respondents were divided into three groups: people who succeed to have a baby following ART (n= 523), those currently undergoing ART (n =199) and people having terminate the ART process without a baby (n = 219). Results are descriptive and are presented in percentage or by their mean ± standard deviation.
Main results and the role of chance: Respondents had already experienced 3.6±4.2 cycles of ART on average. The two main concerns prior to starting ART were the possibility of never become a parent (57%) and self-questioning about their own accountability in prior failure of getting pregnant (46%). For 470 patients (45%), the first specialized consultation occurred less than 12 months after the start of natural attempts. Patients had started their reproductive project 3.8±3.6 years before participating to the survey. Satisfaction rates regarding medical care are above 80% for most items: quality of care (88%), amenities (86%), adequate answers to their questions (85%), availability of medical staff (83%), medical information (83%) and involvement of medical staff (83%). Overall, 77% of respondents reported that examinations needed for monitoring were burdensome or very burdensome. The most prominent feeling in case of ART failure were discouragement (65%), and the most frequent psychological consequences were fatigue (65%), distress (59%), and feeling of unfairness (53%). Important impact on sexual life was reported by 57% of respondents. They pointed out the lack of information about non-medical support (42%), treatments options and modalities (24%), criteria for medical care access and reimbursement (23%).
Limitations, reasons for caution: This was an online survey, potentially biased by the representativeness of study sample. Also, data are only descriptive, and the single-country nature of the study limits the generalization of these findings.
Wider implications of the findings: These findings showed high overall level of satisfaction regarding the medical care received. Nevertheless, the burden of infertility and of ART are not negligible and should be considered. ART units should be encouraged to develop non-medical support for all patients.
Trial registration number: not applicable

P-482 Working conditions and fertility quality of life (FertiQoL): a cross-sectional study in Japan

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Study question: Is there an association between working conditions and quality of life among women undergoing fertility care?
Summary answer: Working conditions for both women and their partners, such as the ease of taking days off, are significantly associated with fertility quality of life.
What is known already: More than 20% of Japanese women undergoing fertility treatment were reported to have resigned from their jobs due to difficulty in coping with work and fertility treatment. However, studies examining the impact of working conditions on fertility quality of life are lacking.
Study design, size, duration: We conducted a cross-sectional survey in Japan in January 2019. Participants were 721 women recruited via an online social research panel.
Participants/materials, setting, methods: Participants included women aged 25-44 years, having paid employment, and undergoing fertility diagnosis and/or treatment. They completed questionnaires to assess fertility quality of life (FertiQoL), job stress based on the demand-control-support model (Brief Job Stress Questionnaire, BJSQ), and working conditions of participants and their partners (e.g., working hours per week, ease of taking work days off rated on 5-point scale). They rated their partner's cooperation during fertility treatment and provided clinical and socio-demographic data.
Main results and the role of chance: Of the 1,223 eligible people, we sent recruitment emails to 958, and 721 completed the survey (75% participation rate). Twenty-nine percent of the participants reported that it was difficult (i.e., very difficult or difficult) to take days off for themselves and 58% reported it was difficult for their partners. The mean (SD) total score on the FertiQoL increased linearly with a higher level of ease to take days off: from 39.3 (15.7) to 57.1 (15.1) for the participants' time-off, and from 47.3 (15.4) to 57.2 (17.6) for their partners' time-off. The proportion of those who received high support from co-workers on the BJSQ was 12%. Multiple regression analysis showed that total FertiQoL score was significantly associated with the following work factors: self-employed (β = 0.10, compared with regular employees), high job demand (β = –0.13), high support from co-workers (β = 0.07), easy to take days off (β = 0.24), easy for partners to take days off (β = 0.10), partner works $\geq$ 50 hours per week (β = –0.08), after adjusting for partner's cooperation (β = 0.23), clinical (e.g., etiology, treatment stage), and socioeconomic factors. The association was evident especially in the Mind-Body, Social, and Treatment Tolerability domains.
Limitations, reasons for caution: We cannot infer causality due to the cross-sectional study design. The use of a social research panel and possible volunteer bias may limit the generalizability of the findings.
Wider implications of the findings: Flexible work arrangements and restrictions on long working hours would improve fertility quality of life, which might help employees balance work and fertility treatment. Considering the low proportion of participants receiving support from co-workers, social support at workplaces could assist women with fertility problem who tend to be psychologically isolated.
Trial registration number: Not applicable.

P-483 Psychological stress at the time of transvaginal ultrasound guided oocyte retrieval does not adversely affect in-vitro fertilization outcomes: A prospective observational study

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Study question: Does psychological stress at the time of transvaginal ultrasound guided oocyte retrieval (TUGOR) adversely affect in-vitro fertilization (IVF) outcomes?
Summary answer: Infertile women experienced stress during TUOGR, however psychological stress did not adversely affect the IVF outcomes.
What is known already: Women felt most stressful during TUGOR in the whole IVF treatment, because of the uncertainty and the pain experienced at the time of the procedure. However, it is unclear

whether physical and psychological stress would adversely affect the IVF outcomes. The majority of past studies measured stress level by the use of questionnaires; the use of stress biomarkers such as salivary alpha amylase (sAA) and salivary cortisol (sCort) may provide additional, objective assessment of the body's response towards stress.
Study design, size, duration: This prospective observational study took place in a university based hospital over a 12 month period. A total of 209 women undergoing TUGOR were recruited.
Participants/materials, setting, methods: Participants were asked to (1) complete two psychological questionnaires (Beck depression inventory-BDI; general health questionnaire-GHQ) before TUGOR, (2) fill in a questionnaire (State trait anxiety inventory–STAI) and collect saliva samples before and after TUGOR, and (3) rate the pain score by visual analogue scale (VAS-P) immediately after TUGOR. Results above the 75 th centile for each measurement were considered to represent high score. The psychological and biochemical stress scores in women with different outcomes were compared.
Main results and the role of chance: The median (25 – 75 percentile) of STAI scores (65, 56 – 71), sAA level (0.9 x 10^5, 0.5 x 10^5– 1.8 x 10^5) and sCort level (10, 7 - 14) after TUGOR were significantly (p = 0.001, p = 0.017 and p = 0.001 respectively) higher than those before TUGOR (56, 49 – 61; 0.8 x 10^5 , 0.5 x 10^5 – 1.3 x 10^5; 7, 5 – 9.8 respectively). There was no differences in age, body mass index, basal follicle-stimulating hormone, the duration of infertility and endometrial thickness between the various groups of subjects (non-conception, conception, miscarriage and live birth group). There was no significant differences in in sAA, sCort, VAS-P, STAI, GHQ, BDI scores between non-conception and conception group, and between live birth and miscarriage group. In additional, there was no differences in the number of oocytes retrieved, mature oocytes recovered and viable embryo produced between women with high or low stress score.
Limitations, reasons for caution: The high stress scores in this study represented only modest increase in stress level; it does not rule out the possibility that more marked increase in stress level could have an adverse effect.
Wider implications of the findings: Although women do find the TUGOR procedure painful and stressful, they should be reassured that a modest increase in the amount of stress does not appear to affect the IVF outcomes.
Trial registration number: Not applicable

P-484 Development of a measure to assess Infertility-related external and internal shame

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Study question: Is there any short, valid and reliable measure to assess the experience of infertility-related shame?
Summary answer: The Infertility external and internal shame scale (I-EISS) is a short, valid and reliable tool to assess the experience infertility-related shame in men and women.
What is known already: Shame is a universal human emotion that plays a critical role in psychosocial functioning and development. Shame can be painful and incapacitating as it involves feelings of inferiority, defectiveness, uselessness and isolation, and has been associated with depressive symptoms in a wide range of populations. Studies on emotional aspects of the infertility experience have often documented that shame is associated with infertility. Indeed, men and women with infertility often report feelings of defectiveness, unworthiness and low self-esteem or inferiority and social isolation, which often leads to difficulties in disclosing their infertility experience.
Study design, size, duration: Cross sectional study. Data was collected through self-report measures using online and paper-and-pencil methods. Inclusion criteria were age (18 years or older), and having an infertility diagnosis. A 8-item questionnaire assessing external (4 items) and internal shame (4 items) was developed to specifically assess infertility related shame, based upon a questionnaire developed by the same research team to assess general external and internal shame.
Participants/materials, setting, methods: Participants (*N*=245) are men (*n*=75, 30.6%)) and women (*n*=170, 69.4%) diagnosed with infertility. Of these, 98 were recruited online (40%) and 147 (60%) were recruited in the Reproductive Medicine Unit of an Universitary Hospital. Mean age is 34.88 (*SD*=0.32) for women and 35.74 (*SD*=0.50) for men. Self-report questionnaires were used to assess external and internal shame, Infertility related stress, depression, and shame. A confirmatory factor analysis was conducted to test factor model of the I-EISS.
Main results and the role of chance: Regarding the factor structure of the measure, a confirmatory factor analysis in a two-factor model was tested. This model revealed an very good fit to the data, with χ^2 (19) = 40.57; p =.003; CFI = .97; RMSEA = .08, SRMR = .04. Reliability was also high (α= 85 for external shame and .91 for internal shame). Criterion validity was assessed examining the correlations of the I-EISS subscale and other measure assessing general shame and results were of r = 0.60, p<0.001 for both subscales. We also tested the association of I-EISS subscales and the Fertility Problem inventory subscales assessing Social concerns (SocC), sexual concerns (SexC) and relationship concerns. External and internal shame were highly correlated with social concerns (r=0.65 and r=0.70, both p < 0.001), sexual concerns (r=0.43 and r=0.63, both p < 0.001) and marital concerns (r=0.42 and r=0.52, both p < 0.001). These results allowed us to establish the concurrent validity of the I-EISS with infertility-related emotional outcomes, namely infertility-related stress.
Limitations, reasons for caution: Due to the small sample size and the cross-sectional design, the results must be analysed with caution. Because sample was self-selected, patients with higher experience of shame may have preclude themselves from participating in the survey and therefore participants may not be representative.
Wider implications of the findings: The I-EISS can be used in research and clinical contexts to assess infertility related-shame and identify patients at risk of psychological malfunctioning. I-EISS holds potential interest for researchers and clinicians being a brief and reliable tool for assessment of a specific emotion that seems particularly relevant in infertility-related psychological adjustment.
Trial registration number: not applicable

P-485 Cycle Apps – help or confusion? Understanding women's perception towards cycle tracking apps

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Study question: How do women perceive the fertility predictions that are provided through calendar-based cycle tracking apps?
Summary answer: Women did not know the accuracy of fertility apps, with some believing calendar app predictions. This led them to question their fertility causing unnecessary stress.
What is known already: Monitoring lifestyle and health factors on their smartphones is now commonplace. Women looking for ways to reduce their time to pregnancy often turn to calendar-based fertility apps when trying to conceive.

Studies have shown that the day of ovulation can vary between women with the same cycle length which means that predictions of apps solely using cycle length are often found to be inaccurate or imprecise. Information on the accuracy of these apps is scarce, making it hard for women to make an informed decision on their use.
Study design, size, duration: This study was a qualitative component of a larger mixed methods study examining the efficacy of the Clearblue Connected Ovulation Test System. We performed 38 qualitative in-depth interviews on women randomly sampled from both study arms and all study outcomes (conceived after 1 or 2 cycles, or did not conceive). All women were provided ovulation tests either during or after study completion. Interviews were conducted May – September 2018.

Participants/materials, setting, methods: Women aged 18-40 who had a smartphone and were actively trying to conceive were recruited from across the UK. Telephone interviews were conducted to discuss their views and experience of trying to conceive. Data was transcribed and analysed by thematic framework analysis. Ethical approval was given from the internal review board.
Main results and the role of chance: The initial trust in calendar-based apps varied, from believing that they would not be on the market if they didn't work, to others using them as a guide or record. Some women did understand their limitations with one saying "I'm wanting to not work off estimations, I want to work on something that is more accurate and more specific to me".

When using ovulation tests women found the results did not always correlate with predictions from calendar-based apps. Comments included ". . .obviously it is incorrect. . .. . .because I was ovulating weeks later", ". . .my fertile time [from the ovulation test] started when the other [calendar-based] app said it would have finished".

Querying the impact of inaccurate predictions women mentioned they would have queried their body rather than the app, "I think I probably would have questioned myself before I questioned an app, it's hard to question technology really, isn't it? Because you think it's got the answers to everything". This may have resulted in delays to pregnancy ". . .would've taken a hell of a lot longer for me to conceive if I had just used the app." "If I hadn't have used the ovulation kit, I don't think I would be pregnant...".
Limitations, reasons for caution: Due to recruiting online, participants were self-selecting and the data reflects those who applied to the study.
Wider implications of the findings: Some women question their bodies before fertility apps. Following incorrect advice from calendar-based apps could prolong their time to pregnancy and cause unnecessary stress. We would recommend that fertility apps provide more information on their accuracy, so women can make an informed choice on how to use them.
Trial registration number: NCT03424590

P-486 Does socioeconomic status (SES) affect anxiety/depression in women undergoing assisted reproduction treatment?

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Study question:

The aim of the study was to investigate whether SES is associated with higher levels of anxiety/depression in women undergoing assisted reproduction treatment.

Summary answer: We found no correlation between SES and anxiety/depression levels among subjects studied.
What is known already: SES affects overall human functioning, including physical and mental health. Financial burden due to fertility treatment contributes to worsening of quality of life and treatment dropout. There are no studies correlating SES and anxiety/depression levels in assisted reproduction patients.
Study design, size, duration: Cross-sectional study conducted between April-December 2018.
Participants/materials, setting, methods:

127 women undergoing fertility treatment in a private clinic were included. They underwent SES evaluation and were divided in 4 groups according to family income. All fulfilled a questionnaire on demographic data, period of infertility, previous treatments. Subjects also fulfilled IDATE anxiety test and Beck Depression Inventory.
Main results and the role of chance: There was no significant association between SES and depression (p=0,39) or anxiety (p=0,67). There was no significant association between number of previous attempts and depression (p=0, 06) or anxiety (p=0,74). However, we found a statistically significant association between period of infertility and depression (p=0,02).
Limitations, reasons for caution: Considering our sample size, more studies are required to confirm these findings.
Wider implications of the findings: To wait for a child in the context of infertility seems to be the most important factor leading to anguish and depression, overcoming the need of successive treatments or financial issues.
Trial registration number: Not aplicable

P-487 Cross-border reproductive care from the Netherlands to Belgium explained by shared-decision-making

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Study question: Can the cross-border movement from Dutch IVF-patients to Belgian clinics be explained by a difference in shared decision-making?
Summary answer: Most Dutch patients travelled to Belgium for shared decision-making (SDM) and experienced SDM on IVF was indeed higher in Belgium as compared to the Netherlands.
What is known already: Dutch patients travel to the Dutch speaking part of Belgium for Cross-Border Reproductive Care (CBRC). Belgian and Dutch clinics offer Dutch patients equally successful reimbursed in vitro fertilisation (IVF). Quantitative research clarified that Dutch patient's most cited reason for traveling is 'higher quality care' (Shenfield et al., Hum Reprod, 2010). Dutch patients clarified in interviews and on internet fora that they expected and experienced more shared decision-making (SDM) in Belgium (Van Hoof et al, Facts Views Vis Obgyn 2014). Dutch patient's expectations and experienced level of SDM in Belgium and the Netherlands had yet to be quantified and compared.
Study design, size, duration: Last winter (2017-2018), we conducted a multicenter retrospective survey on the motivations and experiences of 320 heterosexual couples living in the Netherlands and having traveled to Belgium for IVF between September 2015 and September 2017.
Participants/materials, setting, methods: In total 104 couples completed minimally half of the questions send out by three Belgian IVF-clinics. Two dichotomous questions assessed whether expected SDM and informed decision-making (IDM) motivated couples to travel. The valid and reliable 'SDM-Q9' questionnaire (Kriston et al, Patient Educ Couns, 2010), assessed the levels of experienced SDM (0-45) and IDM on IVF (0-27; the higher, the better). Paired and independent sample t-tests assessed within and between participant differences in SDM and IDM.
Main results and the role of chance: Of the 104 participating Dutch couples (participation rate=33%) having IVF in Belgium, only 32 previously had IVF in the Netherlands (30%). Most couples (n=53/104) travelled because they expected more SDM and/or more IDM in Belgium. The SDM-Q9 reliably assessed the levels of experienced SDM and IDM in our sample (respectively: Cronbach-alpha=0.89 & 0.78; Item Total Correlation $\geq$0.36 & $\geq$0.49). Experienced SDM and IDM on IVF in Belgium, did not differ between the three Belgian clinics (respectively: p=0.904; p=0.627). Dutch patients treated with IVF in both countries (n=32) experienced more SDM and more IDM on IVF in Belgium as compared to the Netherlands (respectively: paired difference=7.1 +/-9.5; p<0.001 & paired difference= 2.0 +/-3.5; p=0.003). Neither of these differences seem due to second rather than first opinion seeking in Belgium, as having had IVF in the Netherlands (n=32) or not (n=72) did not affect the experienced level of SDM or IDM on IVF in Belgium (respectively: 31.8+/- 8.3 vs 34.0 +/- 8.3; p=0.21 & 10.9+/- 2.9 vs 11.9 +/- 2.8; p=0.12).
Limitations, reasons for caution: What drove participation might also drive expectations and experiences of SDM. Focussing on couples who had IVF in both countries, ensured that SDM and not accessibility was compared. Paired t-tests rather than multilevel modelling were appropriate as Belgian clinics did not differ but numbers per Dutch clinic were too small.
Wider implications of the findings: This study proves that SDM rightfully drives clinic surfing across national borders. This novel finding implies that SDM can affect clinic's market share. Whether clinic's ability to provide SDM

also drives national IVF-clinic surfing and even IVF-discontinuation should be examined.

Trial registration number: Not applicable

P-488 Evaluation of depression and anxiety levels in IVF / ICSI cycles of patients with endometriosis; a prospective study

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Study question: Can psychological health levels and scales be important in endometriosis patients during ART ?

Summary answer: Although the depression level is higher in endometriosis patients, it does not cause any changes in pregnancy rates in ART cycles.

What is known already: Pelvic endometriosis negatively affected the quality of life of women and caused significant depressive and anxiety symptoms. Controlled trials have shown that endometriosis can adversely affect women's psychological health and overall quality of life. Although several studies on IVF treatment have shown that psychological distress before and during the treatment did not affect the likelihood of pregnancy, there are not enough studies examining the effect of psychological health levels on the ART cycle in patients with endometriosis.

Study design, size, duration: This study was designed prospectively. A total of 93 women in the IVF/ICSI program at the educational research hospital between October 2017 and March 2018 agreed to participate in the study. Patients were divided into two groups as endometriosis (n:37) and non-endometriosis (n:57) group. Patients were included in the study regardless of ART indication. But the presence of psychopathological disease and chronic systemic disease was defined as exclusion criteria.

Participants/materials, setting, methods: The WHOQOL-BREF short-form quality of life questionnaire, the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI) scale were used to determine the psychological health levels before IVF treatment. Psychological health levels were defined as percentiles, and depression and anxiety scales were categorized through the scores (normal, mild, moderate-severe). In addition, demographic features, stimulation parameters, oocyte-embryo quality, and clinical pregnancy outcomes were recorded. SPSS 25 program was used in the analysis.

Main results and the role of chance: Ninety-three infertile patients underwent IVF treatment and 26 positive pregnancies are achieved. No statistically significant difference was observed between the two groups in terms of age, body mass index, day two FSH, LH and estradiol levels, antral follicle count and infertility reasons (p> 0,05). Moderate-high depression was 8,9 percent in non-endometriosis group and 37,8 percent in endometriosis group. A significant difference was found between endometriosis and non-endometriosis groups in depression scores (p <0,01), but no significant difference in psychological health levels and anxiety (p: 0,897, p:0,058,respectively). A significant correlation was observed between depression and endometriosis (CC: 0,435, p<0,01) In terms of stimulation parameters no significant difference was observed between two groups, except total gonadotropin dose. Total and MII oocyte counts, number of embryos and pregnancy outcome were similar in both groups (p> 0.05).When the effects of psychological health, depression and anxiety scores on pregnancy outcomes were examined, no significant relationship was found.

Limitations, reasons for caution: We can say that one of our limitations is the number of patients. Increasing the number of patients can prevent errors that we cannot foresee.Another issue is that the study was designed as a single center.

Wider implications of the findings: We observed a relationship between endometriosis and depression scores similar to the literature. With wider studies in future, we think that the role of psychological factors in IVF treatment and its relationship with endometriosis can be elucidated.

Trial registration number: not applicable

P-489 The impact of a 12-hour mindfulness-based wellness curriculum on obstetrician/gynecologists (OB/GYN) residents' burnout, mindfulness, and self-compassion.

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Study question: Does participation in a 12-hour mindfulness-based wellness curriculum decrease burnout, increase mindfulness, and improve self-compassion in a group of OB/GYN residents?

Summary answer: Overall self-reported levels of mindfulness and self-compassion increased at a 6-9 month follow-up assessment, but burnout did not change.

What is known already: A lack of physician wellness due to burnout is a serious problem impacting over half of obstetrician/gynecologists (OB/GYN) in the United States. Efforts to reduce burnout in medical training and post-graduate practice settings have focused on increasing resilience through mindfulness and compassion-based programs, as mindfulness and self-compassion are positively correlated with well-being in the general population. There is some evidence that these programs reduce burnout. However, effectively reducing burnout in residency training programs may be limited by inflexible institutional factors such as demanding rotations, extended work hours, stigma associated with admitting burnout symptoms, and limited time for social support.

Study design, size, duration: Fourteen 1st year OB/GYN residents (n=7 in two separate cohorts) completed a 12-hour, in-person mindfulness-based resilience curriculum over a 3-month period that emphasized meditation training, emotional awareness, empathic patient communication, self-compassion, gratitude and value-based living. The curriculum included didactic and experiential components, along with home-based exercises in meditation, gratitude, and value-clarification. The curriculum was implemented at a California medical school between January 2017 and May 2018.

Participants/materials, setting, methods: Assessments occurred prior to the start of the curriculum (T1), at the end of the curriculum (T2), and 6-9 months later (T3). Participants completed the Maslach Burnout Inventory (MBI), the Five Facet Mindfulness Questionnaire (FFMQ), and the Self-Compassion Scale (SCS) during each assessment period. Qualitative focus groups were also conducted with both cohorts of residents following the conclusion of the programs (July 2017, August 2018) and themes were analyzed using a grounded theory approach.

Main results and the role of chance: Paired samples t-tests examined changes in scores from T1 to T2 and T3. Levels of burnout did not change significantly from T1 to T2 or T3. However, overall self-reported levels of mindfulness increased from T1 to T3 ($p<.05$), as did the mindfulness subscale of non-judging inner experiences ($p<.01$). Overall levels of self-compassion also increased from T1 to T3 ($p<.05$), with the specific subscales of self-kindness and self-judgment showing improvements from T1 to T3 (both $p<.05$). Levels of isolation (a subscale on the SCS) significantly decreased from T1 to T2 ($p<.01$), however, no other significant changes from T1 to T2 were observed for mindfulness or self-compassion. Thus, the vast majority of improvements were evident only at the 6-9 month follow-up. The delayed nature of improved mindfulness and self-compassion may indicate a developmental process of skill building that equated to lasting change. It may also be indicative of adjustment to the demands of residency training as residents were now in the second year with more experience and more effective coping strategies. Qualitative reports indicated that increased emotional awareness could make stress worse in the short-term, however, residents also commented that mindfulness training may become more valuable over time.

Limitations, reasons for caution: Due to the small sample size, caution is warranted in generalizing findings. Qualitative focus-groups indicated that increasing emotional awareness could increase short-term stress and burnout. Furthermore, unchanging institutional factors in residency training program (mandatory challenging rotations, inflexible work hours) may obscure the impact of mindfulness-based resilience training on resident burnout.

Wider implications of the findings: This pilot study provides preliminary support that a mindfulness-based resilience curriculum may improve overall levels of mindfulness and self-compassion in 1st year OB/GYN residents. As these traits are related to decreased psychological stress and increased wellness in other populations, larger scale studies to further examine this issue are warranted.
Trial registration number: not applicable

P-490 2018 study of 2,013 donor-conceived people

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Study question: What are the experiences, desires, and needs of the donor-conceived community?
Summary answer: Donor-conceived people desire to know about their origins and to connect with their donor relatives.
What is known already: The Donor Sibling Registry has been connecting, supporting, counseling and studying donor-conceived people for more than 18 years. We know that many donor offspring desire to know more about their ancestry, medical backgrounds, and about their first and second-degree genetic relatives.
Study design, size, duration: In 2009 and again for three weeks in October of 2018 surveys with both quantitative and qualitative questions were collected reporting on the experiences of being a donor-conceived person, including disclosure, terminology, anonymity, missing father/male figure, feelings about being donor-conceived, and curiosity about and contact with previously unknown genetic (donor) relatives.

Participants in 2018 were asked to *not* take the survey again if they already had in 2009. Each participant is, therefore, a unique case.
Participants/materials, setting, methods: 1268 individuals responded to the survey designed for donor-conceived people raised in non-LGBTQ households (HET) (467 in 2009 and 801 in 2018).

745 individuals responded to the survey designed for donor-conceived people raised in LGBTQ households (LGBT) (287 in 2009 and 458 in 2018).

64% of LGBT and 74% of HET respondents were female. 14 LGBT and 69 HET respondents were conceived using an egg donor and the balance were conceived with a sperm donor.
Main results and the role of chance:
Disclosure

69% of LGBT offspring and 32% of HET offspring indicate that they have "always known" about being donor conceived.

In the HET families, over 50% of those under 18 have "always known." This number is considerably less for older cohorts.
Terminology

"How do you refer to the donor? LGBT Sperm Donor Offspring: 53% Donor, 30% Sperm Donor, 18% Biological Father, 14% Donor Dad, 8% Father, 2% Genetic Father, 10% Other

HET Sperm Donor Offspring: 41% Biological Father, 41% Donor, 39% Sperm Donor, 12% Donor Dad, 7% Father, 6% Genetic Father, 8% Other
Anonymity

87% of HET offspring, 69% of LGBT offspring said that their parents used an anonymous donor.

"If your donor is anonymous, do you wish that your parent(s) would have used a willing-to-be-known or known donor?"

59% of LGBT offspring and 73% of HET offspring answered "Yes".
Missing Father/Male Figure

"If you are conceived via sperm donation, and have been raised by a single mom, have you felt something missing from your not being parented by a father/male figure?"

52.6% HET offspring answered yes, 37% LGBT offspring answered yes.
Limitations, reasons for caution: 52% of respondents to the HET survey and 42% respondents of the LGBT survey reported that they are Donor Sibling Registry members.

The data is, therefore, representative of both Donor Sibling Registry members and non-members.
Wider implications of the findings: The reproductive medicine industry can better serve the needs of the donor-offspring community by better understanding their experiences and desires to know more about their origins and to connect with their close relatives.
Trial registration number: None

P-491 Assessing pain-related psychological inflexibility in endometriosis

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Study question: Is the Psychological Inflexibility in Pain Scale (PIPS) adequate for the assessment of psychological inflexibility in women facing a diagnosis of endometriosis?
Summary answer: Results suggest that the Portuguese version of the PIPS is a valid and reliable instrument for the assessment of psychological inflexibility in women with endometriosis.
What is known already: Endometriosis is a chronic, incapacitating condition which may involve experiencing chronic pain. Assessment instruments addressing emotion regulation processes, such as avoidance of pain and cognitive fusion with pain, which are components of psychological inflexibility, in this population are scarce. ThePsychological Inflexibility in Pain Scale was developed to assess these processes related to chronic pain and its psychometrics properties have been tested in other medical conditions implicating chronic pain such as whiplash associated disorders, fibromyalgia and back pain. Assessing these emotion regulation mechanisms allows the identification of relevant targets for psychological intervention with women dealing with endometriosis-related pain.
Study design, size, duration: Participants were recruited 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. Data were collected online through self-report instruments between February 2018 and May 2018. A sub-sample of 88 participants also completed the PIPS-PT 4 week later to the first administration.
Participants/materials, setting, methods: A sample comprising 209 women with an endometriosis diagnosis completed online a sociodemographic questionnaire, the PIPS-PT, the Numeric Pain Rating Scale (NPRS), the Depression, Anxiety and Stress Scales (DASS – 21), the Chronic Pain Acceptance Questionnaire (CPAQ) and the Acceptance and Action Questionnaire - II (AAQ-II). A confirmatory factor analysis was conducted, as well as reliability, and relationship of the PIPS-PT with others measures.
Main results and the role of chance: The PIPS-PT two factor model: (1) avoidance of pain and (2) cognitive fusion, showed the following adjustment indices: $\chi 2/gl = 2.72$, CFI = .92, GFI = .86, TLI = .91, RMSEA = .091, MECVI = 1.68. As for the Cronbach alpha values .94 was observed for the total scale,.94 for the pain avoidance subscale and of .82 for the cognitive fusion subscale. The composite reliability (CR) revealed a value of .95 for the total scale, of .93 for the pain avoidance subscale and of .82 for the cognitive fusion subscale. Test retest reliability showed a correlation of .75 ($p < .001$). The correlation between the two PIPS-PT subscales was of .69 ($p < .001$). The PIPS-PT showed positive correlations with the AAQ-II ($r = .49$; $p < .001$), the Numeric Pain Rating Scale ($r = .51$, $p < .001$), the depression scale ($r = .45$; $p < .001$), the anxiety scale ($r = .38$; $p < .001$) and the stress scale ($r = .43$; $p < .001$) and a negative correlation with the CPAQ ($r = -.60$; $p < .001$).
Limitations, reasons for caution: The online recruitment tends to recruit more educated participants, with easy access to online platforms. Participants diagnosis of endometriosis was self-reported, there was no consulting of medical records.
Wider implications of the findings: The PIPS-PT proved to be a valid and reliable measure for psychological inflexibility related to chronic pain for women with endometriosis. Women with endometriosis-related pain show a high prevalence of psychiatric disorders. The PIPS-PT allows to assess relevant psychological intervention targets. It can be used in clinical and research settings.
Trial registration number: N/A.

P-492 Participant experiences with an evidence-based diet and lifestyle group educational and support program: a feasibility study

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Study question: What are the experiences of participants in a group educational health information program on diet, lifestyle and stress management for fertility?

Summary answer: Overall positive experiences were noted among participants. Themes of trust, the nature of content, group dynamic, commitment, and facilitator expertise emerged as key common experiences.

What is known already: Individuals undergoing fertility treatment often make lifestyle choices that negatively impact their fertility success rates. The experience of infertility can increase psychological, physical, and financial stress on an individual/couple. Recent studies on mindfulness and cognitive behavioural therapy-based interventions, and group-based interventions are of benefit to patients. Given the complex psychosocial and emotional impact that infertility can have on individuals, there is the likelihood that an evidence-based diet, lifestyle and stress-reduction group-based, educational program would be beneficial to participants. The purpose of the current study was to examine the impact of a diet, lifestyle and stress-reduction group-based, educational model of care.

Study design, size, duration: Nineteen participants were enrolled in a 6-week evidence-based educational pilot program providing support related to healthy diet, lifestyle and stress-reduction behaviours in a group setting. Participants were provided information in a lecture-style format and were trained on stress reduction techniques including deep breathing and mindfulness exercises. Participants completed pre and post standardized questionnaires including open –ended options assessing satisfaction, challenges and adherence to different program components provided a framework for program evaluation and participant experiences.

Participants/materials, setting, methods: Participants were current patients of an urban fertility clinic in Toronto where the sessions took place. Of the 19 participants, 13 were female and 6 were male. Twelve participants attended as a couple and 7 attended individually. On completion of the 6-week program participants were asked complete evaluation of the program. Results were analyzed using descriptive statistics (quantitative data) and thematic analysis (qualitative data).

Main results and the role of chance: Survey data was collected from 15 of the 19 participants at the end of the program. Participants attended anywhere from 2-6 sessions; open-ended feedback states that despite commitment to the program, travel and scheduling posed a logistical inconvenience, in some cases adding additional stress. Many responses included different suggestions for scheduling, and number of sessions. Most participants indicated that they were hoping to learn about dietary and lifestyle strategies, including stress reduction techniques to improve their overall health and fertility. Participants rated the material as being very useful (average 4.67 out of 5), and when asked how many of the diet and lifestyle strategies they were able to implement (0=none - 5=all) the median response was 3 (IQR=3-4). Most participants (86.6%) enjoyed the group format of the sessions, although some mentioned that they were apprehensive about this at first; feedback shows that trust in facilitators as well as co-attendees was critical in creating a safe and supportive dynamic. Participants were also exceptionally likely to recommend this program to others (median=5, IQR=5). All appreciated the knowledge and expertise of the facilitators, although divergent views and preferences on the quantity of topics and depth of content were exhibited.

Limitations, reasons for caution: Limitations of the study's findings include;

1. Small sample size
2. 21% loss to follow-up
3. The facilitators and co-participants were likely to influence each participants experience
4. There were no matched controls and no long-term pregnancy follow-up, all of which add elements of bias and subjectivity to the findings

Wider implications of the findings: This feasibility study indicates that participants who are patients of a fertility clinic are willing to participate, and are satisfied with a 6-week group program to learn how to implement positive diet, lifestyle and stress-modification behaviours. This allows for a starting point for future research on this model of care.

Trial registration number: N/A

P-493 A novel and easy method for implementation of donor selection: a new useful algorithm

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Study question: How can we improve the donor selection to increase the resemblance between receptors and their offspring?.

Summary answer: It's necessary to implement new algorithms including biometry distance measurements across family generations to improve donor selection according to the facial resemblance.

What is known already: In addition to the essential medical criteria in donor selection, there are basic criteria in facial resemblances, such as eyes and hair colour.

In order to improve this focus including facial biometric algorithms, several R and Phyton packages are available based on facial recognition and the probability of being the same person. Unfortunately, this approach is not valid as sorting images by biometric distances is needed to lead to a scientifically verified result. So, a new Data Scientist approach using specific and innovating methodologies with Big Data and Artificial Vision Technologies is required.

Study design, size, duration: During the last two years, a Data Science study based on biometry distances, artificial vision technologies and neuronal net algorithms has been developed.

Creation of the new algorithm was based on the study of family trees (n = 108), with an innovative approach using pre-ponderation of facial biometry distances (12,208) and allow establishing measurements on results.

In addition, the new algorithm was compared to the pre-existing facial recognition approach.

Participants/materials, setting, methods: A total of 864 subjects (age 18-60) from 108 families were included with their participant agreement (73% training - 27% validation). All images correspond to 21-50 years old subjects, with a gender distribution 1:1. Several scripts in Python and R were developed to classify 12,208 facial distances of each picture using K-means algorithms to study facial similarity objects. 80 Gb RAM and 60 computing processors were used intensively. Artificial Neural Networks methodologies were used.

Main results and the role of chance: The new algorithm is stable and can reproduce results when iterating validation data. The new prediction model based on biometric distances results is 86,66%(σ2 47.88) if we require all images must be in the right order to get a positive result. The accuracy results improve performance up to 96% when the right order is just required in the 5 images with more resemblance. Comparison between images is independent of the subject gender providing same accuracy results for gender-crossed images as for same-gender.

As final extra testing, when the test was made by the computational algorithm and also by a human team (10 individuals with a majority vote criteria), humans results are 98% in line with the new algorithm.

When doing the same testing with the two most used existing open python scripts based on facial recognition average results provide 56.88% of accuracy(σ2 638.98) in ordering subjects right. When comparing gender-crossed images test shows just a 23.38%(σ2 89.23) of accuracy.

We can conclude that the new algorithm is valid as it has great accuracy in its results and it has widely improved results in the current state of the art.

Limitations, reasons for caution: The algorithm should be used as a decision support application that helps the medical team decide on a donor from among those who already meet the medical and genetic criteria in order to guarantee to find the donor that most resembles the receptor's family.

Wider implications of the findings: This application provides more confidence and reduces receptor stress caused when addressing the decision of accepting a gamete's donation.

The new findings in Artificial Vision when locating objects in images may serve in new time-lapse technology studies of embryos.

Trial registration number: not applicable

P-494 Fertility knowledge and beliefs about fertility: findings from a cross-sectional survey in Tunisia

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Study question: The aim of this study was to evaluate the level of fertility knowledge among the reproductive-aged population.

Summary answer: We evidenced a lack of knowledge with regards to fertility issues in our study population.

What is known already: Fertility knowledge is an important factor that can influence individual and couple's fertility behavior. Numerous reports have highlighted a lack of information about fertility in the general population, especially in relation to risk factors for infertility and treatment beliefs. However, few data are available about such issues among Tunisian population, and Arabic-speaking countries in general.

Study design, size, duration: A 14-items questionnaire on the knowledge of infertility demography (2 items), risk factors (10 items) and treatments (2 items) was completed by a cross-sectional sample, over a period of 3 months.

Participants/materials, setting, methods: The sample (n=150) consisted of 83 female and 67 male with an average age of 32.5±9 years and 45.8±14 years, respectively. Knowledge scores were based on responses to the questionnaire. The validity and reliability of the questionnaire were previously tested. Questionnaires were completed online (74%) and in our fertility testing department (26%).

Main results and the role of chance: Average knowledge score per participant, corresponding to the mean percentage of correct answers on all 14 questions, was 48.9 ± 2% ranging from 0 to 85.7%. The majority of respondents (n = 96, 64%) had a knowledge score between 34 and 66%. The lowest scores were for questions related to infertility prevalence (7%) and to health insurance coverage of fertility treatments (7%). Questions related to the impact of stress and smoking on fertility have had the highest scores (87% and 81%, respectively). Only 36% of respondents have correctly identified the age of fertility decline among women. The score was even lower for age-related fertility decline in men (21%).

Limitations, reasons for caution: The majority of participants have responded online which could indicate a better level of education and thus lead to an overestimated score of knowledge.

Wider implications of the findings: A wider implementation of the questionnaire will allow us to improve its reliability and to identify the major knowledge gaps that have to be especially considered while developing population education programmes.

Trial registration number: No trial registration number

P-495 Consideration of a multi-method assessment approach to the psychological evaluation of gestational surrogates

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Study question: What is the utility of a multi-method assessment approach to screening gestational surrogates (GS) for determining both psychological appropriateness and for pre-surrogacy implications counseling?

Summary answer: Utilizing multiple assessment methods can give more specific information about psychological vulnerabilities that are important to address during implications counseling prior to a surrogacy pregnancy.

What is known already: Psychological assessment of GS candidates requires consideration of complex factors in determining their psychological appropriateness to proceed with mental health professionals often placed in gate-keeping roles. ASRM guidelines recommend psychosocial consultations and psychological testing (where deemed appropriate), but there are no uniform interpretations of these guidelines. Using a single, broad-band self-report measure (such as the MMPI-2 or PAI) is the most common practice among mental health professionals asked to assess commercial arrangements in the US. However, recent research suggests that surrogates consistently underreport and deny problems and their MMPI-2 and PAI profiles are often defensive and difficult to interpret.

Study design, size, duration: This retrospective chart review examined psychological test data from women screened and accepted to become GSs (n = 33) between January, 2010 and March, 2018. Data from two psychological tests were scored and entered into SPSS software. GS test data from these tests were compared as measures of the psychological constructs most important in consideration of a potential surrogate, including Self-Esteem. Resiliency, Self-Efficacy, Problem-Solving/Coping, and Interpersonal Relationships.

Participants/materials, setting, methods: 33 women seen in private practice and psychologically evaluated for consideration as surrogates were administered the Personality Assessment Inventory (PAI) and an abbreviated protocol of the Thematic Apperception Test (TAT) (Cards 1, 2, 3GF, and 13MF). Data were scored using well validated scoring systems. Analyses (t-test and chi-square) examined both their measurement of the psychological constructs of interest and comparison to published normative data. .

Main results and the role of chance: PAI Positive Impression Management (PIM) scores between 44T-56T reflect a respondent presenting themselves realistically. Therefore, PAI profiles deemed least defensive (PIM $\leq$ 56; n = 19) are reported here. Corresponding TATs didn't show significant differences between groups, so all (n = 33) are reported here. Lower Self-Esteem was reflected in PAI Aggression scale scores with the majority (65%) of scores $\leq$ 44, suggesting meekness and difficulties with assertiveness. Likewise, lower Self-Esteem on the TAT (n = 33) was reflected both in Emotional Tone on Card 1 reflecting sadder themes than the normative population ($p < .001$) and "Inferiority" and "Depression" endorsed at the highest rate on Fine's Scoring Scheme. Interpersonal relations were reflected on the PAI Warmth scale, with 32% (n = 6) having t-scores $\geq$ 60. Although this suggests a warm and sympathetic personality style, it reflects potential difficulties with confrontation and conflict. 79% of PAI Treatment Rejection Scale scores were > 50. Scores > 60 (n = 6) reflect individuals who are rejecting of intervention, which might reflect higher levels of self-efficacy but create potential difficulties with implications counseling. Tendencies towards denial of card pull and task demand/difficulty may reflect magical thinking impacting both resiliency and problem-solving skills.

Limitations, reasons for caution: This is a retrospective chart review and TAT scores obtained for present research were not considered in the acceptance or rejection of the GSs in this sample. Statistical analyses were based on the theoretical assumption that published norms represented the general non-patient population and deviations from this assumption are unknown.

Wider implications of the findings: Multi-method assessment may address limitations posed by single measure assessment, particularly given the defensive profiles often seen on self-report measures. Combined analyses may also help clarify psychological vulnerabilities that should be addressed during implications counseling prior to a surrogacy pregnancy to protect the psychological well-being of a gestational surrogate.

Trial registration number: not applicable

P-496 Out of sync: A qualitative examination of male and female partners' role in decision-making in in vitro fertilization (IVF)

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Study question: What role do male and female partners play in the process of reproductive decision-making and how aligned are their expectations?

Summary answer: In most cases, male and female partners hold disparate views on whether treatment decisions should be made jointly or by women alone.

What is known already: Couples face multiple, significant decisions in IVF. However, people's capacity to make decisions decreases in stressful situations. In this scenario, the provision of accurate information and support from clinicians may empower couples to make informed treatment decisions. Active involvement of patients in decision-making is highly desirable, and is predictive of treatment compliance and better psychosocial adjustment. However, most research on reproductive decision-making is conducted with women, rather than men or the couple, as the unit of analysis. This risks overlooking the role and preferences of male partners, as well as couple-level factors that might influence the course of treatment.

Study design, size, duration: Participants were infertile couples who received at least one cycle of IVF recruited from a local fertility clinic in Hong Kong. Two two-hour parallel focus group interviews were conducted with male and female partners separately.

Participants/materials, setting, methods: Focus group interviews were conducted with five couples (*n*=9; one woman attended without partner). The age range of participants is 32-39 for women and 37-41 for men. Two couples report male factor as the cause of infertility, two couples report female factor, and one couple report mixed factors. The number of previous ART cycles performed ranged between zero and seven. Interviews were tape-recorded and transcribed. Verbatim transcripts were analyzed using a thematic, inductive approach.

Main results and the role of chance: Our analysis revealed two major findings. First, whilst men often willingly hand over the right to make important decisions to their partner, women expect men to involve actively and to make decisions jointly as a couple. Second, and consistent with existing knowledge, the physical and emotional needs of women constantly take precedence over that of men. Couples, especially women, think it is desirable when they are 'on the same page' with their partner when making treatment decisions. However, joint decision-making in treatment may be difficult if partners are not aligned in one of four main areas, which are summarised as, strategies used to arrive at decision, perceived importance of childbearing, emotional investment in fertility treatment, and acquiring fertility-related information.

Limitations, reasons for caution: Participants were recruited from one fertility clinic through self-selection. The low response rate, especially for male partners, means our sample may principally comprise couples who have stronger marital relationship, find IVF more palatable, and who are less concerned about disclosure.

Wider implications of the findings: Educational programs should be directed at increasing men's self-efficacy in making informed treatment decisions. Reproductive counselors should better address men's perceived barriers in decision-making and facilitate joint decision-making with the couple as a unit.

Trial registration number: Not applicable.

P-497 What about decisional regret regarding fertility preservation one year after diagnosis? A randomized controlled trial of a decision aid for female cancer patients

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Study question: Does the use of an online decision aid in addition to counseling with a reproductive specialist have an impact on decisional regret?

Summary answer: Decisional regret was low in both groups. The intervention group showed a decrease, whereas the control group showed an increase in decisional regret over time.

What is known already: Female cancer patients perceive deciding whether to opt for fertility preservation (FP) or not as very difficult and emotionally challenging. They report decisional conflict and decisional regret and express the need for more support. Information provision and being aware of their own values is associated with lower decisional conflict and regret. We could demonstrate that the use of our online decision aid (DA), in addition to fertility counseling, lowers decisional conflict and patients show high satisfaction with the additional support.

Study design, size, duration: The study was a randomized-controlled trial including women, who were referred to one of the participating fertility centers after having been diagnosed with cancer. Participants were either assigned to the control group (counseling only) or to the intervention group (counseling and additional use of the online DA immediately after counseling). Recruitment was ongoing for 18 months at eight fertility centers in Switzerland and Germany.

Participants/materials, setting, methods: The online DA was developed by an interdisciplinary team of reproductive specialists, gynecologists and psychologists. Participants were asked to complete an online questionnaire at three time points: after counselling (control group) or after counseling and the use of the DA (intervention group) (T1) as well as one month (T2) and 12 months later (T3). The survey comprised questions about fertility-related knowledge, decisional conflict, decisional regret, attitude towards FP, willingness to undergo FP and socio-demographic data.

Main results and the role of chance: Whereas the main outcome measure of this RCT was decisional conflict, this abstract focuses on one of the second outcome measures, i.e. decisional regret over the course of time (T2 to T3) in those participants with follow-up until one year after diagnosis (N=37).

Both groups showed low scores on the decisional regret scale (from 0 min. to 100 max.) at both time points (T2 and T3), whereby the intervention group consistently reported lower scores compared to the control group. The control group showed an increase of decisional regret from T2 (Mean: 19.00, SD: 13.24) to T3 (Mean 22.00, SD: 20.67). The intervention group, however, showed a decrease of decisional regret from T2 (Mean: 14.12, SD: 11.07) to T3 (Mean: 12.94, SD: 13.24).

Neither at T2 (p=0.113) nor at T3 (p=0.129) the group differences reached statistical significance. The difference in the change over the course of time of each group almost reached significance (p=0.078).

Limitations, reasons for caution: The study included 51 participants at T1, however until the end of all follow-up analysis, there were 14 dropouts to note. Decisional regret is a second measure outcome of this study, as a consequence statistical significant differences could not be expected. Therefore, the results can only be interpreted with caution.

Wider implications of the findings: These longitudinal data complement our previous results that showed a beneficial short-term effect of the DA regarding decisional conflict. The use of the online DA seemed to support patients also regarding decisional regret. The findings all together reinforce the qualification of the DA to be implemented into clinical practice.

Trial registration number: NCT02404883 (clinicaltrials.gov)

P-498 Same-donor offspring networks: Donor-conceived people's experiences with making contact, through group meetings, with same-donor offspring

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Study question: What are the experiences of donor-conceived people with making contact, through group meetings, with same-donor offspring?

Summary answer: While relationships with same-donor offspring are viewed as beneficial, they can cause confusion and questions about how to integrate them into one's life.

What is known already: A recent phenomenon is the contact of donor-conceived people with offspring of the same donor, partly enabled by more openness regarding donor conception, online searches, social media, donor

registers and (online) DNA testing. The relationships of same-donor offspring are commonly viewed as generally more beneficial than connecting with a donor. Yet donor-conceived people can find large numbers of same-donor offspring. So far, the impact of meeting large numbers of same-donor offspring has not been explored.

Study design, size, duration: Qualitative semi-structured interviews with 19 donor-conceived people, registered in the Fiom KID-DNA Database, were conducted between April and June 2018. Participants were recruited from same-donor offspring group meetings (*n*=6) that were organised by Fiom counsellors between May 2017 and February 2018. A total of 82 donor-conceived persons were present at the meetings (30% male, 70% female). 74 persons were contacted by e-mail, 22 wanted to participate in the study of whom 19 persons were interviewed.

Participants/materials, setting, methods: Participants were female, 15-42 years old (*M*= 30; *SD*=8.6), born into a hetero (*n*=12), single (*n*=3) or lesbian (*n*=4) household. The groups of same-donor offspring consisted of 7-18 donor-offspring at time of group meetings, extending to 8-31 at time of interviews. Participants were interviewed about: (1) their motives for searching same-donor offspring, (2) their expectations about the contact and (3) their feelings prior, during and after the group meeting. Interviews were analysed using MAXQDA.

Main results and the role of chance: Most participants (*n*=13) started searching for their donor and had not thought about same-donor offspring upfront, others (*n*=4) were solely interested in contact with same-donor offspring, or (*n*=2) wanted to know if their sisters were full-genetic siblings. The three main motivations to search were: curiosity (*n*=19), the wish to receive medical information (*n*=11), and to the wish to extend their family, arising from feeling different (*n*=5) or missing family (*n*=7). All participants (*n*=19) expected to find resemblances (physically and/or personality-wise), but not all found them (*n*=2). Meetings went together with various emotions: shock and feeling overwhelmed (*n*=14) of being confronted with so many half-siblings; tension (*n*=17) due to insecurity, the unknown and uncomfortable feelings; restless feelings (*n*=12) due to the lack of a framework how and where to situate these new relationships; and satisfaction and excitement (*n*=18). Specific challenges concerned the continuous expansion of the group; and differences in privacy boundaries and information sharing (about the donor). This data shows that while relationships with same-donor offspring are commonly viewed as generally more beneficial than connecting with a donor, they can cause confusion and leave questions about how to integrate these new relations into their lives, as family, kinship, and/or friendship.

Limitations, reasons for caution: Participants are self-selected, and we do not know the reason for non-participation of the other donor-conceived people who participated in the group meetings and if they have similar or different experiences of study participants. All participants were female. Donor-related information is lacking, although 30% of group sessions participants were male.

Wider implications of the findings: These findings may be a starting point for developing needs-orientated counselling for donor-conceived people searching for same-donor offspring. This study identifies issues for further research to explore the meanings of genetic networks: are they family, kinship and/or friendship? These data are important in debates about the offspring limit per donor.

Trial registration number: not applicable.

P-499 Five Country Study of women's attitudes and knowledge regarding fertility

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Study question: What are women's attitudes towards becoming pregnant, how good is their fertility knowledge and does it vary between countries?

Summary answer: Fertility knowledge was poor amongst women in all countries surveyed. US and Chinese women were more focused on trying to maximise pregnancy chances.

What is known already: The demographics of women seeking to conceive has changed, with many women now seeking pregnancy when older. When unsuccessful, this can lead to disappointment, with the sentiment "I wish I had known more about fertility" often given, indicating basic knowledge on fertility is missing. Having a life-plan and a expecting a high degree of control over major events in life is now prevalent and extends to pregnancy planning.

Study design, size, duration: On behalf of SPD, Ipsos Suisse SA surveyed a minimum of 1,000 women in each of five countries: US, UK, Germany, Italy and China. The survey took place between 27th of July to 7th of August 2018 and covered a broad range of fertility and pregnancy themes, including knowledge and attitudes.

Participants/materials, setting, methods: Participants were women aged 20-45 years old who were able to have children and who chose to take part in our survey from Ipsos Panel. Interviews were conducted online.

Main results and the role of chance: Fertility knowledge varied between countries; when choosing the most accurate description of a menstrual cycle from five options, only 14% (U.S), 23% (U.K) 41% (Germany), 26% (Italy) 37% (China) selected the correct answer (first day of bleeding to the day before the next bleeding start. Only 20% (US), 25% (UK), 39% (Germany), 25% (Italy) 19% (China) of participants correctly knew there were 3-6 fertile days when women are able to get pregnant during a menstrual cycle. It was interesting that at least a quarter of women in the UK (29%) and US (25%) thought intercourse on any cycle day could lead to pregnancy; the proportion was lower for Germany (14%), Italy (14%) and China (8%).

Attitudes to becoming pregnant were assessed by asking women currently trying or planning pregnancy, which statement reflected their opinion best "I want to maximise my chance of getting pregnant as soon as possible (using methods to identify my most fertile days, taking supplements, etc)" or "prefer to let nature take its course and wait". US (74%) and Chinese (61%) were more likely to want to maximise chances than UK (55%), Germany (54%) and Italy (54%).

Limitations, reasons for caution: Participants were selected from the Ipsos on-line panel. Given the sample composition, the findings cannot be considered truly representative of this target audience. In China, the majority of participants resided in East (33%) and South Central China (32%) who are more affluent that the general population of this country.

Wider implications of the findings: There is a basic gap in the fertility knowledge among many women. Especially worrying was the proportion of women who believed that pregnancy was possible following intercourse on any day of the cycle, as this could lead to mistiming of intercourse when trying for a baby, and failure to conceive.

Trial registration number: Not applicable

P-500 Psychosocial, medical and practical factors affecting patients' fertility experience in a NHS setting. The importance of counsellors exploring beyond emotional factors

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Study question: What were the patients difficulties, as expressed to the counsellor, in relation to the impact of, the rigours of treatment, physical health, practical life management, relationship issues and emotional wellbeing.

Summary answer: 50% of respondents reported their work/lifestyle and relationships were impacted adversely. Whereas treatment funding, waiting times, uncertainty, tiredness, sleep and sexual problems ranged between 25-30%.

What is known already: Distress, stress and the physical burden of fertility related issues are well known. In addition, there is increasing awareness of psychosocial and relationship pressures which can impact on patients quality of life (QOL). In promoting QOL in patients we need a deep understanding of their difficulties from different aspects. However there is little knowledge into what kind of psychological approach, specialist training and understanding, duration of therapy, can best benefit patients with reproductive problems.

Study design, size, duration: A retrospective analysis of fertility patients' experience was undertaken from January 2013 to December 2018. At the end of each year all patients seen by the counsellor were posted a client experience form. A stamped addressed envelope was included. A total of 1,388 were posted to 972 addresses, 60% responded totalling 843 questionnaires. As 43%

attended as a couple two forms were sent to the same address. The remaining respondents' had one form posted.

Participants/materials, setting, methods: The 'patient experience questionnaire' was adapted from the American Cancer Society (ACS). All of the sample were National Health Service (NHS) patients who had been seen by a member of the medical team in reproductive medicine. Responses were anonymous and sent, not to the counsellor, but to an administrator within the department. All questionnaires were sent with a stamped address envelope. The data was imported onto an Excel data base by an administrator.

Main results and the role of chance: The main difficulties reported were in areas of work/lifestyle and relationships; which impacted 50% of the respondents. The findings also highlighted sleep and sexual problems. Furthermore treatment funding, waiting times, uncertainty, tiredness, ranged between 25-30%. Of these respondents 52% were undergoing fertility treatment, 15% were suffering from/had been diagnosed with premature ovarian insufficiency, 10% had experienced recurrent miscarriage, less than 7% were pursuing gamete donation, undergoing fertility preservation, suffered from Turners Syndrome or had male factor issues. The respondents' emotional feedback reflected 15% stress, fears/worries and sadness versus 10% reported either low mood, feelings of being out of control and frustration, 7% reported lowered confidence and increased anger and finally 5% felt a lack of support, shame and low concentration.The referral source was 74% by a doctor, 10% made a self referral, 8% nurse, 6% other and 2% by an Andrologist.

In conclusion, fertility counsellors adopting an eclectic approach beyond the emotional factors may have far a reaching benefits to patients'.

Limitations, reasons for caution: The questionnaire adapted was from American Cancer Society from a different patient population and was not validated for fertility patients.

Wider implications of the findings: The high numbers of the sample reporting relationship and sexual concerns might indicate a place for fertility counsellors to be trained in couples' work and/or psychosexual awareness. Counsellors need increased awareness and skills with helping patients' reduce stress and manage work/lifestyle. In addition, sleeping difficulties might usefully be addressed.

Trial registration number: Not applicable.

P-501 Psychological impact and functional recovery after a Spontaneous Hemoperitoneum in Pregnancy (SHiP) in women with endometriosis.

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Study question: What is the psychological impact and functional recovery of a Spontaneous Hemoperitoneum in Pregnancy (SHiP) event?

Summary answer: SHiP had an impact on daily life. All women received psychological help. Lower scores on social functioning, vitality, pain and general health perception were found.

What is known already: Spontaneous Hemoperitoneum in Pregnancy (SHiP) is a rare, but life-threatening complication of pregnancy. SHiP occurs predominantly in the third trimester of pregnancy and is associated with adverse pregnancy outcomes (Brosens et al., 2012). Although the exact etiology is still unknown, endometriosis seems to be the major risk factor for SHiP. Recently the largest case series in literature was published (Lier et al., 2017) describing 11 Dutch cases of SHiP between 2010 and 2015. All women with SHiP were diagnosed with endometriosis. No previous studies have investigated the psychological impact and functional recovery of women after SHiP.

Study design, size, duration: An observational, prospective, follow-up study of our case series (Lier et al.,2017) was performed. Informed consent was obtained from nine patients. Interviews were conducted and questionnaires were completed in 2016 and 2017.

Participants/materials, setting, methods: Inclusion criteria were a history of SHiP. Participants completed three questionnaires (Utrecht Coping List (UCL), Impact of Event Scale (IES) and RAND-36 and had a semi-structured face-to-face in-depth interview which was audiotape recorded. Quality of life was described through an interview (focusing on psychological and functional aspects) and measured with a quality of life questionnaire (RAND-36). Quantitative data were analyzed by using descriptive statistics and interviews were analyzed thematically with a framework approach.

Main results and the role of chance: Seven women were interviewed individually and eight women completed the questionnaires. Saturation in the interviews was achieved meaning that no new information was gathered in the last available interviews. In the interviews women either described an anxious reaction (n=2) or a survival mode mindset (n=5) at moment of the SHiP event. In the period after SHiP one woman experienced problems with attachment to her child, however at the moment of interviewing all women bonded with their child adequately. SHiP had a major impact on daily life for all women (RAND-36). All women received psychological help after the SHiP event. Two women quitted their job and two other women did not work for almost a year. Three women were temporarily limited in daily activities after the event. At moment of interviewing, two women still had functional limitations of the SHiP event. They are both tired quickly. Coping strategies described by the women in the interviews correlate well with the answers of the UCL, except for one woman. The SHiP event developed a new perspective on the important things in life (benefit-finding) in two cases and one woman refrained from conceiving again after the SHiP event.

Limitations, reasons for caution: As SHiP events are rare, the available research population is small. However, saturation was achieved in the in-depth interviews. There could be participation bias. All questionnaires were analyzed through descriptive analysis because of the limited population. The analysis of the interviews were based on researchers interpretations.

Wider implications of the findings: By using these results, better support and follow up after SHiP can be given. The results may contribute to appropriate counseling concerning the psychological and functional impact of SHiP and could lead to a more extensive follow up to recognize the need for psychological help.

Trial registration number: not applicable

P-502 The use of generalized estimating equation model in the analysis of the correlation between life quality and pregnancy outcomes during IVF treatment

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Study question: If there is any association between quality of life and pregnancy outcomes among embryo transfer cycles of infertile women?

Summary answer: Generalized estimating equation analyses can prospectively evaluate the association between quality of life and IVF pregnancy outcomes among infertile women.

What is known already: Generalized Estimating Equations models with adjustment for the correlation among repeated measures (quality of life and pregnancy) can minimize the potential biases commonly seen in longitudinal cohort studies, especially for analyzing IVF data with multiple cycles.

Study design, size, duration: This longitudinal cohort study included 686 infertility women with 1,205 embryo transfer cycles from 2012 to 2017.

Participants/materials, setting, methods: Quality of life was measured using FertiQoL tool before embryo transfer procedure. The context of FertiQoL includes a Core module (mind/body, emotional, relational, and social domains) and a Treatment module (treatment environment and tolerability domains). The higher scores of FertiQoL indicate better quality of life. Generalized estimating

equation analyses were used to assess the association between FertiQoL scores and IVF outcomes, with various factor adjustments across multiple embryo transfers for an individual person.
Main results and the role of chance: The lowest scores were noted in the emotional domain of the core module and the tolerability domain of the Treatment module. Univariate generalized estimating equation analyses showed increased pregnancy rates are associated with higher FertiQoL scores. Multivariate generalized estimating equation analyses found when one unit score increases in the emotional domain, the ongoing pregnancy and live birth rates significantly increased by 2.4% and 2.6%, respectively ($p < 0.05$).
Limitations, reasons for caution: Because we did not manipulate the impact of emotional change before embryo transfer procedure for the prediction of subsequent pregnancy outcomes, randomized control trial is recommended to confirm the results of this study.
Wider implications of the findings: These results demonstrate that FeriQoL score, especially emotional domain before embryo transfer, is useful in clinical practice to predict subsequent pregnancy outcomes among women undergoing embryo transfer cycles.
Trial registration number: not applicable

P-503 The impact of unsuccessful PGT-HLA treatment(s) on the family and the ill child.

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Study question: How are families doing after failed PGT-HLA and how do they look back on the PGT-HLA experience?
Summary answer: For all children an alternative treatment was found. Despite PGT-HLA failure, the treatment was empowering because parents felt they tried everything to save their child.
What is known already: The intention of people who apply for PGT-HLA (Preimplantation Genetic Testing combined with Human Leukocyte Antigen) is to save their ill child by means of a transplantation using haemotopoietic stem cells retrieved from the umbilical cord blood of the compatible newborn sibling. This new application of PGT-HLA raised numerous questions. While health and psychological well-being of children born after PGT is generally reassuring, little is known about families who experience an unsuccessful trajectory and are not able to save their ill child through PGT-HLA. Therefore, a semi- structured interview was applied to the couples in this situation.
Study design, size, duration: This study is part of a follow-up study of 162 couples treated with PGT-HLA in our centre between 2000-2016, 104 couples ended up without a PGT-HLA child.

Between September 2018 and January 2019, 61/104 families with an unsuccessful PGT-HLA treatment were approached by phone. 23 couples were lost to follow-up, 16 couples did not respond even after leaving several messages. Until now, only 22/61 (36%) couples were actually reached.
Participants/materials, setting, methods: Information was obtained by means of a semi-structured interview applied to the couple and with the following topics: the health-status of the ill child, experience of the PGT-HLA treatment, the impact on the partner-relationship, reasons for quitting with the PGT-HLA treatment and openness towards the ill child. In 2 cases the father instead of the mother was the interviewee. In one of these cases the mother had deceased and further details could not be gathered.
Main results and the role of chance: As to the **experience of PGT-HLA treatment,** only in 2/21 cases the financial burden was mentioned as the most stressful aspect. The practical and logistical aspects were mentioned 6 times and in 1 case the couple got relational problems during treatment. The foremost disturbing treatment aspects were however the psychological (76%) (e.g. fears about low success rates, waiting period between oocyte pick-up and embryo-transfer and pregnancy notification) and the physical impact (62%).

On average, PGT-HLA treatment was ceased after 3.09 times (range 1-6). The reasons to **stop treatment** were: psychological (43%) and physical (38%) burden, maternal medical reasons (38%) in combination with an alternative treatment for the ill child (48%). Four couples mentioned their relational issues (break-up, partner not allowing a subsequent treatment) as a reason to cease treatment.

Fifty-two percent of the children were aware of the PGT-HLA trajectory of their parents. According to the parent(s), children were able to recognize efforts that were made by the mother/parents and none of them expressed any problems with parents having ceased treatment. In most cases mothers felt they did everything they could to help their child. None of the parents expressed any regrets and 81% would recommend treatment to others.
Limitations, reasons for caution: Given that only a small number of families were interviewed in this study, the generalizability of these preliminary findings is limited.
Wider implications of the findings: People applying for PGT-HLA have high hopes on saving their ill child. Unfortunately, in most cases, the treatment will not succeed. Nonetheless, PGT-HLA was regarded as a positive, empowering experience generating a feeling of having tried the maximum to help their child. This information should be integrated in PGT-HLA counselling.
Trial registration number: Not applicable

P-504 Mediemo App - is tracking the emotional signature of patients undergoing IVF treatment useful?.

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Study question: Can we use a medical app to ascertain the emotional signatures of patients undergoing IVF cycles?
Summary answer: Through mobile technology, women undergoing IVF are empowered and willing to share their mood, allowing effective and low cost retrieval of vast emotional data.
What is known already: Infertility affects 1 in 6 couples; every year, over 60,000 cycles of IVF or ISCI performed in the UK. Assisted reproductive technologies (ART) are complex and stressful for patients, with 30% of couples ending treatment prematurely because of psychological burden. The period of waiting for the outcome of a pregnancy test further amplifies the stress and anxiety. Although ART is becoming increasingly prevalent, factors to address psychological burden or even harm within IVF units is limited. The use of medical technology in empowering women to record mood data and to further develop targeted coping strategies has not yet been explored.
Study design, size, duration: This was a prospective longitudinal study from May 2017 to December 2018. Every patient undergoing IVF/ICSI treatment at the Complete Fertility centre were offered the use of the Mediemo app as part of their care plan. Data was collated from all women who participated in using the Mediemo app.
Participants/materials, setting, methods: From May 2017 to December 2018, all women undergoing IVF (fresh or frozen embryo transfer) or IUI at the Complete Fertility Centre in Southampton, UK were given the opportunity to download and use the MediEmo mobile phone application. The application has a main feature known as 'medication timeline', an interface that allows patients to be reminded of their medication doses and timing. An additional component of MediEmo application allows patients to record their daily mood
Main results and the role of chance: Women during an IVF or IUI cycle are engaged in the MediEmo app and are voluntarily sharing their state of mind and mood, during potential episodes of stress. Over 30,000 mood samples have been recorded for women undergoing IVF at Complete Fertility. 43.4% of mood data was entered during the stimulation phase, 20.1% were entered in the 'luteal phase' (period after oocyte retrieval) and 34.1% were recorded either prior to or after the IVF treatment cycle. Further analysis is underway to delineate the key characteristics that influence the uptake of the app and the relevance of the emotional signature in relation to the clinical outcome.
Limitations, reasons for caution: The MediEmo application was only made available to women undergoing IVF/IUI treatment and so only women's and not the partner's mood has been recorded. However, we recognise the importance in the emotional state of the partner.
Wider implications of the findings: We have shown feasibility in the use of a medical mobile application in monitoring mood amongst women undergoing

IVF. This allows the development of targeted coping strategies deliverable through the medical app to help support patients through a potentially psychologically harmful IVF process.
Trial registration number: NA

P-505 Fertility Awareness among Malay women in KualaTerengganu: a cross sectional study

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Study question: What is the quality of knowledge towards fertility awareness among laywomen in comparison to healthcare worker (HCW)?
Summary answer: Level of fertility awareness knowledge was comparable between both groups but higher in HCW (67.9% and 68%) than laywomen (65.2% and 59.7%).
What is known already: It has been observed that delayed childbearing has lead to increasing incidence of infertility. Although there has been increasing awareness to seek treatment, there is limited data on the level of knowledge in fertility awareness and affecting factors.
Study design, size, duration: A cross-sectional study conducted in 2016, that included laywomen attending public health education exhibition and a cohort of HCW that comprised doctors and nurses working in our department of O&G.
Participants/materials, setting, methods: Self-Administered questionnaires were distributed after consent to participate was obtained. Information on age and fertility, lifestyle factors influencing fertility and knowledge on fertility and treatments of infertility were recorded from 150 laywomen and 200 HCW. Results were analysed in each sections of age factor, lifestyles and fertility knowledge then further comparison between both groups were made.
Main results and the role of chance: Main ResultsMajority of the respondents were adults between age of 26-35(58.7%) with background education at diploma/degree level. Half (51.4%) of both groups agreed that increasing age negatively affect fertility. Majority (66.7%) of women studied were aware of lifestyle factors influencing fertility. More than two-thirds (66%) demonstrated knowledge about fertility and its treatment. Less than half (44%) of both groups of women were aware that fertility assessment may be initiated after 12 months of trying to conceive. 54% of laywomen were aware of their fertile periods as compared to 71.5% of HCW.
Limitations, reasons for caution: Laerger study including diverse group of respindents including infertility patients is required to further assess population's fertility awareness.
Wider implications of the findings: There is already considerable knowledge of fertility awareness amongst laywomen and healthcare provider. Further knowledge update and capacity building among healthcare provider is essential to further equip them to incrrease fertility awareness among laywomen to reduce infertility related to lack of fertiltiy awareness.
Trial registration number: Not applicable

P-506 The role of genetic counselling in reproductive decision making: an overview of the literature

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Study question: What is the impact of genetic counselling on reproductive decision making?
Summary answer: Genetic counselors play an important role in reproductive decision making and can be involved in different ways.
What is known already: The National Society of Genetic Counselors defines genetic counseling as *the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates the interpretation of family and medical histories, the education about inheritance, testing, management, prevention, resources and research and counseling to promote informed choices and adaptation to the risk or condition*. While genetic counseling was primarily conceptualized as a form of reproductive counseling, genetic counselors have now extended their experience to other non-reproductive areas such as cardiology and oncology (Resta, 2018).
Study design, size, duration: This poster provides a review of the current available literature regarding the role of genetic counseling in reproductive decision making. The Science Direct database was searched using 'genetic counselling', 'reproduction' and variant spellings. Only studies in English and published between 2008 and 2019 were included. Articles were screened to ensure they were peer reviewed and published in an academic journal.
Participants/materials, setting, methods: In total 2623 studies met the inclusion criteria, further selection of only review, research and data articles led to 1310 studies. An initial review of these abstracts resulted in a selection of a small number of articles.
Main results and the role of chance: It is well known that reproductive disorders are an important component of human diseases, affecting 10-15 % of couples both men and women. The main genetic factors in human reproductive disorders are the chromosome abnormalities (Cozaru et al., 2012). The negative psychosocial impact of reproductive disorders has been repeatedly described. Stanhiser and Steiner (2018) state that infertility and miscarriages are associated with a significant psychological burden. Furthermore, LoGiudice & Massaro (2018) state that an in vitro fertilization (IVF) treatment is a stressful process which often has a negative impact on woman's psychological health. Women undergoing such a treatment experience often feelings of anxiety, depression and distress (Kahyaoglu Sut & Balkanli Kaplan, 2015).

Despite this negative psychosocial impact, research has shown the positive impact of genetic counseling. Two reviews of genetic outcome studies from Madlensky et al. (2017) and Athens et al. (2017) concluded that patients can benefit from receiving genetic counseling. Genetic counselling can lead to an increase of knowledge, perceived personal control, positive health behaviors and patient satisfaction. Moreover, it can result in a decrease of anxiety, worries and decisional conflict.
Limitations, reasons for caution: Caution is indicated since recent genetic counseling outcome studies have mainly focused on cancer genetic counseling. Furthermore, many of the studies included in these described reviews lack rigorous methodologies and use different scales or other assessments for the same outcome (Resta, 2018).
Wider implications of the findings: These limitations suggest that further genetic outcome studies are needed, specifically in the reproductive field. Furthermore, genetic technologies are evolving leading to an expansion of applications in the reproductive field. Genetic counselors can play an important role in preconception family history evaluation, donor screening, preimplantation genetic diagnosis/screening and carrier screening.
Trial registration number: not applicable

P-507 Stress and the impact on assisted reproductive technology (ART) outcomes

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Study question: To determine if psychological stressors had an impact on IVF/ICSI treatment cycle outcomes including miscarriage rates.
Summary answer: This study suggests that stressors do not impact greatly on the chance of conception from ART but may be implicated in increased rates of miscarriage.
What is known already: There is a small number of studies assessing the effect of stress on ART outcomes. A meta-analysis by Matthiesen et al found a small but significant effect of stress and anxiety on clinical pregnancy rates, but not for livebirth rates. There is more robust data however on the effect of stress on early pregnancy success. There is evidence to suggest the high perceived stress in early pregnancy is associated with increased rates of miscarriage. Immunological imbalances have been linked to miscarriage in those who reported high perceived stress.
Study design, size, duration: A survey-based study of 320 women recruited prior to commencing ART and followed prospectively for treatment outcome during the study period 2013- 2015. The survey on emotional/psychological wellbeing to measure specifically perceived stress, emotional well-being, maternal social support and outlook. The women were asked to grade their responses according to a specified scale.

Participants/materials, setting, methods: Women attending Waterstone Clinic, Cork, were invited to complete the survey in advance of treatment. A paper- based study was distributed to women attending the clinic and a further group of women were invited to complete an online version of the survey in advance of commencing their IVF/ICSI cycle. The reposes were collated and assessed according to outcome which included negative pregnancy test, positive pregnancy test and first trimester miscarriage.

Main results and the role of chance: 320 women completed the survey with a mean age of 36 years ± 3.4 years.

290 (90%, 290/320) proceeded to ART treatment of which 58.2% (n=169/290) conceived. Analysis of individual life stressors in the preceding 12 months, including job stress or serious financial problems did not reveal significance in terms of conception. A small number had experienced serious illness in the preceding 12 months which demonstrated significance in terms of not achieving pregnancy.

Overall, there were high rates of emotional wellbeing, very high rates of maternal support and medium to low levels of perceived stress and this did not differ amongst those who conceived and failed to conceive from treatment.

The women were further analysed according to livebirth and pregnancy loss (biochemical pregnancy and miscarriage). Individual life stressors revealed a higher rate of pregnancy loss amongst those who reported a stressful/demanding job ($p < 0.05$). A number of patients reported non-specified stressful life events and separation/divorce in higher numbers amongst the pregnancy loss group, reaching statistical significance, however the numbers are small thus limiting interpretation.

Psychological factors had no impact on the risk of pregnancy loss amongst the group. Those who achieved livebirth were less likely to have higher perceived stress.

Limitations, reasons for caution: The limitations of the study are the small numbers in some of the positive findings, thus limiting interpretation. Stress is multifactorial and therefore difficult to measure objectively.

Wider implications of the findings: There is evidence from the fertility cohort that stress, particularly job-related stress, is associated with higher chance of pregnancy loss. Conversely, those who achieve livebirth are less likely to report high perceived stress. This suggests that there may be a role for stress management in early pregnancy.

Trial registration number: Not applicable

POSTER VIEWING
REPRODUCTIVE (EPI)GENETICS

P-508 Chromosomal abnormalities occurrence in human blastocysts and detail

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Study question: What is the incidence of whole and segmental aneuploidies in human blastocysts?

Summary answer: Among abnormal blastocysts, almost all chromosomes had either trisomy or monosomy. The frequency of segmental aneuploidy was less than whole aneuploidy.

What is known already: Human blastocysts have a high frequency of aneuploidy and cannot be identified by traditional morphological methods. In recent years, it has been found that chromosomal abnormalities also occur when there is segmental gain or loss.

Study design, size, duration: This study was conducted between September 2017 and November 2018. Chromosomal copy number data was obtained from 42 blastocysts of 13 IVF patients, who requested chromosome testing for aneuploidy due to repeated implantation failure and/or recurrent pregnancy loss.

Participants/materials, setting, methods: Whole genome amplification was performed on TE biopsied from day5 or day6 blastocysts of IVF patients who requested chromosome testing for aneuploidy. The samples were processed and analyzed for their chromosome composition using microarray comparative genomic hybridization (aCGH).

Main results and the role of chance: Chromosomal abnormalities were detected in 69.0% of the analyzed samples. Among the chromosomal abnormalities, 39.3% involved a single chromosome, 35.7% two chromosomes, and 10.7% three and more chromosomes (maximum 6). Among the abnormal blastocysts, almost all of the chromosomes had either trisomy or monosomy. The trisomy/monosomy ratio approximated 1:1. Additionally, 21.4% of blastocysts also involved segmental aneuploidies.

Limitations, reasons for caution: Cytogenetic analysis was performed on TE biopsied from blastocysts. However, it is unclear as to whether the biopsied sample is representative of the true incidence of whole and segmental aneuploidy of the entire blastocyst.

Wider implications of the findings: This data shows details of the chromosomal abnormalities in repeated implantation failure and recurrent pregnancy loss. This data is useful for providing information on patient's future treatment options.

Trial registration number: non

P-509 Chromosomal analysis of early fetal losses in relation to embryonic heart rate on day 27-29 following in vitro fertilization-embryo transfer

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Study question: Do early pregnancy losses (EPLs) with different karyotypes differ in embryonic heart rate (EHR) on day 27-29 following in vitro fertilization-embryo transfer (IVF-ET)?

Summary answer: The EHR differs significantly among EPLs with different karyotypes. When a slow EHR is detected, there is a higher likelihood of abnormal karyotype.

What is known already: Approximately 15% of all clinically recognized pregnancies end in miscarriage during the first trimester. Even after EHR is detected, the pregnancy loss rate remains 3–4%. Chromosomal abnormalities cause more than half of EPLs. Transvaginal sonography (TVS) enables us to identify the EHR to document fetal viability at very early time points. The first TVS examination is routinely arranged on day 27-29 after IVF-ET, and the EHR could be detected in most normal cases. The EHR, whether absent or slow, has been shown to have associations with EPLs. But the relationship between EHR and different karyotypes were seldom studied.

Study design, size, duration: The chromosomal data of 159 women who underwent dilation and curettage (D&C) from January 2012 to December 2016 for missed abortion were analyzed retrospectively. Chromosomal analyses were performed with chorionic villus. Written informed consent was obtained from all participants before D&C and cytogenetic tests. The absence or presence of embryonic cardiac activity and its rate were recorded during TVS examination in each patient.

Participants/materials, setting, methods: All included patients experienced singleton EPLs and underwent TVS examination on day 27-29 after ET. According to the karyotype results, the EPLs were divided into normal karyotype group (A, n=76) and abnormal karyotype group (B, n=83). The EHR was recorded by the M-mode imaging and was calculated using the software of ultrasound machine. The EHR and its distribution in EPLs were compared between 2 groups. The EHR of EPLs with different karyotype were also analyzed.

Main results and the role of chance: Except the maternal age of group B was significantly higher (31.1 ± 4.9 vs. 33.2 ± 5.6, $p = 0.014$), other characteristics were similar between 2 groups ($P > 0.05$). The EHR was markedly lower in group B compared to group A (106.2 ± 14.8 vs. 101.1 ± 16.2 bpm, $p = 0.044$). The 2 groups were similar regarding EHR distribution ($p = 0.313$). However, most cases in group A (32.9%) had an EHR among 111-120 bpm, while 101-110 bpm was more common in group B (31.3%) . Autosomal trisomy was the most common abnormality in group

B. Trisomy 15 (12.0%), trisomy 14 (9.6%) and monosomy X (9.6%) were the top 3 abnormalities. The EHR differs significantly among EPLs with different karyotypes (P = 0.003). Trisomy 13 (115.7 ± 8.7 bpm) and normal karyotype (115.5 ± 7.0 bpm) had the fastest EHR, while trisomy 8 (72.5 ± 10.1 bpm) with the lowest EHR. 82. 6% cases with viable abnormal karyotype had an EHR over 100 bpm. Additionally, the EHR in EPLs with viable abnormal karyotype was significantly higher than those with non-viable karyotype (107. 7 ± 13. 4 vs. 98. 6 ± 16. 7 bpm, P = 0. 022).

Limitations, reasons for caution: Maternal cell contamination is a major problem. We didn't exclude the female losses due to the sample size was not large enough and there were limited cases of some rare chromosomal abnormalities. Additionally, other variables associated with EPLs such as embryo and yolk sac were not studied here.

Wider implications of the findings: The findings suggest that an early slow EHR might be included as an additional parameter in the screening for chromosomal abnormalities. It might be useful in clinical practice for providing miscarriage clues and guidance for future pregnancies. However, additional studies involving larger patient populations are needed to further corroborate it.

Trial registration number: None

P-510 determination of mitochondrial DNA levels in human blastocysts as a predictor for embryonic implantation potential

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Study question: Is there correlation between the quantity of mitochondrial DNA (mtDNA) in trophectoderm (TE) biopsy samples and female age, embryo morphology, ploidy and blastocyst implantation rate?

Summary answer: Elevated mtDNA levels, above a threshold (0,004), are associated with the advanced reproductive age, embryonic aneuploidy and implantation failure.

What is known already: At present, even the transfer of euploid embryos with high morphological grade cannot guarantee pregnancy achievement. Therefore, it is necessary to find better methods of embryo selection leading to the improvement of ART outcomes. Recently it has been proposed, that the quantity of mtDNA in TE cells can serve as a new biomarker of embryo viability. For instance, was founded statistically significant increase mtDNA copy number in advanced reproductive age and aneuploid blastocysts, also higher mtDNA quantity associated with reduced implantation potential. However, for better understanding of mitochondrial biogenesis the subsequent randomized controlled trials are needed.

Study design, size, duration: This prospective cohort study involves the analysis of relative mtDNA levels in 106 blastocysts obtained from 50 couples undergoing preimplantation genetic testing for aneuploidy. The patients included in the study were divided into 2 groups: I- average female age 32±2.8 years (25-34 years), II – 38.6 ±3.6 years (35-45 years). The study performed from February to November 2018.

Participants/materials, setting, methods: DNA from the trophectoderm samples were amplified and subjected to aneuploidy analysis using array comparative genomic hybridization. MtDNA was assessed in the same whole genome amplification products using quantitative PCR.

Main results and the role of chance: Out of the 106 obtained blastocysts 38 embryos were diagnosed as aneuploid, and 68 as euploid. The aneuploid blastocysts (n = 38) contained significantly higher levels of mtDNA in all age groups, vs. euploid embryos (n = 68) (P = 0.003). A positive correlation of the relative level of mtDNA in the trophectoderm of embryos with the patients' age (P = 0.0038) was revealed. The ROC analysis allowed to establish a threshold value for the mtDNA level - 0.004, exceeding of the threshold predict implantation failure with a sensitivity of 76.8% and specificity of 74.9%. The 41 euploid blastocysts had been transferred, 27 led to ongoing pregnancies, an overall implantation rate was 65.8% (27 / 41). Out of the transferred embryos, 32 blastocysts contained levels of mtDNA in the normal range, thus the implantation rate for the euploid embryos with a subthreshold mtDNA level was 84.4% (27/32). The remaining 9 blastocysts contained mtDNA quantities above the threshold value and none of these led to a pregnancy (9/41). The difference between the implantation rates for embryos with normal/low and elevated mtDNA quantity was statisticaly significant (P < 0.0001).

Limitations, reasons for caution: Our study was carried out in a relatively small subset of participants and obtained embryos. Therefore, the results obtained cannot be readily extrapolated on other groups of patients and need to be confirmed in larger trials.

Wider implications of the findings: This study showed that mtDNA quantification can serve as an independent biomarker and predict the implantation potential of euploid blastocyst, hereby, allows to increase the effectiveness of IVF treatment, including treatment in advancing female age group.

Trial registration number: N/A

P-511 Examination of the Relationship between Mitochondrial DNA (mtDNA) Content Analysis using Next-Generation Sequencing (NGS) and the Morphological Assessment of Embryos and Aneuploidy

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Study question: We examined whether the mtDNA read count obtained, along with the NGS result, could be used as a more reliable index of embryo for transfer.

Summary answer: There was no difference in mtDNA content based on age, aneuploidy or pregnancy outcome; there was a significant difference based on embryo morphological assessment (P<0.05).

What is known already: Recently, mtDNA content has been reported to be related to patient age, morphological assessment of embryos, euploid and implantation rate. In our hospital, NGS has also been used in preimplantation genetic testing (PGT). The data on mtDNA content was analyzed in this study.

Study design, size, duration: The data on 150 embryos were obtained from 15 patients who underwent PGT between 2014 and November 2018. The mean age of patients at the time of oocyte collection was 35.8±3.01 years. Embryo transfer was performed in 23 cycles in 14 patients after PGT.

Participants/materials, setting, methods: Mitochondria score (Ms) (mtDNA read count/total chromosome read count×10000) was calculated from total chromosome read count and mtDNA read count obtained by NGS. Correlation between patient age, embryo morphological assessment, chromosomal abnormalities, pregnancy and Ms was examined by median testing. In embryo assessment via Gardner grading, those with morphological grading better than/equal to 4BB were regarded good embryos. Pregnancy judged positive if a gestational sac (GS) was confirmed after embryo transfer.

Main results and the role of chance: There was no difference noted between a group of patients under 37 years (<37 years, n=104, median=9.91) and a group of patients aged 38 years or more (≧38 years, n=46, median=10.32). There was no difference between a group of patients with chromosomal abnormalities (n=91, median=10.98) and a group of patients without chromosomal abnormalities (n=59, median=9.76). When patients were categorized according to pregnancy outcome after embryo transfer, there was no difference between those who became pregnant (n=13, median=10.03) and those who did not (n=10, median=8.54). When embryos were categorized according to morphological assessment, there was a significant difference between good embryos (n=129, median=9.82) and poor embryos (n=21, median=13.01) (P<0.05).

Limitations, reasons for caution: There were no reasons for caution or limitations.

Wider implications of the findings: In this study, it was suggested that Ms might be correlated to the morphological assessment of embryos. There was no correlation between Ms and pregnancy. Because of the scarcity of data, we should accumulate more data and reexamine the results in the future.

Trial registration number: None

P-512 Mosaic embryo rate decreases with maternal age but mitotic error remains constant in embryos derived from euploid oocytes.

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Study question: Which is the true incidence of the different types of embryo mosaicism (euploid and aneuploid) and how does it correlate with maternal age?

Summary answer: Euploid-mosaic rate decreases with age, but remains constant relatively to euploid rate. Instead, the aneuploid-mosaic rate is constant, but drops with regard to aneuploid rate.

What is known already: One of the main consequences of next generation sequencing (NGS) implementation is the greatest power to detect mosaic samples. It is well known that mosaicism arises from chromosome segregation mistakes during mitotic divisions. Several studies about the relationship between age and mosaicism indicates that, even though incidence of euploid mosaicism varies with age, mitotic errors are constant. Thus, the total mosaicism rate (euploid and aneuploid) is theoretically always the same and non-correlated with oocyte age. However, this argument is based on the hypothesis that euploid and aneuploid oocytes have an identical behaviour related to mitotic errors during their aging.

Study design, size, duration: This is a retrospective study that includes 899 blastocyst stage embryos from 252 preimplantation genetic testing cycles (PGT) performed between July of 2017 and December of 2018 and analysed through NGS. All these embryos were cultured under the same conditions, with the same one-step media and in a time-lapse incubator.

Participants/materials, setting, methods: After PGT-A results, the embryos were classified in four groups: euploid; aneuploid; euploid-mosaic (embryo with at least two cell lines, one euploid); and aneuploid-mosaic (embryo with at least two different aneuploid cell lines).

Cases were clustered according to maternal age, following the recommendations of the *Society for Assisted Reproduction Technology*: Egg donor (<35), <35, 35-37, 38-40, and >40 years old. Chi-square test was performed to compare the mosaicism rates between the different age groups.

Main results and the role of chance: Our data show that euploid-mosaicism rate decreases while oocyte age increases (egg donor= 26.9%, <35= 21.9%, 35-37= 24.5%, 38-40= 16.5%, >40= 3.3%), being statistically significant above 40 years old ($p<0.05$). Nevertheless, if we consider that euploid-mosaic and euploid embryos have the same origin (euploid egg), the mosaicism rate of them remains invariable through all age groups (33.7±7.6%; p= 0.323). Otherwise, the aneuploid-mosaic rate of embryos derived mainly from aneuploid oocytes decreases with age (egg donor= 22.9%, <35= 23.1%, 35-37= 17.2%, 38-40= 12.0%, >40= 4.9%), being statistically significant above 40 years old ($p<0.05$).

These results make us think that mitotic errors happen differently depending on ploidy and age of the oocytes. While embryos arising from euploid oocytes have always the same mitotic errors, embryos originated from aneuploid oocytes are more susceptible to mitotic errors the younger the egg is. Another plausible reason could be that aneuploid mosaic embryos are less able to achieve a biopsiable stage the older the oocyte from which they come.

Confronting previous publications, this study proves that embryonic mosaicism have different behaviour depending on the ploidy and the age of the oocyte.

Limitations, reasons for caution: This study is based on the capacity to detect mosaicism in 4 to 8 cells of trophoectoderm biopsy. Therefore, the representativity of the embryo biopsy is limited, and we could be underestimating the real incidence of mosaicism.

Wider implications of the findings: Our results demonstrate that mitotic error remains invariable in embryos derived from euploid oocytes. Thus, mosaicism in embryos that can be transferred is not correlated with age. However, a different age-depending dynamic of euploid and aneuploid mosaicism has been detected, so they should not be grouped.

Trial registration number: Not applicable

P-513 Optimizing clinical exome design for recessive genetic conditions in preconception carrier screening: insights from 14,125 exomes

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Study question: It is possible to define the optimal preconception carrier screening (PCS) panel based on disease prevalence, severity and penetrance using a large data set of 14,125 exomes?

Summary answer: Exome sequencing achieves high sensitivity in the detection of highly penetrant childhood-onset recessive disorders and provides useful data for developing preconception carrier screening (PCS) programs.

What is known already: Approximately 30 in 10,000 children suffer from one of the 1,300 known recessive genetic conditions. These data suggest that 1-2% of couples are at high risk of having an affected child. PCS aims at revealing recessive genetic conditions in healthy individuals in order to provide them with genetic risk information and assist them with reproductive decision-making. Current debate on PCS application focuses on which gene/mutation panel maximises clinical sensitivity and cost-effectiveness while minimising counterproductive effects. Lack of large translational research data from genomic sequencing hinders proper evaluation of reproductive risk in healthy individuals and optimization of PCS gene-panel design.

Study design, size, duration: De-identified ES data from 14,125 samples were used to define gene-condition level and aggregate fetal risk. Gene-condition pairs were included based on severity, penetrance, inheritance pattern, age of onset, and strength of gene-disease association according to ClinGen framework. Modelled disease fetal risk (MDFR) was based on specific inheritance pattern and simulated parental populations. Conditions with MFDR >1 per million were included. The diagnostic impact of actionable secondary findings (SF) detection (ACMGv2-recommended genes) was investigated.

Participants/materials, setting, methods: Clinical ES was performed on gamete donors (5,845) and infertile couples (8,280) without any known family history of inheritable genetic conditions. 4,813 genes were analysed using TruSight One Sequencing Panel (Illumina). Variants were classified as pathogenic (P); likely pathogenic (LP); uncertain significance (VUS); likely benign (LB); or benign (B) based on ClinVAr records. Multiple methodologies were employed to detect pathogenic variants undetectable with sequencing (HBA, SMA, GBA, GJB2; DMD and X-Fragile for females only).

Main results and the role of chance: Among the 14,125 samples analysed, 52.3% showed at least one positive carrier result. The average number of P/LP variants was 0.74 per individual, ranging between 0 and 7 variants. Donors and patients, as well as male and females, showed similar carrier burden (P=NS), suggesting that recessive genes causing severe and early onset diseases are not related to fertility. ES achieved high clinical sensitivity for highly penetrant childhood-onset disorders (1 out 280 conceptions) through the analysis of 127 selected gene-condition pairs. Significant contributions to MDFR were observed from rare (carrier rate <1:100) and X-linked conditions (15.4% and 52.2% of total MDFR, respectively). Subgroup analysis identified 32 of 776 couples at increased risk (4.1%; 95%CI=2.8-5.8) for one of the 127 conditions analyzed. Two additional couples were found at increased risk for very rare conditions beyond 1 in a million of prevalence (Adenylosuccinate lyase deficiency MIM: 103050 and Microcephaly, epilepsy, and diabetes syndrome MIM: 614231) when both members of a parental pair were treated as a unit and the search for reproductive risk was extended to the entire exome content. Finally, 7.8% of the participants showed at least one pathogenic variant for genes included in the updated ACMGv2 list of medically actionable SF.

Limitations, reasons for caution: One major limitation to our conclusions involves the lack of ethnic diversity in our large cohort (mainly of Caucasian). However, the disease-specific frequencies provided should allow comparison with ES data collected in other preconception populations with different ethnicities. The current ES protocol lacks CNVs and non-coding pathogenic variants analysis.

Wider implications of the findings: Our results support the use of ES for PCS, which maximises clinical sensitivity especially when aiming at providing a test applicable to a broad population with different ethnicities or in consanguineous relationships. Future studies are required to investigate the clinical impact of reporting of SF in reproductive medicine practice.
Trial registration number: none.

P-514 46,XX male? Reality, PGT-A misdiagnosis or laboratory quality management error?

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Study question: Does a mid-term ultrasound of a confirmed male fetus derived from a single non-mosaic euploid 46,XX blastocyst transfer definitely imply a laboratory error occurred?
Summary answer: Sex determination errors from non-mosaic embryos are rare, but not when random meiotic X translocations of a SRY gene causes an uncommon XX male syndrome.
What is known already: Blastocyst biopsy (BL-Bx)/preimplantation genetic aneuploidy testing (PGT-A) have been shown to be subject to false-negative diagnosis due to mosaicism. We have previously shown that the BL-Bx technique can contribute to mosaic diagnoses. Wrong sex determinations are extremely rare based on the precision of NGS tests. Errors in the sex of offspring are typically associated with a laboratory handling/identification error. Conversely, viable aneuploidy of sex chromosomes is not unusual as it pertains to Klinefelter(<1:1000) or Turner's syndrome(<1:5000). Less common(1:20,000) is the random meiotic translocation of the SRY gene onto the sperms' X chromosome, causing a female to be born biologically male.
Study design, size, duration: Following a "wrong sex" ultrasound confirmation at 18-20 weeks, a retrospective case study analysis was performed. All aspects of embryo handling in the patients IVF/ICSI cycle and subsequent thaw/biopsy ("thawopsy") procedure were evaluated for potential error and confirmation of witness identification. Furthermore, the timing and procedural order/paperwork of other same day, BL-Bx/vitrification/FET cases was scrutinized for possible embryo mix-up. In addition, the patients' NGS tests were reviewed and rerun to assess accuracy of outcome.
Participants/materials, setting, methods: An infertility couple performed an ICSI cycle with blastocyst vitrification in May 2013. A successful term male birth followed and in May 2018 the couple chose to perform a thawopsy of their residual embryos. In June 2018 they performed a single euploid ET using a 6AA Day 6 46,XX blastocyst with a non-mosaic NGS profile. During the second trimester of pregnancy, a perinatologist performed ultrasound exams of the fetus and additional genetic testing (NIPT, amniocentesis).
Main results and the role of chance: A 35 year old patient entered her second trimester with a predetermined female pregnancy, only to be told and shown at 20 weeks, that the fetus had a penis. The distraught patient contacted the REI physician, whom informed the IVF Lab looking for answers to how such an error could occur. A detailed investigation of patient records revealed that the initial cycle produced 25 mature oocytes, 21 zygotes and 13 blastocysts (1 transferred and 12 vitrified) and that all embryo handling events had been witnessed, as were the subsequent thawopsy procedures. Of the 7 embryos re-biopsied/re-vitrified, 5 euploid (2-46,XY and 3-46,XX) blastocysts were confirmed. In retrospect, the only fact not fully detailed to alleviate any doubt in potentially mishandling another patients' male embryo post-biopsy was the exact time of vitrification. As we typically vitrify within 45 min post-biopsy, we had confidence that the prospects of a mix-up 90 min later was highly doubtful. Post-repeated ultrasound, the NIPT outcome was "inconclusive", which we assumed meant it did not support the ultrasound scan findings. Finally, in October 2018, the amniocentesis finding was conclusive revealing the rare XX male syndrome condition.
Limitations, reasons for caution: It is imperative that laboratories implement strict quality management practices involving witness verification and time/date details for all embryo handling/movement events to confidently assess and troubleshoot potential lab errors. As we integrate routine genetic testing, we must inform patients about the risks of potential rare events, besides issues of mosaicism.
Wider implications of the findings: When faced with a potential misdiagnosis, it is comforting to everyone when quality management efforts effectively confirm the elimination of lab error. Furthermore, we must anticipate incorrect mosaic profile interpretations, as well as unusual/rare developmental events to best inform and support our patients.
Trial registration number: None

P-515 Embryo culturing conditions and non-invasive preimplantation genetic testing for aneuploidy detection (NI-PGT-A); an observational study

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Study question: Do embryo culturing conditions during IVF impact results obtained for non-invasive preimplantation genetic testing for aneuploidy (NI-PGT-A) of spent embryo culture media?
Summary answer: Replacing culture media during IVF improves overall concordance rates between the embryo biopsy and spent embryo culture media for NI-PGT-A.
What is known already: Challenges to the use of NI-PGT-A include achieving high concordance between the spent embryo culture media and embryo biopsy results, and also the ability to distinguish contaminating maternal DNA from the embryonic DNA. Variable rates of concordance have been reported to-date, but this may stem from the absence of standardized culturing conditions or molecular testing methodologies. Optimisation of either or both components will likely result in the highest accuracy of NI-PGT-A. The accumulation of embryonic and contaminating DNA in spent embryo culture media will be determined by culture conditions, however a robust amplification technology will be crucial for NI-PGT-A.
Study design, size, duration: Spent embryo culture media was collected and stored at -20°C by clinics (under their ethics approval) from single embryo culture droplets following biopsy of the embryo for PGT-A. Specific culturing conditions, including droplet size, day of biopsy, and whether the media was changed, were disclosed along with the biopsy PGT-A result at the time of sample processing. All samples were processed by a single laboratory.
Participants/materials, setting, methods: Spent embryo culture media samples (n=170) from 10ul-60ul culture droplets were whole genome amplified (WGA) using DOPlify® kit reagents (PerkinElmer). Next generation sequencing libraries and sequencing was performed according to the standard PG-Seq™ kit 48 sample protocol on a MiSeq sequencer (Illumina). Data was bioinformatically aligned to hg19 and analysed using PG-Seq™ kit software. WGA DNA yield, NGS metrics, and whole chromosome aneuploidy concordance with the PGT-A result for the embryo biopsy were determined.
Main results and the role of chance: Results were collated for each set of culturing conditions. Whole genome amplification using DOPlify® kit reagents resulted in the amplification of 78-100% of spent embryo culture media samples (WGA failure rate 0-22%). Ploidy concordance with the embryo biopsy ranged from 33-55% for autosomal chromosomes and 47-53% for sex chromosomes using a continuous culturing system (n=3 protocols), compared with concordance rates of 60-95% and 50-97% respectively when media was changed during the 5 day culturing period (n=4 protocols).
Limitations, reasons for caution: Limited numbers of media samples were available for each individual culturing protocol and researchers performing the data analysis were not blind to the culturing conditions or PGT-A biopsy results. Maternal DNA contamination of media may be underestimated, as it can only be detected when the embryo in culture was male.
Wider implications of the findings: Specific embryo culturing protocols and/or technology to determine the level of external DNA contamination are likely to improve concordance for NI-PGT-A. A subsequent study using DOPlify® kit reagents (PerkinElmer) is planned to be undertaken with researchers blind to the embryo culturing conditions and PGT-A results.
Trial registration number: -

P-516 Prevalence and properties of copy number variation (1-10 Mb) in preimplantation blastocysts

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Study question: Can next generation sequencing (NGS) accurately detect small segmental aneuploidy ((1-10Mb) using 3-5 cells and what is the prevalence and properties of SSA in human preimplantation blastocysts?
Summary answer: Our method can effectively detect SSA, the incidence of SSA was about 4.7% and a certain frequency was pathogenic in human preimplantation blastocysts.
What is known already: Copy number variations (CNVs) are a major contribution to the genome variability and can be either benign or pathogenic, depending on their location and genetic content et al. Pathogenic CNVs including microduplication and microdeletion syndromes are frequently associated with intellectual disability, multiple congenital autistic spectrum disorders and other phenotypic findings. Previous studies have indicated NGS-based method for preimplantation genetic testing (PGT) could accurately detect chromosome aneuploidy and segmental aneuploidy above 10 Mb. However, little is known about the NGS-based SSA detection of human preimplantation blastocysts.
Study design, size, duration: Six human embryonic stem cell lines with known CNV < 10 Mb and 158 preimplantation genetic testing for structure recombinant (PGT-SR) blastocysts from 35 patients were used to build and evaluate methodology. 4212 blastocysts from 1735 patients who requested PGT for aneuploidy(PGT-A)were used to evaluate prevalence and properties. The study was approved by the ethics committee (LL-SC-SG-2014-011).
Participants/materials, setting, methods: limited stem cells and biopsy samples were subjected to PicoPLEX WGA amplification and low-coverage sequencing were performed using the BGISEQ-500 sequencing platform. An average of 20 million reads were obtained for each sample. Further, genome-wide CNV analysis of the parental genomic DNA were performed to determine whether SSA were inherited or de-novo. The Clinical interpretation of the detected CNVs was based on the guidelines of American College of Medical Genetics and Genomics.
Main results and the role of chance: Using improved bioinformatic methodology, the picoPLEX WGA combined NGS can accurately detect SSA > 1Mb, the sensitivity and specificity were more than 90%.

From 4212 blastocysts from 1735 PGT-A cycles, 191 blastocysts were seen to carry 196 SSA (47 were 4-10Mb and 149 were 1-4Mb). Among 47 4-10Mb SSA, 28 were loss and 19 were gain. All 28 4-10Mb loss were de-novo CNV and pathogenic or likely pathogenic CNV(PCNV), all located the end of the chromosome. Among 19 4-10Mb gain, five from two couples were inherited from parents and were classified as variants of uncertain significance (VOUS), the remaining 14 4-10Mb gain, 11 were PCNV and 3 were classified as VOUS CNV. Among 149 1-4Mb CNV, 60 were loss and 89 were gain. 116 parental gDNA available were used to determine inherited or de-novo. The results suggested 45 (38.8%) were de-novo and 71(61.2%) were inherited from parents. Among 71 inherited 1-4Mb SSA, 27 were loss and 44 were gain. 29.6% (8/27) inherited 1-4Mb loss were PCNV, however, most inherited 1-4Mb gain were VOUS or benign. Refer to 45 de-novo 1-4Mb SSA, 18 loss out of 26 were PCNV and only 3 gains out of 19 were classified as PCNV.
Limitations, reasons for caution:

The de-novo SSA needed further confirmed using other credible methods. The clinical interpretation of the SSA was challenge,especially the de-novo SSA, as the phenotypic consequences of some SCNVs were quite variable and their impact on human health depends on factors that are not yet known.
Wider implications of the findings: Increasing the detection resolution to 1Mb is valuable for excluding the transfer of blastocysts with PCNV and non-priority transfer the blastocysts with VOUS-CNVs.
Trial registration number: not applicable

P-517 Efficacy of nuclear transfer to prevent the inherited mitochondrial pathology

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Study question: Can mitochondrial transfer in ART provide embryos without the risk of mitochondrial pathology?
Summary answer: Nuclear transfer procedures showed high efficiency in describes case. But further investigations and long term follow up of children is required.
What is known already: Mitochondrial pathology is characterized by maternal inheritance. Women may be hidden mutation carriers, but in some cases the risk for descendants may reach up to 100%. Due to the uneven distribution of mitochondria during cell division, the practice of preimplantation testing of embryos has significant limitations, and prenatal diagnosis may be unacceptable. The first baby after nuclear transfer procedures was born in 2016 (J. Zhang et al., 2017).
Study design, size, duration: The study included a married couple with a confirmed hidden woman's carriage of Leigh syndrome mutation (37 years old). The couple had a history of birth of a girl with Leigh syndrome. For ethical reasons, prenatal diagnosis and donation of oocytes were not considered for further childbirth.
Participants/materials, setting, methods: The patient's mutation carrier status has been confirmed in independent laboratories (mtDNA 9176 T>C, Heteroplasmy ~ 40%). The child was diagnosed with confirmed Leigh syndrome (Homoplasmy 99.4%). The couple had 2 controlled ovarian stimulations followed by nuclear transfer procedures. Embryo biopsy was performed on Days 5-6 of development. Preimplantation genetic embryo testing PGT-A (NGS: Veriseq, Illumina) and heteroplasmy testing of pathological mutation (NGS, coverage of >1000x) were performed.
Main results and the role of chance: During the first stimulation, 3 blastocysts (euploid female embryo and two aneuploid embryos) were obtained. After consultation with the patients, it was decided not to use the female embryo for the embryo transfer. During the second stimulation, 6 blastocysts, incl. 3 aneuploid and 3 euploid (males) were obtained. Heteroplasmy of euploid embryos for pathological mutations: 0.51 ± 0.02%, 2.96 ± 0.05 and 3.32 ± 0.06%. The embryos obtained during ART from a patient with a mtDNA mutation carrier status were characterized by a low level of heteroplasmy of mtDNA mutation and had a low risk of mitochondrial pathology development. The patient is preparing for embryo transfer.
Limitations, reasons for caution: Nuclear transplantation in human is still experimental and highly controversial procedure. Further investigations and long term follow up of children is required.
Wider implications of the findings: Nuclear transplantation could be used in prevention of mitochondrial diseases, preimplantation embryonic lethality, repeated implantation failure and in investigation of nuclear-cytoplasmic interactions.
Trial registration number: Clinical trial is registered by Shupyk National Medical Academy of Postgraduate Education of Ukraine. Registration number: is not used.

P-518 Segmental insertions and monosomies are linked to developmental arrest.

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Study question: Which genetic abnormalities will prevent blastocyst formation?
Summary answer: Embryos from which the day 3 cleavage stage blastomeres have segmental insertions or monosomies, have a significantly decreased chance to reach the blastocyst stage.

What is known already: A major negative selection against aneuploid cells happens during the transition from the morula to the blastocyst stage. When analyzing different morphological or time-lapse parameters, they do not allow us to discriminate between euploid and aneuploid blastocysts. Besides the morphokinetic parameters, chromosomal abnormalities of day 3 cleavage stage embryos have been analyzed for their detrimental effect on development. After fluorescence in situ hybridization (FISH) for a selected number of chromosomes, it was noted that trisomies, extensive mosaicism and monosomy X or 21 reach the blastocyst stage more often.
Study design, size, duration: A single center retrospective study was performed between April 2016 and January 2017. Patients requiring Preimplantation Genetic Testing for Aneuploidies (PGT-A) by Next Generation Sequencing (NGS) were included. All embryos were cultured in a time-lapse imaging system and biopsy was performed on day 3 of development, which allowed us to perform a fresh embryo transfer as embryo vitrification is prohibited. Segmental or whole chromosome insertions and deletions were registered.
Participants/materials, setting, methods: Of the 285 embryos biopsied on day 3, the embryo arrest was defined at the blastocyst stage if the embryo failed to cavitate 118 hours post-injection. A logistic regression model was applied using the time to cavitate as the response variable and the different mutations as the explanatory variables, and a p-value <0.05 was considered significant. The reliability of the model was tested by plotting the sensitivity and specificity in a ROC curve.
Main results and the role of chance: After single blastomere biopsy, the 285 cleavage stage embryos were further cultured until day 5 of development. A total of 103 (36.1%) embryos were euploid and 182 (63.9%) were aneuploid. There was a significant difference in the developmental arrest between euploid and aneuploid embryos (8.7% versus 42.9%; p=0.0001). Segmental insertions and monosomies were found to have a statistically and clinically significant effect on developmental arrest (p=0.0163 and p=0.0075), while chromosomal insertions and segmental deletions were not found to have a statistically significant effect on developmental arrest. In case of segmental insertions, an increase of one extra segmental insertion in any given chromosome increases the odd of arrest by 159%. For chromosomal monosomies, the odd will only increase by 29% for every extra chromosomal monosomy. The area under the ROC curve, indicating the ability of our model to correctly classify embryos with arrest based on their chromosomes, was 0.6573.
Limitations, reasons for caution: Besides the retrospective design of the study, a higher number of embryos is needed to detect which individual chromosomes show a more pronounced effect on developmental arrest.
Wider implications of the findings: The results of this study reinforce the use of day 5 biopsy. Not only will euploid embryos have a higher chance to develop to the blastocyst stage, the genetic result obtained with trophectoderm samples is also more reliable as compared to day 3 cleavage stage biopsies.
Trial registration number: Not Applicable

P-519 Clinical outcomes of comprehensive 24-chromosome preimplantation Genetic testing in couples with balanced reciprocal or Robertsonian translocations

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Study question: To determine the clinical value of comprehensive 24-chromosome preimplantation Genetic testing (PGT) in couple with balanced reciprocal or Robertsonian translocation.
Summary answer: PGT for Male carriers had better clinical outcomes than the female carriers in both balanced reciprocal or Robertsonian translocation.
What is known already: Balanced translocation is one of the more common structural rearrangements of chromosomes. The two most common types are Robertsonian translocation and reciprocal translocation, which are associated with reproductive risks, such as infertility, spontaneous abortion and the delivery of babies with mental retardation or developmental delay. There is controversy over whether PGT can improve the pregnancy outcome.Some studies suggested the transfer of chromosomally normal/balanced embryos following PGT significantly reduce the risk of an affected pregnancy and decrease the risk of miscarriage. However, some research demonstrates that natural conception offers similar pregnancy outcomes compared with IVF-PGT.
Study design, size, duration: A retrospective study from October 2011 to October 2018, a total of 12338 blastocysts originating from 3076(2917) oocyte retrieval cycles from 2568(2410) couples with one of the partners carrying reciprocal or Robertsonian translocations were investigated (2263 reciprocal translocations cycles and 654 Robertsonian translocations cycles). The mean maternal age was 30.3±4.1 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. The euploid embryo and unbalanced embryo rate were analyzed according to the translocation pattern, the carrier's gender and maternal age.
Main results and the role of chance: Of the 3075 oocyte retrieval cycles, 188 cycles had no embryos to biopsy. Among 2910 biopsied cycles, the average biopsied blastocysts number was 4.2 and 2099 cycle resulted in one or more euploid embryos. We calculated the rate of euploid and unbalanced translocation in male and female carriers respectively. For reciprocal translocations, 32.0% (1475/4610) embryos in female and 37.5% (1476/3941) in male was euploid ($p<0.01$). The Rates for unbalanced translocation was 56.6% (2599/4610) and 49.9% (1967/3941) respectively in female and male carriers, ($p<0.01$). For Robertsonian translocations, the euploid embryos rate was 42.9% (542/1262) and 57.9% (666/1144) in in female and male carriers, The Rates for unbalanced translocation was 40.2% (507/1262) and 17.2% (197/1144) in female and male carriers, with significant difference ($p<0.01$). Further, we compared the results between maternal age$\leq$35 years and >35 years,>35 years group had less biopsied blastocysts(4.4 vs 2.9) and higher risk(25.6% vs 45.3%)of no euploid embryo compared with the $\leq$35 group ($p<0.01$). So far, out of 1590 ET cycles, the clinical pregnancy rate, miscarriage rate and live birth rates (LBR) per ET was 63.0% (1001/1590), 18.2% (182/1001) and 51.2% (814/1590) respectively.
Limitations, reasons for caution: About 30% oocyte retrieval cycles had no ET especially in women >35 years and reciprocal translocation.
Wider implications of the findings: Male carriers had better clinical outcomes than the female carriers both in balanced reciprocal or Robertsonian translocation. Patients should be aware of the high risk of no embryos ET, particularly in women >35 years and reciprocal translocation. This study may provide data for genetic counseling of balanced translocation carriers.
Trial registration number: This study was supported by the National Key R&D Program of China (2018YFC1003100).

P-520 Whole genome amplification (WGA) allowing combined PGT-A and PGT-M for detection of heteroplasmy and estimation of mtDNA mutation load in embryos.

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Study question: Can data generated from embryos biopsied for PGT-A provide genetic information for the detection of heteroplasmy and estimation of mutation load at mitochondrial disease sites?
Summary answer: The PG-Seq^{TM} kit allows for excellent coverage of the mtDNA in biopsy samples from embryos and allows detailed calculations of heteroplasmy to be performed.
What is known already: A large number of pathogenic mitochondrial DNA (mtDNA) mutations have been implicated in a variety of diseases. As mtDNA is maternally inherited, women with pathogenic mtDNA mutations are likely to have affected children, the severity depending on the heteroplasmy proportion. A systematic meta-analysis showed that there is a $\geq$ 95% chance of being unaffected at a mutant level of $\leq$ 18% (Hellebrekers et al, 2012). PGT-M can be used to identify embryos with mutation loads below this phenotypic

threshold. The PG-Seq™ kit yields superior coverage of mtDNA and may allow combined PGT-A and PGT-M for mtDNA diseases from single biopsies.
Study design, size, duration: Two cell lines, a reference and "mutant", with mitochondrial single nucleotide variants (SNV) were selected. Six 5-cell samples from each cell line were whole genome amplified (WGA). The replicates were pooled before the cell lines were mixed in proportions ranging from 0% to 100% (mutant), in increments of 10%. Libraries were prepared for each of the pools prior to sequencing using the MiSeq instrument (Illumina).
Participants/materials, setting, methods: SNV sites with < 10x depth across all samples were removed, leaving 31 SNV sites. The proportion of mutant SNV at each site was calculated and analysed. The data were corrected for variance introduced by pipetting error in pooling. These corrections were only applied to samples with proportions of 40%, 50%, and 60%, and subsequent analyses were restricted to these data. To model data at different read depths we combined data across sites.
Main results and the role of chance: The average (± sd) mitochondrial read count for cell line samples was 5037 (± 1083). After filtering, the average (± sd) depth of coverage for the cell line samples at the SNV sites was 25.8x (± 12.5). Combining SNV data to achieve a read depth typical of a standard PG-Seq™ kit 48 sample run using a single embryo biopsy (500,000 reads), the observed SNV proportion was within 25% of the expected proportion in all cases. Combining SNV data to achieve a read depth of 5x a standard PG-Seq™ kit 48 sample run, the observed SNV proportion was within 11% of the expected proportion in all cases. This means that in any case where a heteroplasmy is detected at 7% or lower, including no detection, the mutant load should be below the phenotype threshold. In summary, the PG-Seq™ kit has the potential to be used for combined PGT-A and PGT-M for mtDNA diseases, although accuracy is dependent on read depth. Read depth at mtDNA can be increased by increasing sequencing throughput or by alternate means such as using PerkinElmer's Target Sequence Enrichment protocol.
Limitations, reasons for caution: Estimates of accuracy with increased depth were based on modelled data rather than experimental data; experimental data is needed for validation. Due to variable but consistent depth of coverage across the mtDNA, some sites will be more suited for analysis by this method than others.
Wider implications of the findings: The superior amplification of mtDNA achieved by the PG-Seq™ kit may allow combined PGT-A and PGT-M for mtDNA disease analysis. Combining PGT-A and PGT-M in this novel way would streamline a laboratory testing workflow. Further enrichment of mtDNA could be achieved using PerkinElmer's Target Sequence Enrichment protocol.
Trial registration number: -

P-521 Comparative proteomics of Seminal Exosomes reveal deregulation of cell signalling and defects in chromatin remodelling in Recurrent Pregnancy Loss Patients

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Study question: Do seminal exosomal proteins hold an answer to anomalous sperm maturation leading to aberrant embryo formation as a cause of recurrent pregnancy loss (RPL)?
Summary answer: Compromised exosomal proteome expression involved in sperm maturation may be responsible for failed paternal gene expression leading to aberrant embryo formation and miscarriage in RPL.
What is known already: Spontaneous recurrent pregnancy loss (RPL) most often investigated from the women's perspective. However, recent evidences show the involvement of male factor as a plausible cause particularly in idiopathic RPL. Exosomes are vesicles derived from cells. Seminal exosomes derived from epididymis and prostate carry a distinct repertoire of regulatory RNAs and proteins necessary for sperm maturation and function. Though exosomes are known to carry proteins involved in energy pathways, protein metabolism, cell growth and maintenance, there is paucity of data on the role of seminal exosomal proteins in spermatozoa in general and RPL patients in particular.
Study design, size, duration: This prospective study consisted of male partners of RPL patients (n=21) with no female factor abnormality as revealed by gynaecologic investigation including karyotyping; and age matched fertile healthy volunteers (n=21). All samples were collected during 2016-2018 after getting institutional ethical approval and written consent from the participants.
Participants/materials, setting, methods: Seminal ejaculates were collected by masturbation after 2-3 days of sexual abstinence and analysed according to World Health Organization criteria. Exosome were isolated by ultracentrifugation and characterized by western blot, transmission electron microscopy, and nanoparticle tracking analysis followed by LC-MS/MS analysis. The expression profile of proteins present in exosomes was further examined using bioinformatics tools to elucidate the possible regulatory pathways.
Main results and the role of chance: Of the 998 proteins detected in the proteomic data set, 939 were from control and 935 from RPL group. A total of 447 differentially expressed proteins were detected of which 385 under expressed and 62 over expressed in RPL while 63 were exclusive to control and 59 exclusive to RPL. STRING protein-protein interaction analysis revealed that the major pathways dysregulated in RPL are immune response (HSA:168256 ; false discover rate p=2.67e-28), signalling proteins (HSA:376176 ; false discover rate p=3.04e-22), chromatin packaging and remodelling (GO:0031497; false discover rate p=2.78e-05), protein folding and apoptosis (HSA:109581; false discover rate p=5.93e-06). The present findings thus corroborate the previous idea with relation to immune dysfunction and DNA fragmentation in RPL that needs to be validated through Western Blotting and compared with proteome profile of spermatozoa to elucidate plausible biomarkers of male factors of RPL.
Limitations, reasons for caution: The validation of identified DEPs was not done as well as analysis of spermatozoa proteome. Further, the non-coding regulatory RNAs in the exosomes were not analysed.
Wider implications of the findings: The result of this pilot study implies the importance of exosomes in sperm maturation and function, particularly in RPL. Further validation may lead to identification of candidate biomarkers for determination of male factors in idiopathic RPL.
Trial registration number: Not applicable

P-522 TGFβ/SMAD signaling regulates transzonal projection (TZP) formation in growing follicles of the mouse ovary

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Study question: Do TGFβ signalling mediators SMAD3/4 regulate TZP formation by the granulosa cells during follicular development?
Summary answer: SMAD4 regulates the number of TZPs, whereas SMAD3 regulates expression of genes encoding proteins implicated in formation of filopodia.
What is known already: Shortly after the initiation of follicular development, the generation of an extracellular layer called the zona pellucida (ZP) physically separates the oocyte from the proliferating granulosa cells (GCs) that surround it. Since GC-oocyte contact-dependent communication is essential for oocyte development, GCs generate filopodia, termed transzonal projections (TZPs), which penetrate the ZP and establish contact with the oocyte plasma membrane. Growth-differentiation factor (GDF) 9, an oocyte-secreted TGFβ-family ligand, increases TZP number as well as the steady-state levels of *Myo10* and *Fscn1*, which encode proteins implicated in filopodial assembly. However, the mechanism by which GDF9 acts is not known
Study design, size, duration: Ovaries obtained from day-4 and day-12 mice (enriched in early and mid-growth follicles, respectively) were used for the quantification and localisation of SMAD-pathway and TZP-related factors during follicular development. The tamoxifen-inducible *Cre-ER*/floxed *Smad4*$^{+/+}$ knockout (KO) system was used in 4-day ovaries and 12-day granulosa-cell oocyte complexes (GOCs) in culture to delete the *Smad4* gene.
Participants/materials, setting, methods: Quantitative RT-PCR and immunofluorescent staining of ovarian histological sections were used to measure relative mRNA and protein levels. ChIP-qPCR was used to detect SMAD3 and SMAD4 binding to *Myo10* and *Fscn1* promoter regions. Following tamoxifen (1 µg/ml) treatment in *Cre-ER*/floxed *Smad4*+/+ KO ovaries and GOCs and subsequent incubation, western blotting to measure the protein levels of SMAD4, SMAD3, MYO10 and FSCN1; Image J software was used to quantify TZP number, GC number and oocyte diameter
Main results and the role of chance: Quantitative RT-PCR and immunofluorescent histological sections of ovaries revealed a significant increase in relative

mRNA and protein levels of Fscn1 and Myo10 between day-4 and day-12 of age. SMAD4 protein was depleted by ~50% in the tamoxifen-treated *Cre+/floxed Smad4* neonatal ovaries and GOC cultures. This was accompanied by a striking decrease in the number of TZPs in both early-growth follicles of day-4 ovaries and GOCs of day-12 ovaries, although oocyte diameter was unchanged. In contrast, MYO10 and FSCN1 protein levels were not significantly altered. Consistent with this, ChIP-qPCR revealed that SMAD3, but not SMAD4, bound to the promoter region of Fscn1 and Myo10, suggesting that SMAD3 may play regulate Fscn1 and Myo10 expression in GCs independently of SMAD4.

Limitations, reasons for caution: Experiments were performed using ovaries and GOCs maintained *in vitro*, which may not reproduce the physiological conditions *in vivo*. Deletion of the Smad4 gene did not occur in all cells. All experiments were performed using mouse,therefore further work is required to determine whether the same mechanisms operate in other species.

Wider implications of the findings: These results identify part of the mechanism by which the growing oocyte induces neighbouring granulosa cells to generate new TZPs. Understanding the molecular pathways regulating TZP formation may help identify novel markers of follicle/oocyte quality and provide insight about the causes of age-related and age-independent infertility.

Trial registration number: Not applicable

P-523 Paternal Age Has No Impact on Aneuploidy Rate in Embryos Derived from Young Egg Donors

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Study question: Is there a correlation between paternal age and the frequency of chromosomal aberrations in embryos derived from young oocyte donors?

Summary answer: There was no association between paternal age and aneuploidy rates in embryos derived from donated oocytes after adjusting for donor, sperm, and IVF cycle characteristics.

What is known already: Embryo aneuploidy is among the major factors contributing to poor in vitro fertilization (IVF) outcomes. The incidence of aneuploid embryos increases with maternal age. Oocyte donation overcomes the effect of maternal age on embryo aneuploidy. There is controversy regarding the impact of paternal age on embryo aneuploidy. Several studies have reported higher percentages of aneuploidy than expected in embryos derived from young oocyte donors. This ranges from 4-59% in published studies. Whether this higher than expected prevalence is related to advanced paternal age is still uncertain.

Study design, size, duration: A retrospective cohort study performed at the CReATe Fertility Centre, Toronto, Canada, between January-December,2017. Clinical and demographic data from 201 egg donation cycles for 246 patients, where preimplantation genetic testing for aneuploidy (PGT-A) was performed, was obtained. A total of 1248 blastocysts from these cycles were analyzed. PGT-A results from d5/6 trophectoderm biopsy obtained by next-generation sequencing(NGS) were available for 1185(98%) of embryos. Cycles using donors >33 years were excluded from the final analysis (n=30).

Participants/materials, setting, methods: Embryos from IVF-PGT-A ovum donor antagonist cycles were allocated to three groups according to paternal age: Group A 27-39 years (n=592); Group B 40-49 years (n=496); Group C ≥50 years (n=97). Multiple logistic or ordinal regressions were conducted to evaluate euploidy, aneuploidy, mosaicism, and blastocyst formation rates between paternal age groups where statistically appropriate. Analyses were adjusted for oocyte donor age, AMH, number of oocytes retrieved, semen characteristics, and number of embryos per patient.

Main results and the role of chance: 1185 embryos from 246 male patients included in the analysis had PGT-A results classifying them as euploid, aneuploid or mosaic (10Mb resolution; 30%-70% mosaicism). Fresh sperm was used in 51%, while frozen in 49% of patients. None of the patients had a male factor infertility diagnosis and there was no difference in semen parameters (concentration and motility) and stimulation protocol between the study groups.

No significant differences between paternal age groups A, B and C were found in euploidy rates (67.06%, 68.7%, 69.07%, respectively; p=0.812), aneuploidy rates (13.6%, 13.3%, 14.4%, respectively; p=0.953) or mosaicism rates (19.2%, 17.9%, 16.4%; respectively, p=0.749). Similar results were found when the rates of euploidy, aneuploidy and mosaicism in the three paternal age groups were analyzed at the level of treatment cycle (batch of embryos/patient cycle). Multiple regression analyses for embryos found older oocyte donor age to be significantly associated with mosaicism (OR 1.06, 95% CI 1.002-1.11; p=0.04). AMH levels of 20-80 pmol/L were found to be significantly associated with blastulation rate (OR 1.04, 95% CI 1.01-1.07; p=0.008). Analyses at the treatment cycle level demonstrated AMH levels >80 pmol/L to be significantly associated with both mosaicism (p=0.038) and aneuploidy (p=0.042) (n=172).

Limitations, reasons for caution: The main limitation is the retrospective nature of this study; however, multiple regression analyses were conducted to limit potential bias by adjusting for donor, sperm, and oocyte characteristics.

Wider implications of the findings: The results of the study may assist medical fertility practitioners when providing preconception counseling to patients using oocyte donation and their male partners, particularly for couples in which the male partner belongs to an older age group.

Trial registration number: not applicable

P-524 Genetics of infertility: Diagnostic interests of a custom designed panel for the analysis of 51 genes involved in non-syndromic human infertility

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Study question: Panels of infertility genes are now available. What is the relevance to offer them as a diagnostic tool for infertility patients?

Summary answer: According to our preliminary results, it is possible to define precisely the causative mutation for the defined phenotype by using custom designed panel.

What is known already: Infertility is a global healthcare problem which affects men and women equally. In research, the focus has shifted from single gene mutation studies to genome wide analyses. This has allowed, during the last decade, to identify a fast growing up list of human genes involved in infertility. However with the exception of few genes, the described mutations are rarely detected, most of them stayed as a familial mutation or individual cases. Therefore, the impact on human fertility still needs to be confirmed for the majority of these genes. Furthermore the prevalence of these mutations in specific population is unknown.

Study design, size, duration: In order to scan the 51 identified genes involved in different form of non-syndromic infertility, a genetic diagnostic test based on high throughput sequencing technology has been designed. The panel encloses 35 genes for male infertility, 16 genes for female infertility and 6 common control SNPs . As an initial study, a cohort of 125 French patients, with a well-defined infertility phenotype, has been planned to study.

Participants/materials, setting, methods: Male patients identified with teratozoospermia, asthenozoospermia, spermatogenic failure or total fertilization failures were recruited. For female infertility, women diagnosed with premature ovarian insufficiency/ failure (POI/POF) or with oocyte maturation arrest were included in the study. DNA was extracted from peripheral blood

and library was prepared using SureSelectQXT Target Enrichment system. Multiplex sequencing has been performed on Illumina NextSeq 550 with 2x150bp reads for total 51 genes. Variant analysis has been achieved using VaRank tool.

Main results and the role of chance: The panel size is 187 kb comprising 883 regions . Control SNPs were used to confirm identity of each patient. So far, 38 males and 4 females were analyzed after sequencing. Mean coverage was 495X and 99.4% of target bases were succesfully sequenced for >30X . Results revealed de nevo mutations in two genes related to defined infertility phenotype in three patients which were confirmed by Sanger sequencing. However two genes in the panel, one of them identified for female infertility and the other for male infertility, shows unexpectedly high amount of variations for unrelated phenotypes. This puts a question mark on the implication of these two genes in human infertility with the specific phenotype for which they have been identified.

Limitations, reasons for caution: The main limitation is the number of patients' analyzed so far. In order to challenge the clinical interest of such diagnostic tool, a larger group of patients need to be analyzed. Further works on the two genes for which lots of variations have been found has to be done.

Wider implications of the findings: The precise diagnostic is important for adapting the best treatment and counsel not only to patients but to their spouse and relatives. This study allows us to designate gene mutations which have significant impact on specific infertility phenotype on a restricted cohort of French patients.

Trial registration number: Not applicable

P-525 Thaw, biopsy and refreeze procedure for PGT-A analysis of previously cryopreserved embryos

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Study question: To compare the clinical pregnancies after thaw, biopsy and refreeze (TBR) procedure versus biopsy of fresh embryos for PGT-A analysis.

Summary answer: Our data show that previously cryopreserved embryos can be screened with the PGT-A procedure after thaw, trophectoderm biopsy and refreezing with vitrification.

What is known already: Patients with cryopreserved embryos have not been able to access embryo screening techniques due to the poor survival rates after refreezing with the slow-freeze protocol. In recent years, the vitrification procedure has become widely used in assisted reproduction procedures. Vitrification has enormous advantages over slow-freeze techniques due to the high efficiency, reliability and excellent pregnancy rates after thaw. Vitrification of embryos also appears to be suitable for the refreezing of embryos previously thawed, although there is little data on this subject. In this work, we have tested whether the refreeze technique is suitable for screening cryopreserved embryos for PGT-A.

Study design, size, duration: The study is a retrospective analysis of data between 2016 and 2017. Group A patients had previously cryopreserved embryos were treated with the TBR procedure. Group B patients elected for PGT-A on fresh embryos in the same period (group B).

Participants/materials, setting, methods: Patients aged between 20 and 50 years old, with a body mass index between 19 and 30 were included. Embryos were created with either IVF or ICSI. Cryopreserved and fresh embryos were biopsied and frozen on day 5 or 6, with a minimum quality of 3Cc required (Gardner criteria). Vitrification and thaw of embryos followed Kitazato protocols. Chi-squared test was used for statistical analysis with $P<0.01$ considered significant.

Main results and the role of chance: Overall, 24 cycles of thaw biopsy and refreeze were performed (group A). 126 cycles of PGT-A were performed in group B. A total of 85 embryos were analysed in group A (TBP procedure). 25 of these (29.4%) were normoploid after PGT-A, 57 were aneuploid (67.0%) and 3 had no result obtained (3.5%). In group B, 453 embryos were analysed of which 168 were normoploid (37.1%), 271 aneuploid (59.8%) and 14 had no result obtained (3.0%). After receiving results, 7 patients had embryo transfer in group A and 5 clinical pregnancies (71.4%) were obtained. Of these, 4 achieved a live birth. In group B, 22 cycles of frozen embryo transfer were performed after results were obtained and 7 clinical pregnancies resulted from these transfers (31.8%), with 6 live births. Our results show that the thaw biopsy and refreeze approach is efficient and safe to be applied for previously cryopreserved, undiagnosed blastocysts, as it ultimately allows the transfer of euploid blastocysts and good clinical outcomes.

Limitations, reasons for caution: This study was limited by the retrospective nature of the study, and its small group size. Prospective data on the outcomes of embryo transfers is needed to determine the true efficiency of the technique.

Wider implications of the findings: This study demonstrates that the thaw biopsy and refreeze protocol is suitable for the PGT-A analysis of patients with previously cryopreserved embryos.

Trial registration number: None

P-526 Impact of semen quality on blastocyst mosaicism in preimplantation genetic testing for aneuploidy

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Study question: Does semen quality impact the rate of mosaic blastocysts in preimplantation genetic testing for aneuploidy (PGT-A) cycles?

Summary answer: Compromised sperm quality is positively associated with increased rates of mosaic blastocysts analyzed in PGT-A cycles.

What is known already: PGT-A has long been proposed as an approach for selection of euploid and viable embryos in order to improve treatment efficiency and reduce time to pregnancy. The origins of embryo aneuploidy are well understood, in the large majority of cases being derived from chromosome segregation errors during maternal meiosis. Much less is known on possible intrinsic (inherent to reproductive cells) or extrinsic (e.g. in vitro manipulation) factors that may be responsible for embryo mosaicism. Understanding the causes of embryo mosaicism would be therefore beneficial to formulate more precise prognoses and potentially to better preserve embryo quality.

Study design, size, duration: Retrospective observational study carried out between May 2013 and December 2017, on 340 PGT-A cycles. Study groups were identified according to semen quality: i) severe male factor (SMF), (post-preparation total count <0.1 M/ml sperm); ii) ICSI (sub-optimal post preparation semen characteristics: total motile spermatozoa <2 M/ml; total sperm motility <70%; total sperm rapid motility < 20%, normal sperm morphology <30%); iii) non-male factor (NMF). Performance and rate of mosaic blastocysts of different groups were compared.

Participants/materials, setting, methods: Irrespective of semen quality, all available oocytes were fertilized by ICSI to prevent contamination of biopsied cells by non-embryonic material (cumulus cells, non-fertilizing sperm attached to the zona pellucida). Embryos were cultured to the blastocyst stage (day 5-6) and, where possible, subject to biopsy to collect 3-7 trophectoderm cells to be analyzed for PGT-A. Genetic testing was carried out by array comparative genomic hybridization (aCGH).

Main results and the role of chance: Twenty-two, 173 and 146 cycles were responded to the criteria of the SMF, ICSI and NMF groups, respectively. Age differences between embryo transfer (ET) cycles were not statistically significant. Cycles with blastocysts suitable for genetic testing were 17, 110 and 87 for the same groups, respectively. The rate of euploid blastocysts was 48.1%, 45.9% and 42.9%, respectively ($P>0.05$). Implantation rates of euploid embryos were 30.8%, 30.1% and 39.0%, respectively ($P>0.05$). Live birth rates per treatment cycle were 58.3%, 40.5% and 48.8% in the SMF, ICSI and NMF groups, respectively ($P>0.05$).

Importantly, the rates of mosaic blastocysts were inversely associated with sperm quality being 7.7%, 3.6% and 0.5% in the SMF, ICSI and NMF groups, respectively (SMF vs. NMF, $P=0.0012$; ICSI vs. NMF, $P=0.03$). A significantly higher mosaicism rate in the SMF group was also observed in comparison with all other cycles taken together ($P=0.008$).

Limitations, reasons for caution: The retrospective design and limited numerosity of the study call for more extensive investigations.

Wider implications of the findings: The results indicate that compromised sperm quality is positively associated with increased rates of mosaic blastocysts. This is in line with the hypothesis that sub-optimal characteristics of fertilizing sperm impact the fidelity of chromosome segregation. Sperm assessment appears therefore relevant to a more precise prognostic assessment in PGT-A cases.

Trial registration number: None.

P-527 To examine concordance and mosaicism in oocytes and embryos

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Study question: Analysing concordance and mosaicism in oocytes and embryos to predict the optimal stage of development to perform biopsy to maximise clinical outcome in a PGT-A program

Summary answer: Biopsy for PGT-A assessment most accurately predicts embryo ploidy when done at the blastocyst stage

What is known already: ESHRE PGD Consortium best practice guidelines 2005 found biopsy of polar body (PB) 1 to be acceptable, PB1 and 2 combined to be recommended, blastomere in cleavage stage embryos (day 3) recommended and trophectoderm cells in blastocyst (day 5/6) acceptable. Updated guidelines in 2011, found that due to evolving technologies/techniques and increasing knowledge of mosaicism, biopsy at the day 3 stage was no longer recommended.

HFEA Code of Practice published January 2019, there is no evidence that blastomere biopsy on cleavage stage embryos is safe and effective, trophectoderm biopsy on blastocysts shows conflicting evidence and further research is required

Study design, size, duration:

1. Oocyte donors (5) had oocytes collected and PB1 biopsy from mature oocytes. ICSI performed using donor sperm and PB2 biopsy from zygotes. Embryos were cultured to day 3 and disaggregated.
2. Frozen/vitrified day 3 embryos (34) and blastocysts (21) were thawed/warmed. Blastomere biopsy (day 3 embryos) mural/polar trophectoderm biopsies (blastocysts developed from day 3 embryos and warmed blastocysts) were performed. The ICM was isolated via immunosurgery.

Biopsies, blastomeres and ICM's were sent for genetic testing

Participants/materials, setting, methods: Oocyte and sperm donors were recruited for this project, which was approved and licensed by the HFEA. Oocyte collection, ICSI and biopsy/tubing were carried out as per clinical protocols.

Disaggregation was performed by laser ablation of the Zona Pellucida to release the blastomeres.

Thawing/warming and biopsy/tubing were carried out as per clinical protocols. Immunosurgery using anti-trophoblast antibody and baby rabbit complement isolated the ICM.

Genetic testing was performed by aCGH or NGS

Main results and the role of chance:

1. 65 oocytes collected, maturity rate 92% (60) and fertilisation rate 40% (24). All 24 embryos had PB1 and 2 biopsy and were disaggregated on day 3.
2. Amplification rates for PB1 were 95.8% (23), PB2 79.2% (19) and disaggregation 96% (191).
3. 18 embryos included in concordance analysis. PB1 and 2 concordance rate was 44% (8), 3 aneuploid and 5 euploid. The non concordance was aneuploid:euploid (4) and euploid:aneuploid (6).
4. 16 embryos included in PB1 to blastomere concordance analysis (where >= 70% blastomere concordance). Concordance rate was 56.3% (9). 31.3% (5) were euploid:aneuploid non concordant.
5. Mosaicism was 87.5% (21) with 48% (10) being aneuploid/euploid.
6. The thawing/warming survival rate was 76.5% (26) for day 3 embryos and 71% (15) for blastocysts. 38.5% (10) of the day 3 embryos developed into blastocysts.
7. 19 embryos had a complete set of tubed samples (+- blastomere, mural/polar trophectoderm and ICM) analysed.
8. Amplification rates for blastomeres were 0 (4), mural trophectoderm 42% (8), polar trophectoderm 68% (13) and ICM 63.2% (12).
9. 6 blastocysts were included in the concordance analysis.

Concordance rate was 100% (for mural/polar trophectoderm and ICM). With 50% (3) showing euploid concordance and 50% showing aneuploid concordance. The type of aneuploidy was 100% concordant.

Limitations, reasons for caution: The amplification rates for the blastomere/trophectoderm biopsies and the warming survival rates for blastocysts were inexplicably lower than those in clinical cases during the same time period (90% and 95% respectively).

Wider implications of the findings: Trophectoderm biopsy of blastocysts gave robust concordance data, and perhaps reflects poor reliance upon Day 3 biopsy. A successful PGT program requires robust freezing and frozen embryo transfer. Our 95% vitrification/warming survival rates, 90% amplification rates and pregnancy rates comparable to fresh embryo transfers provides for dependable PGT-A service.

Trial registration number: not applicable

P-528 Pre-conceptional maternal exposure to cyclophosphamide results in modifications of DNA methylation in F1 and F2 mouse oocytes: evidence for transgenerational effects

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Study question: Does pre-conceptional maternal exposure of mice (F0) to cyclophosphamide (CPM) induce modifications of DNA methylation of imprinted genes in F1 and F2 mouse oocytes?

Summary answer: CPM pre-conceptional administration causes alterations of methylation in F1 germline, which are trans-generational inherited by F2, which has not been directly exposed to CPM.

What is known already: CPM, an agent widely used in cancer therapy, alters the ovarian reserve, is mutagenic, teratogenic and embryolethal. Conceptions early after CPM exposure result in a high rate of pregnancy failure and high malformation rate. By contrast, potential effects of maternal exposure to CPM on the health of offspring conceived during the "safe" late period following CPM exposure have never been investigated. A crucial factor for oocyte competence is correct methylation of imprinted genes during gametogenesis. Methylation of maternally imprinted genes including IGF2R e PEG3 is completed in the fully-grown oocyte, whereas paternally imprinted genes including H19, remain unmethylated.

Study design, size, duration: 32 CD-1 young female mice (F0) received vehicle, Crocetin or AS101 for 15 days prior to a single dose of CPM (100 mg/kg) and 12-weeks after CPM were mated with untreated males. Mean number of pups (F1) per mouse, weight at birth, weaning and at 2 month of age were recorded. At this age F1 females were mated and the mean number of pups (F2) per mouse and weight at birth and weaning were recorded.

Participants/materials, setting, methods: Pools of at least 200 healthy germinal vesicle (GV) oocytes from 21-day old mice of each experimental group were processed for DNA extraction. DNA was bisulfite-converted and H19, Peg3 and Igf2r promoters were amplified by PCR. Pyrosequencing reaction was run on a PyroMark Q96ID (Qiagen) and CpGs methylation analysis was conducted by the PyroMark CpG software (Qiagen). The methylation for each

amplicon was calculated as the median of methylation status of each analyzed CpG.

Main results and the role of chance: Twelve-weeks after CPM, all F0 mice got pregnant after mating and gave birth to F1 pups. F1 offspring born from CPM mice presented growth retardation as demonstrated by reduced weight at weaning (Ctrl-F1 vs. CPM-F1: 10.88±0.79 g vs. 6.42±0.58 g, P<0.001) which was recovered at adult age. Pre-conceptional maternal exposure to fertoprotectors AS101 and Crocetin prior to CPM prevented this growth retardation in F1 (AS101-F1: 9.06±0.92 g, Crocetin-F1: 11.11±0.58 g, not significant to Ctrl-F1, P<0.001 to CPM-F1). GV oocytes obtained from 21-day-old CPM-F1 mice presented a reduced methylation of maternally imprinted genes Igf2r (Ctrl-F1 vs. CPM-F1: 69±3.79% vs. 8±2.03%, P<0.05) and Peg3 (Ctrl-F1 vs. CPM-F1: 78±3.61% vs. 59±2.33%, P<0.05) and increase methylation of paternally imprinted gene H19 (Ctrl-F1 vs. CPM-F1: 5±2.19% vs. 28±173%, P<0.05). Nevertheless, F1 mice from all experimental groups were fertile and gave birth to healthy F2 pups. The alterations of maternally imprinted genes observed in CPM-F1 oocytes were present also in oocytes generated by F2 mice (Peg3, Ctrl-F2 vs. CPM-F2: 86±3.61% vs. 61±4.04%, P<0.05; Igf2r, Ctrl-F2 vs. CPM-F2: 90±2.60% vs. 54±5.78%, P<0.05). Pre-conceptional maternal exposure to AS101 and Crocetin prior to CPM was not able to fully counteract alterations in oocyte imprinting of F1 and F2 offspring.

Limitations, reasons for caution: This pilot study needs further investigations of the long-lasting effects of methylation changes at DMRs on the health of next generations, implying possible risk for adult onset of metabolic or neurological pathologies.

Wider implications of the findings: Pre-conceptional CPM maternal exposure influences the health of offspring conceived during the "safe" period following treatment and affects the competence of offspring's oocytes. Our data warns on possible long-term effects on the health of next generations. Supplementation with fertoprotective agents may induce epigenetic long-lasting effects, which requires further investigation.

Trial registration number: not applicable

P-529 Chromosomal aberrations and aneuploidy features in IVF blastocysts and abortion villus

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Study question: What are the chromosomal aberrations and aneuploidy features in blastocysts following IVF and first trimester pregnancy losses?

Summary answer: The chromosome aberration spectrum shows specific characteristics in blastocysts and abortive villi, indicating specific chromosomal abnormalities could affect certain developmental stage of embryos.

What is known already: Chromosomal abnormalities in human embryos is one of the main reasons that lead to developmental arrest and implantation failure. Studies have shown the types of chromosomal abnormalities are incongruous between recurrent implantation failure (RIF) and recurrent miscarriage (RM) patients, for instance, more diploid/heterozygous chimeric embryos appearing in RIF group. In natural conceptions that reach clinical recognition, 35% of human pregnancies are aneuploidy for which easily causes abortion. Miscarriage caused by trisomy abnormalities ranks top, with rare cases of miscarriage by chromosomal structural abnormality.

Study design, size, duration: This is a retrospective study, and was ethically approved by our hospital in China. We analyzed the data of 220 PGS cycles during 2017 to June 2018, after ovarian stimulation cycles. Totally, 1014 blastocysts were genetically analyzed. Meanwhile, we also analyzed 1724 abortion villus data. These data were collected and statistically analyzed.

Participants/materials, setting, methods: No average age of the females were found to be significantly different between the two groups (32.99 in PGS and 33.02 in abortion).SNP array was applied in both the blastocysts and early abortive villi tests. Comparison in chromosomal aberrations and aneuploidy were performed by Chi-square and F-test.

Main results and the role of chance: ①Normal chromosome rates were significantly different between blastocysts and abortive villi, 60.85% and 43.27% respectively(P < 0.001).②Comparatively, higher chromosomal aberration in abortive villi were found to be trisomy (27.71% vs 66.46%), sex chromosome abnormality (3.02% vs 7.98%) and polyploidy (1.00% vs 7 .57%), showing significant different. In addition, higher abnormalities in blastocysts were discovered in the types of complex aberrations (27.96% vs 11.55%), monosome (22.17% vs 0.72%), and fragment abnormality (16.88% vs 5.21%).③In view of the constituent of chromosomal abnormalities, chromosome X monosome is significantly higher in early abortive villi.④According to the International System for Human Cytogenetic Nomenclature (ISCN), group comparison of chromosome monosome between blastocysts and early abortion villi: Abnormalities in blastocysts were more common in group G (39.00% vs 28.87%; P=0.011), and that in early spontaneous abortive villi were in group E (22.72% vs 32.12%; P=0.007).

Limitations, reasons for caution: PGS samples were collected from IVF patients with ICSI fertilization methods. Abortion samples were obtained from patients with natural and IVF pregnancy. Different sources of samples may have an impact on the results. But in practice,it is impossible to detect embryonic chromosomes in natural pregnancies.

Wider implications of the findings: To compare the chromosomal abnormality spectrum in IVF blastocyst and abortive tissues, help to explore the mechanisms that could affect embryo development and implantation. Findings in the chromosomes susceptible to damage embryo development, also help clinical assessment in prenatal diagnosis, reducing abnormal fetus incidence.

Trial registration number: no

P-530 Demystifying the effect of paternal age on blastocyst ploidy status

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Study question: Does paternal age affect euploidy rate in blastocysts analysed in PGT-A treatment cycles?

Summary answer: The effect of paternal age on blastocysts euploidy rate was not statistically significant, after controlling for maternal age.

What is known already: It is well established that embryo aneuploidy increases with advanced maternal age and this has been mainly attributed to chromosomal nondisjunction that occurs during meiosis II of oogenesis. The existence of a similar paternal age effect is still controversial and inconclusive

Study design, size, duration: Sinle centre retrospective cohort study evaluating the effect of paternal age on euploidy rate was performed. Preimplantation genetic testing was carried out on 5-10 blastomeres and analysis was done by Next Generation Sequencing (NGS) via a single genetic provider (Cooper Genomics).The study included a total of 1877 biopsied blastocysts from 436 preimplantation genetic testing for aneuploidy (PGT-A) cycles performed between January 2016 to December 2017. Study was conducted at a UK centre, CRGH London.

Participants/materials, setting, methods: Patients undergoing PGT-A treatment for the specified period were included. Euploidy rates were calculated for different paternal and maternal age groups. Paternal age was grouped as follows (<40,40-44,45-50,>50yrs). Maternal age was grouped according to the HFEA-UK (Human Fertilisation and Embryology Authority) age categories.Data was age controlled and analysed. Analysis of variance (ANOVA) was required for continuous data, continuous variables were compared using the Student's T test, and logistic regression analysis was performed.

Main results and the role of chance: Main indication for PGT-A was advanced maternal age (56.3%); clinically presenting as recurrent implantation failures and/or previous miscarriages. Mean paternal age was 41.11+/-6.6 years. Mean maternal age was 38.48+/-4.1 years. The euploidy rates for the paternal age groups (<40,40-44,45-50,>50yrs) are 33.8%, 27.5%, 14.9% and 16.5 % respectively. When controlling for maternal age, paternal age effect on euploidy rate was not statistically significant for all age categories. When maternal age is <35 yrs there was no statistical difference in eupliody rates whether the paternal age was <45 or > 45 years; OR=0.72 (0.3-1.7).

Logistic regression model analysis of all variables (regardless of age categories) revealed no correlation between paternal age and euploidy status OR 0.92 (0.7-1.2), p-value 0.63. On the other hand, advanced maternal age

was negatively related to blastocysts euploidy rates OR 0.45 (0.2-0.6), p-value 0.0001.

Limitations, reasons for caution: The study is a retrospective cohort study, further randomised clinical trials are required to confirm findings. The effect of semen parameters on ploidy status were not analysed as ICSI was the mode of insemination for all cases and controls.

Wider implications of the findings: This is the first large prospective study to analyse the effect of advanced paternal age on blastocyst ploidy status. The results are reassuring suggesting that older men can still have a positive reproductive outcome without the concern of having a higher risk of chromosomal abnormalities.

Trial registration number: Not applicable.

P-531 Follicle Diameter Predicts Oocyte Maturity and Blastocyst Formation but not Blastocyst Euploidy

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Study question: Are the oocytes within larger follicles better candidates for pregnancy as judged by the incidence of blastocyst euploidy?

Summary answer: The ploidy of biopsiable blastocysts was not related to the diameter of the follicle from which the oocyte was retrieved.

What is known already: Although oocyte maturity and blastocyst formation are known to be associated with follicle diameter, there has been no study relating the incidence of blastocyst euploidy to follicle diameter. The purpose of this study was to examine follicle diameters, the oocytes from those follicles and the embryos that result from those oocytes to see if there is an association between follicle diameter and the quality of oocytes as judged by blastocyst formation and blastocyst ploidy.

Study design, size, duration: Twenty-two oocyte donors (ages: 24.5 + 3.5 yr) whose oocytes would be inseminated in vitro using intracytoplasmic sperm injection (ICSI) were enrolled in this study. Retrieved oocytes and embryos arising from follicles of known diameter were analyzed for associations between follicle diameter and oocyte/embryo quality.

Participants/materials, setting, methods: Follicles, measured ultrasonographically, were aspirated one-at-a-time. Oocytes were cultured individually in sequential media (Quinn's Advantage, SAGE) associating follicle diameter with each oocyte. Trophectoderm biopsies of blastocysts on days 5/6 were tested for ploidy using next generation sequencing (NGS, CooperGenomics, NJ). Maturity, fertilization, blastocyst formation and ploidy were analyzed according to the oocyte's follicle diameter. Comparisons were made using cumulative histograms, rolling averages and receiver operator characteristic (ROC) curves and areas under the curves (AUC).

Main results and the role of chance: Of 330 retrieved oocytes, 315 (96.1%) had an associated follicle diameter. Of the oocytes with follicle diameters, 255 (80.4%) had a polar body (MII), and 60 (18.9%) were immature: 31 (9.8%) with a visible germinal vesicle (GV stage) and 29 (9.1%) with neither a polar body nor a visible germinal vesicle (MI). The incidence of MII oocytes was significantly associated with larger follicle diameters (ROC AUC = 0.87; $P < 0.0001$). The incidence of GV oocytes was significantly associated with smaller follicle diameters (ROC AUC = 0.96; $P < 0.0001$). Among MII oocytes there was no association with follicle diameter for the appearance of 228 oocytes with 2 pronuclei (2 PN). Among 2 PN's the development of 94 biopsiable blastocysts (TE Bx) exhibited a small but significant association with larger follicles (ROC AUC = .59; P = 0.01). From the 94 biopsiable blastocysts, 51 were determined to be euploid by NGS. The incidence of euploidy among biopsied blastocysts was not significantly associated with follicle diameter (ROC AUC = 0.51).

Limitations, reasons for caution: Follicle diameter is an excellent predictor of oocyte maturity and is a significant albeit weak predictor of the ability of fertilized oocytes to become biopsiable blastocysts. The incidence of euploidy is indistinguishable for biopsiable blastocysts arising from small, medium or large follicles and is unrelated to follicle diameter.

Wider implications of the findings: The relationship between follicle diameter and maturity may help to predict the number of mature oocytes for egg banking. There is no reason to preferentially retrieve follicles of a particular size when attempting to maximize the incidence of euploid blastocysts other than by improving maturity and/or blastocyst formation.

Trial registration number: not applicable

P-532 Karyotype analysis of blastocyst chromosomes derived from three pronuclear fertilized embryos using preimplantation genetic testing-aneuploidy (PGT-A)

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Study question: The rate of blastocyst formation from three pronuclear (3PN) zygotes is generally low. Do blastocysts derived from 3PN zygotes exhibit triploid or normal chromosomes?

Summary answer: Several chromosome patterns were observed, including triploidy, diploidy, euploidy and aneuploidy. Triploidy appeared at a higher rate than expected.

What is known already: Zygotes possessing 3PN have been reported to occur at a low rate in both c-IVF (conventional in vitro fertilization) and ICSI (intracytoplasmic sperm injection). Several hypotheses exist to explain such aneuploidies, but the underlying causes in human assisted reproductive technology (ART)-derived zygotes remain unknown. Furthermore, whether the chromosomal karyotype is normal or not, is also unclear. Here, we used next generation sequencer (NGS) to analyze the chromosome karyotype of blastocysts derived from 3PN.To the best of our knowledge, there is only very small report usage of NGS for analysis of chromosome karyotype.

Study design, size, duration:

Study 1:

This study compared the incidence of 3PN under c-IVF and ICSI treatment cycles. Between January 2014 and December 2017, we observed a development and formation morphology of blastocysts through 7005 oocytes in vitro culture.

Study 2:

All 1013 blastocysts were vitrified and required embryos were thawed, and in final study, 14 blastocysts which derived original 3PN from ICSI were analyzed the chromosome patterns by using NGS.

Participants/materials, setting, methods: The experimental 3PN zygotes were derived from embryos for which informed consent was obtained for disposal. These zygotes were cultured until the blastocyst stage and observed using time lapse imaging. Laser biopsy was used to separate cells from trophectoderm.

Whole genome amplification was carried out using the SurePlex DNA Amplification System (Illumina Inc., San Diego, USA), and further chromosome profiles were analyzed by the VeriSeq-PGS kit (Illumina).

Main results and the role of chance:

Study 1:

The overall incidence of 3PN zygotes was 380/7005 (5.4%), of which 296/4499 (6.6%) occurred under c-IVF and 84/2506 (3.4%) under ICSI. Therefore, the rate of 3PN was higher under c-IVF than ICSI treatment. Blastulation was successful in 39/296 (13.2%) 3PN zygotes obtained from IVF and in 20/84 (23.8%) 3PN zygotes from ICSI.

Study 2:

We biopsied 3PN blastocysts obtained only from ICSI, at a success rate of 14/14 (100%). The ICSI 3PN blastocysts yielded 6/14 (42.9%) normal (euploidy) embryos, 6/14 (42.9%) confirmed or suspected triploid embryos, and 2/14 (14.3%) suspected diploid embryos. Only one euploid embryo was identified as XY. As second polar bodies were observed 43% after ICSI by time lapse cinematography, it is suggested that release of the second polar body may be involved. However, the cause of 3PN zygote formation is unclear.

Limitations, reasons for caution: Further studies are required to conclusively identify the mechanisms responsible for 3PN zygote formation and the high rate of triploid embryo formation**.** Likewise, whether or not euploid embryos can form normal blastocysts is unknown. We were unable to identify normal diploid and triploid chromosome profiles by NGS.
Wider implications of the findings: Here, we report for the application of NGS for identification of euploid/aneuploid/triploid blastocysts derived from 3PN zygotes. It is a clue to elucidate the mechanism of occurrence of triploids.
Trial registration number: Not applicable.

P-533 Does the oocyte vitrification affect the aneuploidy and mosaicism rate in the blastocysts?

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Study question: The main objective of our study was to evaluate if the blastocysts from vitrified/warmed oocytes have more aneuploidies or mosaicism than those from fresh oocytes.
Summary answer: There were no differences in the aneuploidy and mosaicism rates among the blastocysts from vitrified/warmed oocytes and the blastocysts from fresh oocytes.
What is known already: Oocyte vitrification is a common practice in IVF treatments both in cycles with autologous oocytes and in cycles with donated oocytes. This procedure allows to increase the number of blastocysts to analyze in a PGT-A cycle by accumulating vitrified oocytes from different ovarian stimulations in poor responder patients. Moreover, in oocyte donation programs, it can make easier the coordination with the recipient patient. In a robust cryopreservation oocyte program, vitrified oocytes can lead to a pregnancy rate similar to fresh. Nevertheless, there are a paucity of data about aneuploidy and mosaicism rates in blastocysts from vitrified oocytes.
Study design, size, duration: Preimplantation genetic testing for aneuploidy (PGT-A) was performed in 225 oocyte donation cycles in couples who demanded chromosome analysis of the embryos from January 2017 until December 2018. A total of 797 blastocysts were analyzed (644 obtained from fresh oocytes and 153 from vitrified/warmed oocytes). The trophoectoderm biopsies of day 5 and 6 blastocysts were analyzed by NGS. Cycles from males with an abnormal aneuploidy rate in the sperm were not included.
Participants/materials, setting, methods: Embryo analysis were performed using Veriseq NGS (Illumina), with previous whole genome amplification (Sureplex, Illumina). The analysis was performed using the BlueFuse Multi software (Illumina). The main measures were the aneuploidy rate (whole and segmental aneuploidy) and the mosaicism rate (whole and segmental mosaicism) evaluated in the two sources of oocytes. Other measures evaluated were implantation and pregnancy rate of euploid blastocyst. The differences between groups were evaluated by logistic regression and chi-square (SPSSv20.0).
Main results and the role of chance: A total of 797 blastocysts from donate oocytes (153 from vitrified oocytes and 644 from fresh) were analyzed to identify euploid embryos. The aneuploidy and mosaicism rates obtained were comparable in embryos coming from vitrified/warmed oocytes and from fresh oocytes (30.1% vs. 29.8%, p=0.881 and 20.3% vs. 25.3%, p=0.250, respectively). Also, no differences were observed when we separated segmental errors and entire chromosome errors. The incidence of segmental aneuploidies was 7.2% in blastocysts from vitrified oocytes vs. 5.4% in blastocysts from fresh oocytes (p=0.339), while the incidence of segmental mosaicism was 6.5% in vitrified oocytes and 7.6% in fresh oocytes (p=0.580). To evaluate the clinical outcomes, a total of 159 cycles were analysed (94.3% of which were single embryo transfer). The average age of the donors was 25.7 years. There were no significant differences between euploid blastocysts from vitrified oocytes and fresh oocytes regarding to the implantation rate (OR 0.521, 95% CI 0.245-1.107), pregnancy rate (OR 0.562, 95% CI 0.264-1.194), biochemical miscarriage rate (OR 1.194, 95% CI 0.307-4.650) and clinical pregnancy rate (OR 0.514, 95% CI 0.240-1.102).
Limitations, reasons for caution: This study was limited by the small sample size. Larger study is needed to ensure that the percentage of aneuploidies and mosaicism detected in blastocysts are similar independently to the source of oocytes.
Wider implications of the findings: There are few data about aneuploidies in embryos from vitrified/warned oocytes, so this study broad the published data showing that the oocyte vitrification does not increase the aneuploidy or mosaicism rate in embryos. Moreover, this study confirms that the oocyte vitrification is a safe practice in terms of clinical outcomes.
Trial registration number: Not applicable

P-534 A fast and non-invasive workflow for the detection of clinical outcomes on mass spectral data from urine: Application to aneuploidies in high-risk pregnancy.

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Study question: Is it possible to develop a fully automated computational tool for the detection of aneuploidies from mass spectral data of high-risk pregnancy from the UK?
Summary answer: First-trimester pregnancy urine subjected to computer-based MALDI-TOF mass spectral pattern analysis identifies trisomy 21 and 18 in a single rapid cost-effective screening test.
What is known already: MALDI-ToF generated urine mass spectra is a non-invasive approach shown to have the potential for mass market diagnostic or screening tests, with relevance to obstetrics and reproductive medicine. However, urine mass spectra of large data sets are too complex and time-consuming to be tackled with conventional statistical methods. Thus, automated computational-based workflows are needed. The previous study on a small data set of urine mass spectra from high-risk pregnant in the UK has shown promising results forscreening of aneuploidies. This was based on identified mass spectra differences between pregnancies that resulted in non-aneuploid and aneuploid children with trisomy 21.
Study design, size, duration: A computational workflow was developed during a period of 6 months to perform automated mass spectra quality assessment, comparative analysis, and generation of predictive models. Workflow robustness for pre-processing and quality control was tested against 10000 mass spectra. The workflow was further applied to 438 mass spectra of high-risk pregnant urine, previously collected from King's College Hospital (UK). Down syndrome and trisomy 18 pregnancy-outcomes were the tested groups, whereas non-aneuploid the control group.
Participants/materials, setting, methods: Urine was collected prospectively from patients attending a high-risk antenatal clinic at Kings College London, identification blinded and analysed at MAPDiagnostics Laboratory using a Shimadzu instrument. The computational workflow was developed in python by the bioinformatics team of MAP Sciences. Predictive models based on identification of mass spectral patterns were systematically generated using a subset of the data (training set). Models were tested retrospectively by accessing sensitivities and false positive rates on the remaining data.
Main results and the role of chance: The computational workflow was able to process 10000 urine samples in a total of 4 hours. The computational workflow was accurate in quality control decisions on urine spectra, in agreement with independently made manual inspection of 3000 samples. On a small subset of the data with known clinical outcomes, the computational tool identified multiple characteristic patterns for Downs and trisomy 18, when compared with non-aneuploids and considered to be high-risk pregnancies. Based on these patterns, and using the tool, 200 predictive models were systematically generated for the identification of Downs syndrome and trisomy 18. Several models showed good performance with sensitivities up to 100% and false positive rates < 12%. Several specific spectral changes correlated with gestational age and, in general, the performance of the models was improved when designed for the 13th week of gestation. In addition, the performance of the models also depended on the m/z window tolerance for comparisons.
Limitations, reasons for caution: Limiting factor in this study was the total number of known outcomes from pregnancies analysed in the UK population (n < 250). In addition, gestational age is a critical spectral pattern

determinant and thus dating samples to the nearest week of gestation is needed.

Wider implications of the findings: Fast and fully automated tool for extracting diagnostic potential from urine without relying on traditional biomarkers. This tool saves time, money and human resources which enables fast and cheaper screening tests for larger populations worldwide regardless of economic status.

Trial registration number: not applicable

P-535 Reproductive outcomes after PGT cycles in Turner's Syndrome

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Study question: What are embryo chromosomal abnormality rates and reproductive outcomes in women with Turner's Syndrome using their own oocytes after assisted reproduction ?

Summary answer: Embryo chromosomal abnormality rates were 34%,sex chromosomal abnormality rates were 6.9%.Livebirth rates,clinical pregnancy rates and miscarriage rates of thawed cycles after PGT were 42.86%,57.81%,13.5%.

What is known already: Most patients of Turner's Syndrome suffer from gonadal dysgenesis and result in pubertal delay or failure,infertility and premature ovarian failure.Chances of spontaneous pregnancy in Turner's Syndrome are rare,pregnancy outcomes are poor due to increasing risk of miscarriage.Mosaic cases present milder phenotypic abnormality.

Study design, size, duration: This is a retrospective study including 76 Turner's Syndrome women went ART in china from 2013 to 2018.Data from 100 PGT cycles ,followed 65 thrawed cycles and 203 embryos were analysed.

Participants/materials, setting, methods: Patients were devided into three groups according to mosaic cell line ratio,G1≤10%,10%<G2<50%, G3≧50%.Ovary reserve markers and embryo chromosome abnormality rate,sex chromosome abnormality rate,clinical pregnancy rate,miscarriage rate,live birth rate after thawed cycles were evaluated between groups.Chromosome abnormality between Turner's Syndrome patients and women in normal genotype went PGT cycles were compared.

Main results and the role of chance: Live birth rates,clinical pregnancy rates and miscarriage rates of thawed cycles after PGT in Turner's Syndrome patients were 42.86%,57.81%,13.5%.There was no statistical difference between embryo total chromosome abnormality rates in Turner syndrome patients and those of patients went PGS treatments due to repeated abortion or poor pregnancy history(34%vs33.05%).Sexual chromosomal abnormalities include monosomic, trisomy, and fragment abnormalities were more common in embryos of Turner's syndrome(6.9%vs1.07%).45,X mosaic cell ratio of Turner's Syndrome women has no effect on embryo chromosome abnormality probability, sex chromosome abnormality probability, and clinical pregnancy rates,live birth rates,miscarriage rates of PGT followed-frozen embryo transplantation.

Limitations, reasons for caution: Limitations include the retrospective study design and the heterogeneity of the included patients. Additionally, genotype of lymphocytes in blood may be different from ovary.

Wider implications of the findings: PGT is essential for women in Turner's Syndrome using their own oocytes in ART due to high risk of sex chromosome abnormality.The reproductive outcomes of thawed cycles after PGT treatment in patients with Tuner's Syndrome are acceptable.

Trial registration number: na

P-536 Identification of a novel gene responsible of the Oligoasthenoteratospermia (OATs) phenotype in a Tunisian consanguineous family

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Study question: Is it possible to identify the causative genetic mutation responsible of an OATs phenotype in a Tunisian consanguineous family via whole exome sequencing?

Summary answer: A homozygous variation in a conserved promoter region of an autosomal gene was identified by using whole exome sequencing in the two affected brothers.

What is known already: Infertility is a global health problem affecting 48.5 million couples worldwide**.** A male factor is found in approximatively 20 to 30% of infertile couples. Due to the complexity of the reproduction process, 40 to 50% of cases remain idiopathic with 15% having a genetic etiology. OATs is a known cause of infertility in men. Patients show a combination of sperm abnormalities characterized by a reduced motility, number and an altered morphology. Until now, only one gene NANOS1 have already been related to this phenotype.

Study design, size, duration: This study was carried out on a consanguineous Tunisian family comprising seven siblings including five brothers and two sisters. The parents are first degree cousins; the father passed away and the mother is alive. Two brothers showed the OATs phenotype, the two others are fertile and one with unknown fertility status. One sister is fertile and the other one is handicapped with unknown fertility status.

Participants/materials, setting, methods: We obtained blood samples from the two affected brothers, two fertile brothers, the mother and the fertile sister. Genomic DNA was extracted using the FlexiGene DNA Kit (Qiagen, France) according to the manufacturer's instructions. Whole exome sequencing was performed for the two infertile brothers by IGBMC Sequencing Platform (GenomEast) via Illumina technology. Sequencing results were analyzed with *Varank* software developed by our collaborator.

Main results and the role of chance: Whole exome sequencing results revealed a homozygous variation in the promoter region of a gene on chromosome 6. The variation (c.-61 G>A,p?) is in a conserved region through evolution (Human to Elephant) suspected to be a transcription factors (POL II) binding sequence [CGCGGAAGC (WT)/CGCAGAAGC (MT)]. The wild-type protein is part of the complex regulating N6-methyladenosine (m^6A) formation known to play a role in human fertility. Knock out mice for two other subunits of the m^6A complex show an infertility phenotype with an altered spermatogenesis; one knock out results in the OATs phenotype in the mice. Functional studies are being performed to analyze the effect of the variation on the gene transcription. Preliminary results using reporter gene technique based on Luciferase activity showed a decrease in transcription between the Wild Type and the mutated gene.

Limitations, reasons for caution: Our study is limited to one family only and we still have to screen a cohort of patients presenting the same phenotype. The segregation of the variation is being controlled for the family members with Sanger Sequencing.

Wider implications of the findings: Our findings identified a novel gene responsible of the OATs phenotype. This study provides a better understanding of the physiology of male spermatogenesis as well as an advance in the field of diagnosis of infertility.

Trial registration number: Not applicable

P-537 GSTM1 and GSTT1 deletion is a risk factor for infertility in women

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Study question: Is GSTM1 and GSTT1 deletion associated with the development of female infertility?
Summary answer: Yes, GSTM1 and GSTT1 null genotypes, alone or in association, are associated with an increased susceptibility to infertility development in women.
What is known already: Infertility is characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sex or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner. GSTM1 and GSTT1 belong to the Glutathione S-transferases family, that catalyze the conjugation of oxidative stress products, environmental toxins, carcinogens and reactive electrophiles. Individuals with a homozygous deletion at the locus of these genes do not exhibit any functional enzymatic activity, which may lead to increased oxidative stress and consequently an increased likelihood of developing infertility, or pathologies that are known infertility causes.
Study design, size, duration: A case-control study was designed to investigate the association of GSTM1 and GSTT1 gene polymorphisms with female infertility. Case subjects, 143 women with infertility established as women under 39 years of age, were enrolled between October 2015 and July 2018. 95 fertile female controls, with no previous history of infertility, no previous history of gynecological pathologies compatible with infertility, and no previous history of IVF treatments, were selected in Obstetrics Consultation.
Participants/materials, setting, methods: Blood was collected by venous puncture in ethylenediamine tetraacetic acid (EDTA)-containing tubes, as part of the routine analyzes. Genomic DNA was extracted using a DNA extraction kit and stored at 4°C. Genotyping for GSTM1 and GSTT1 was performed using polymerase chain reaction (PCR)-based methods. The presence of the wild-type and/or the null alleles was analyzed by multiplex PCR together with co-amplification of a fragment of beta-globulin gene as a positive control.
Main results and the role of chance: We found an increased risk of developing endometriosis associated with GSTM1 null genotype (OR 0.149; 95% CI 0.064-0.348; p<0.001) as well as GSTT1 null genotype (OR 0.392; 95% CI 0.178-0.863; p=0.017). We also observed an increased prevalence of polycystic ovary syndrome (PCOS) associated with deletion of GSTM1 (OR 0.304; 95% CI 0.139-0.663; p=0.002). We found an increased risk of premature ovarian failure associated with GSTM1 null genotype (OR 0.371; 95% CI 0.166-0.831; p=0.013) as well as GSTT1 null genotype (OR 0.397; 95% CI 0.175-0.900; p=0.023). We verified an increased prevalence of tubal pathology associated with deletion of GSTM1 (OR 0.182; 95% CI 0.077-0.431; p<0.001) and GSTT1 (OR 0.424; 95% CI 0.185-0.970; p=0.034). A strong association of GSTM1 null genotype with female infertility, regardless of the cause was found (OR 0.277; 95% CI 0.159-0.482; p<0.001) as well as the GSTT1 null genotype (OR 0.430; 95% CI 0.243-0.761; p=0.003). The two-way combination of GSTM1 and GSTT1 null genotypes resulted in an increased susceptibility to infertility development (OR 0.113; 95% CI 0.046-0.282; p<0.001).
Limitations, reasons for caution: The genotyping method does not identify homozygous wild-type and heterozygous individuals but classifies as "present" individuals with one or two copies of the relevant gene, and individuals with homozygous deletions as "null". 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 very disparate results. The association of GSTM1 and GSTT1 null genotypes with endometriosis was observed in other studies, but others disagree. The association of GSTM1 null genotype with PCOS, is not corroborated by another study.
Trial registration number: Not applicable.

P-538 Is there a difference in the efficacy of recombinant and urinary purified FSH hormones in patients with different FSHR genotype? A study on 3268 cycles

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Study question: Is the FSHR genotype Ser680Asn correlated with the effectiveness of recombinant (rFSH) or urinary highly purified FSH (HP-FSH) in terms of oocytes retrieved and pregnancy rate?
Summary answer: No difference was observed in number of retrieved oocytes, pregnancy rate and take-home babies in relation to the FSHR genotype and the FSH source
What is known already: In 2015 a study on 382 cycles and 191 egg donors (Lledo et al), demonstrated that there was a difference in terms of retrieved oocytes according to the *FSHR* genotype. It is already known that this polymorphism can modify the response to FSH during ovarian stimulation. HP-FSH was more effective than rFSH in patients with the *FSHR* genotype 680 Ser/Ser; rFSH was more effective in patients with the genotype Ser/Asn and no difference was seen in the group with the genotype Asn/Asn. A large study showing a difference in pregnancy and delivery rate by FSH type is still missing
Study design, size, duration: Our study includes 3268 cycles (fresh and thawing) performed in our center in the last 10 years. All female patients were tested for *FSHR* genotype. Cycles were divided according to FSH type: 694 with Merional (HP-FSH), 606 with Menopur (HP-FSH), 1240 with Gonal F (rFSH) and 728 with Puregon (rFSH). We compared the efficacy of the different FSH in the 3 genotype groups. Outcomes were oocyte yield, metaphase II oocytes, pregnancy rate and delivery rate
Participants/materials, setting, methods: 3268 cycles, fresh and frozen, coming from 2094 *FSHR*-genotyped patients were enrolled in this retrospective study. There were no exclusion criteria. Genotyping of the *FSHR* Ser680Asn polymorphism was performed by Taqman analysis. Stimulation was achieved by conventional long or short protocols. The results were evaluated for the three genotypes in two groups: female patients < 38 years and ≥38 years. Statistics were performed using a chi-squared test
Main results and the role of chance: 30% of patients were *FSHR* Asn/Asn (n = 624), 47% were Ser/Asn (n= 988) and 23% were Ser/Ser (n = 482). No difference was observed for oocyte retrieval, pregnancy or delivery rate comparing the different FSH sources in the different genotype groups.

No difference was observed for oocytes retrieval in patients < 38 years and ≥38 years (mean value 10.5 for <38 years and 9.2 for ≥38; p>0.05). The pregnancy rate (biochemical + evolutive + delivery) in women <38 years was higher with rFSH (43% with rFSH versus 39% with HP-FSH, p = 0.043). No significant difference between rFSH and HP-FSH was observed in this group by considering only the delivery rate. In women ≥38 years the delivery rate was slightly increased using the HP-FSH: 16% with HP-FSH versus 12% with rFSH, but this difference was not statistically significant (p=0.24)
Limitations, reasons for caution: Despite the high number of patients and cycles, it is a retrospective study where different stimulation protocols have been used. Prospective and randomized studies should be performed
Wider implications of the findings: No major differences in efficacy of hormonal ovary stimulation was observed between HP-FSH and rFSH in a large, 10 year retrospective study
Trial registration number: not applicable

P-539 Ep300-mediated crotonylation of EGFR in cumulus cells is essential for the human oocyte maturation in vitro

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Study question: Is crotonylation modifications in cumulus cells essential for the human oocyte maturation in vitro?
Summary answer: Crotonylation of EGFR in cumulus cells is essential for the oocyte maturation in an Ep300-dependent manner.
What is known already: IVM (oocyte maturation in vitro) is an attractive approach forinfertile women, especially the ones with PCOS(polycystic ovarian syndrome) and cancers, due to minimal even no FSH and hCG use. Protein post-translational modifications are crucial epigenetic mechanisms regulating a variety of biological events. Lysine crotonylation is a newly identified posttranslational modification on a series of histone and non-histone proteins.
Study design, size, duration: By analyzing online data (GEO profiles) and detecting clinical samples from the IVM, we detected the level of crotonylation during the oocyte maturation in vitro and confirmed the key modulator of crotonylation in the process. Cell experiments and mechanism exploration were performed to evaluate the role of crotonylation in cumulus cells expansion by

increasing crotonylation level by exogenous sodium crotonate or modulator of crotonylation alteration by siRNAs and specific activator.

Participants/materials, setting, methods: Cumulus cells from human IVM cycles were used to detected the crotonylation level during human IVM by PCR and protein dot blot. Cumulus cells and normal 293T cells were used to evaluated the effects of increasing crotonylation level on cell proliferation and apoptosis by CCK-8 assays and apoptosis markers detection. Mice IVM model was used to measure the effect of crotonylation increase on oocyte maturation in vitro.

Main results and the role of chance: Here we report that crotonyltransferase Ep300 is increased in cumulus cells during human IVM, and associated with cumulus cells expansions. Increased expression of Ep300 mediates enhanced total crotonylation level in cumulus cells. Cell experiments show that increased crotonylation level by exogenous sodium crotonate regulates the cumulus cell proliferation, apoptosis and oocyte maturation in vitro, as well as Ep300 alteration caused by siRNAs and specific activator. We further confirm that EGFR is crotonylated by interacting with Ep300 and crotonylated EGFR mediates the cell functions of increasing total crotonylation. Moreover, crotonylated lysine residue hinders the EGFR degradation and enhances the ATP binding with EGFR, thus activates EGFR pathway.

Limitations, reasons for caution: The effects of crotonylation on oocyte maturation were only evaluated on mice model but not human oocytes.

Wider implications of the findings: Our results reveal that crotonylation of EGFR in cumulus cells is essential for the oocyte maturation, and Ep300 is a candidate target for improving the culture of human oocyte in vitro.

Trial registration number: not applicable

P-540 Mosaicism in human blastocysts and technical factors of laser trophectoderm biopsy

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Study question: Is there any correlation between technical aspects of laser trophectoderm (TE) biopsy and the incidence of mosaicism?

Summary answer: There is no correlation between technical aspects such as mechanical type of biopsy, operator, lengths or number of laser pulses, and the incidence of mosaicism

What is known already: Currently, TE biopsy has become as a gold standard for preimplantation genetic testing (PGT). However, the use of new diagnostic techniques, such as Next Generation Sequencing (NGS), which better discriminate and quantify the genetic material amount, increases the possibility of identifying embryonic mosaicism. Estimating the incidence of mosaicism in human blastocysts is a difficult challenge, due to technical and biological limitations. Different laboratories reported a wide range of mosaicism rate and the involvement of technical factors is still debated. The purpose of this study is to determine whether mosaicism rate is affected by any technical aspects of TE biopsy

Study design, size, duration: A multicentric (two laboratories) prospective observational cohort study involving 53 patients (age= 33.6±7.2) and 192 TE biopsies, was performed between September and December 2018 (3.8±0.4 biopsied embryos/patient). Blastocyst's morphological characteristics and technical factors were recorded. The primary outcome was mosaicism rate correlated with blastocyst's features and technical factors. Chi-square tests were used to assess the association of the selected parameters with the observed mosaicism. Logistic regression analysis was performed to correct the confounding factors

Participants/materials, setting, methods: Laser assisted hatching was performed on Day 3 and culture prolonged up to Day 5/6. Blastocysts were individually vitrified after biopsy. Blastocyst's biopsies were performed by five experienced embryologists who set the procedure according to TE characteristics. TE samples were sent for PGT of aneuploidies (PGT-A) using NGS. The day of TE biopsy, blastocysts quality and expansion (Gardner score), laser pulses number and length, and TE biopsy mechanical technique (flicking or pulling) were recorded

Main results and the role of chance: PGT-A data reported 57 euploid blastocysts (29.7%), 44 mosaic (22.9%), 84 aneuploid (43.8%), 3 inconclusive (1.6%) and 4 not amplified (2.1%). Although some studies consider the impact of technical variation as one possible cause contributing to overestimate the incidence of mosaicism in embryos, it has not yet been demonstrated. In this way, our results show that none of the biopsy technical factors analyzed correlate with the incidence of mosaicism: biopsy technique (flicking= 26.6%; pulling= 26.6%), laser pulse lengths (400ms= 26.2%; 500ms= 20.7%; 600ms= 27.3%), number of laser pulses (<3= 22.1%; 4 to 6= 22.5%; >7= 37.5%). On the contrary, some authors have previously correlated this latter feature with an increase of the aneuploidy diagnosis probability in biopsies performed with more than 10 laser pulses.

About the inherent characteristics of biopsied blastocysts, no significance variation of the mosaicism rate was correlated with blastocyst's quality (excellent= 22.6%; good= 20.6%; regular= 24.2%; poor= 25.9), blastocyst's expansion (≤4= 20.5%; 5= 29.6%; 6= 18.8%) or the day of TE biopsy (5= 22.6%; 6= 23.2%)

Limitations, reasons for caution: These are preliminary results. For the moment, the low number of the collected samples is an important limitation. Moreover, PGT-A mosaicism data refer only to blastocysts resulted as euploid/aneuploid mosaics. For a robust association between mosaicism and technical aspects of TE biopsy, aneuploid/aneuploid mosaics should be included

Wider implications of the findings: In our setting, we have not found any implication of technical parameters of the biopsy and the incidence of mosaicism. Considering our observations, the incidence of mosaicism seems to be only influenced by biological factors, or other technical limitations

Trial registration number: none

P-541 STR and SNP markers for PGT-M: bioinformatic identification, availability and informativity

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Study question: The aim was to compare a PCR-based STR approach versus SNP array regarding the number and informativity of genetic markers for PGT-M.

Summary answer: The coverage of SNP and STR markers is gene locus-dependent. Therefore, the number of genetic markers should be carefully assessed before selecting the analytical method.

What is known already: Preimplantation genetic testing (PGT-M) gives couples at risk of transmitting a monogenetic disorder a possibility to have healthy offspring. The embryo's genotype can be determined by PCR-based direct mutation testing combined with linkage analysis by using short tandem repeats (STRs). Recently, single nucleotide polymorphism (SNP) microarrays and next generation sequencing were introduced for PGT-M. These high-throughput whole genome methods do not require any family- or disease-specific design. However, allelic drop-out can affect up to 80 % of SNPs spotted on a SNP array. Additionally, pretesting of parents and one close relative is necessary to evaluate the number of informative SNPs.

Study design, size, duration: 1) The numbers of potentially informative STRs and SNPs at the corresponding gene locus and 2 Mb flanking regions were compared in six monogenic disorders. The distribution of SNPs on the karyomapping platform (Natesan et al. 2014) was used as a reference. 2) Additionally, for two families, one affected with cystic fibrosis and another with a familial adrenoleukodystrophy, the informativity of STR and SNP markers as well as allelic drop-out rates were examined.

Participants/materials, setting, methods: 1) To identify the number and location of potential and known STR repeats, a python script was developed. Five UCSC Genome Browser tracks were filtered and STRs present in at least two categories were counted. 2) Genomic DNA of selected family members was amplified with either a multiplex PCR assay followed by capillary electrophoresis (ABI 3730XL) or multiple displacement amplification and the karyomapping protocol (Illumina Inc.). The statistical analysis was carried out by paired t-test.

Main results and the role of chance: The use of bioinformatics drastically reduced the likelihood of missing some genetic markers and significantly shortened the time of their identification. The overall number of available SNP markers was significantly higher. However, for two out of six analysed disorders (cystic fibrosis and spinal muscular dystrophy) the difference in STR and SNP marker coverage was marginal. Secondly, although only one-fifth of all

available STRs were selected for genotyping, the number of informative STR and SNP markers inside and around the *CFTR* gene was comparable. In case of adrenoleukodystrophy, no informative genetic markers were detected within the *ABCD1* gene. Furthermore, the distribution of SNPs on the karyomapping array was non-uniform, encompassing only 16 informative maternal SNPs in the 3' flanking region. Finally, the analysis of at least 50 single buccal cells demonstrated that the mean amplification efficiency per cell over all analysed STR markers equalled 93.8 % for cystic fibrosis and 98.7 % for adrenoleukodystrophy. Moreover, the allelic drop-out rate for both disorders was low (2.5 %). These results indicate that for some monogenic disorders, the PCR-based approach is favourable.

Limitations, reasons for caution: So far, the informativity of STR markers was analysed for two monogenic disorders and 20 % of available STRs were assessed.

Wider implications of the findings: Implementation of bioinformatics significantly reduces the time required for the identification of STRs. For loci with a low number of available SNP markers or higher recombination rates, conducting traditional STR linkage analyses may still be advantageous. Therefore it is useful, to compare *a priori* the number of available genetic markers.

Trial registration number: not applicable

P-542 Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilization or intracytoplasmic sperm injection

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Study question: What is the effectiveness and safety of preimplantation genetic screening (PGS) in women undergoing IVF or ICSI treatment?

Summary answer: This updated Cochrane systematic review shows there is no increase in (cumulative) live birth rate by performing PGS in an IVF/ICSI treatment.

What is known already: PGS is being offered to patients to select embryos for transfer in an IVF/ICSI treatment to improve treatment effectiveness in terms of live birth rate. It was demonstrated that the first generation of PGS, using cleavage-stage biopsy and fluorescence in situ hybridization (FISH) for the analysis, was ineffective in improving live birth rates. Newer forms of PGS were developed that perform the procedure at other stages of embryo development and use a different method of genetic analysis. These new forms of PGS have been introduced into routine clinical practice, however effectiveness of this practice still needs to be established.

Study design, size, duration: We performed a systematic review and meta-analysis of relevant randomized controlled trials (RTCs). We searched the Cochrane Gynaecology and Fertility Group Specialised Register of controlled trials, the Cochrane Central Register of Controlled Trials (CENTRAL) , MEDLINE, Embase, CINAHL, PsycINFO, WHO clinical trials register, and clinicaltrials.gov from inception to November 2018. RCTs comparing IVF/ICSI with PGS versus IVF/ICSI without PGS were eligible for inclusion. Two review authors independently selected trials and extracted data.

Participants/materials, setting, methods: The primary outcome was cumulative live birth rate (CLBR). Secondary outcomes included live birth rate (LBR) after a maximum of one transfer, per women randomized and miscarriage rate, per women randomized. Studies that only reported results on women reaching an embryo transfer and studies where multiple ovum pickups were allowed to collect multiple oocytes, were excluded due to bias by design in favor of PGS.

Main results and the role of chance: For this update,1281 titles and abstracts were screened, eleven studies (2076 women) with sample sizes ranging from 47 to 408 were included. Ten studies performed cleavage- or blastocyst stage biopsies followed by FISH- analysis. Based on one study (408 women), results showed that PGS reduced CLBR compared to no PGS in IVF/ICSI treatment (odds ratio(OR) 0.58, 95% confidence interval (CI) 0.35-0.98; moderate-quality evidence). LBR was decreased in the PGS group (OR 0.67, 95% CI 0.54-0.84, 10 studies; 1668 women; high-quality evidence). There was no difference in miscarriage rate between PGS and no PGS (OR 1.03, 95%, CI 0.75-1.41; 10 studies; 1668 women; moderate-quality evidence). One study (396 women) performed PGS on polar bodies using array comparative genomic hybridization (aCGH) and showed no difference in CLBR (OR 1.05, 95% CI 0.66-1.66; moderate-quality evidence) and LBR (1.10, 95% OR 0.68-1.79; moderate-quality evidence), however the miscarriage rate was lower in the PGS group (OR 0.45, 95% CI 0.23- 0.88; moderate-quality evidence). There were no studies on PGS as currently performed in routine clinical practice, i.e. using blastocyst stage biopsy and one of the new methods for the genetic-analysis, that could be included in the review.

Limitations, reasons for caution: The number of RCTs is limited, and those available are heterogeneous in study population, methods of genetic analysis and timing of biopsy.

Wider implications of the findings: This Cochrane systematic review confirmed earlier findings of harm by using early PGS methods. There was no improvement of live birth rate by PGS using polar body biopsy and aCGH for the analysis. PGS should only be considered in studies designed to demonstrate its effectiveness.

Trial registration number: Not a trial

P-543 Can asking hypothetical questions about being the product of sex selection, provide information regarding the impact on parent's use of pre-conception sex-selection?

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Study question: Do attitudes regarding pre-conception sex selection change when one imagines being the product of such technology?

Summary answer: Personalizing questions change perspective. The impact of social sex selection has not been researched. Effects of sex-selection may be necessary to consider in implications counseling.

What is known already: Today the majority of fertility clinics in the US offer pre-implantation genetic technologies that allow for pre-conception sex selection to avoid a medical condition, facilitate family balancing, and cater to social preferences. Previous surveys have inquired about sex selection attitudes in general and mostly within the infertility population. Arguments of reproductive autonomy vs bio-ethical practices have been discussed but no survey to date, has asked about if the *individual* were the product of sex selection. Despite being the result of conjecture, asking this question is important. To date, the social consequences of such practices have not been emphasized or researched.

Study design, size, duration: Participants (n=352) responded to an IRB approved, self-administered, web-based, non-validated survey designed by the author and collected through Survey Monkey®. Solicitation for subjects were conducted for 4 $^{1}/_{2}$ months in 2014 between April through August. Statistical analysis was performed using a series of t-tests with selected groups for comparison.

Participants/materials, setting, methods: Participants were predominantly females (n=269) between the ages of 18-32. Questions were asked about sexual preference (89.5% identified heterosexual), professional degrees held (25%), religious upbringing (61% Christian), and any former pregnancies (19%). Not all questions were answered. Survey questions related to reproductive history, attitudes regarding sex-selection technology, preferences for use, and individual's hypothetical perspective of being the product of such technology. Surveys were distributed via Facebook, email, and college campus posts. Responses were de-identified.

Main results and the role of chance: Most questions required a Positive, Negative or Unsure/Neutral response. Responses were collapsed to create binary data with a 2-part question: Unsure/Neutral and Definite. The second part to the question required a Positive or Negative response. Overall, 68% favored sex-selection for medical reasons; 9% were against this and 23% were unsure. Overall, 29% favored sex selection for social reasons; 47% were against this and 24% were unsure. Women (50.9%) were significantly more likely to be against social sex selection.

When asked, hypothetically, how they might feel if they discovered their sex was chosen, 58% reported they would have neutral feelings. Of the other 42%, 8% of the men and 25% of the women indicated they would feel positively; 13% of the men and 52% of the definitive women imagined they would feel negatively. When asked if they would want to know about their sex being chosen, 30% were unsure. 45% n=104 indicated they would want to know; and 55% n=126 said they would not want to know. Women were significantly more likely than men ($p<.05$) to feel negatively about knowing they were the product of sex selection.

Limitations, reasons for caution: This was a web-based survey potentially creating selection bias. Participants responded if they were interested and those not interested, it is assumed, did not respond. Questions posed on the effects of sex selection were hypothetical. As such, true feelings of actual children of sex selection are unknown

Wider implications of the findings: The advancement and progressive options of novelreproductive technologies brings new decisions for patients. Patients need to consider the use of the technology and the possible impacts for their offspring. This research is designed to facilitate conversations in implications counseling with patients to aid their decision making and parental preparation

Trial registration number: NA

P-544 Tunable copy number algorithm parameters improve the detection and accuracy of small CNV event calling in PGT-A

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Study question: What are the copy number algorithm parameters that influence the detection and accuracy of small copy number variation (CNV) event calling in PGT-A?

Summary answer: Reduced tile size baselines and custom sensitivity settings enable improved detection of small CNV events in the range of 1.81 Mb to 3.36 Mb.

What is known already: PGT-A can be required to detect the unbalanced products of reciprocal translocations in embryo biopsies without a priori information since carriers of a balanced reciprocal translocation with derivative chromosomes may be undiagnosed prior to engaging in IVF. The resultant embryos from balanced reciprocal translocation carriers may have segmental unbalanced chromosomal aberrations that have the potential to be of various sizes and locations across all chromosomes. As a result, it is important to determine the analysis parameters and resolution limitations of PGT-A workflows to detect putative segmental unbalanced products from translocations and other segmental aneuploidies in preimplantation embryos.

Study design, size, duration: Analysis parameters for a NGS-based PGT-A assay were optimized for sensitivity and specificity using samples sequenced between September to December 2018. The dataset consisted of a range of event sizes including four whole chromosome events (45,XX,-21; 47,XY,+18; 48,XXX,+21), 12 segmental CNV events (del(5p) 32Mb, dup(9p) 11Mb; dup(16p) 3.66Mb; dup(22q) 14.8Mb; del(4p) 26.9Mb; del(5q) 2.31Mb, dup(6p) 20.97Mb; dup(3q) 99.1Mb, del(9p) 1.81Mb; del(5p) 30.1Mb, dup(6p) 2.19Mb, del(14q) 1.19Mb), and two euploid cell lines (46,XY; 46,XX).

Participants/materials, setting, methods: Using 4-5 cell inputs picked under a microscope, three replicates per cell line were processed using the protocol for the Ion ReproSeq™ PGS Kits for the Ion GeneStudio S5™ System (Thermo Fisher Scientific) covering: whole genome amplification, library preparation, template preparation, and sequencing. Ploidy calling and size accuracy for segmental CNV events were assessed using aneuploidy analysis workflows in Ion Reporter software v5.10 with transition penalty and baseline tile size parameter adjustments.

Main results and the role of chance: Different aneuploidy analysis parameters were compared for sensitivity, positive predictive value (PPV) and size calling accuracy. Algorithms assessed included decimal ploidy value copy number calling (mosaic) and integer ploidy value copy number calling (non-mosaic) with parameter adjustments (transition penalty (TP) and baseline tile size). The sensitivity for the detection of all events ranged from 68.8%-93.8%. The greatest sensitivity was observed for a non-mosaic workflow with TP= -3 and baseline tile size of 0.2Mb; all events were called except for del(14q) 1.19Mb. In contrast, the least sensitive workflow had a PPV of 100% using a non-mosaic workflow with TP=-3 and baseline tile size of 2Mb; however, only events >3.36Mb were called.

Assessing the size accuracy of calls for events ≥11Mb, a non-mosaic workflow with TP= -2 and baseline tile size of 0.5Mb called events with the best concordance to the expected sizes with a maximum size discrepancy of 10%. Investigating breakpoint accuracy, this workflow called events that overlapped with annotated CNVs with an average of 6% of the called event size that did not correspond to the annotated region for the eight events with positional information (del(9p) 1.81Mb Del(9), dup(6p) 2.19Mb, del(5p) 2.53Mb, dup(6p) 20.97Mb, del(4p) 25Mb, del(5p) 30Mb, dup(3q) 99.1Mb).

Limitations, reasons for caution: The range of CNV events assessed was small with a size distribution that had gaps and effects of sequencing depth were not studied. For a better understanding of the resolution that can be achieved, more cell lines with a greater range of CNV sizes on different chromosomes should be studied.

Wider implications of the findings: Sensitivity, PPV, and accuracy of small CNV event calling in PGT-A can be greatly influenced by the adjustment of copy number algorithm parameters. This study provides guidance on the workflow parameter settings needed to achieve calling of CNV events at a particular size threshold.

Trial registration number: None

P-545 Relationship between Plakophilin-3 (PKP3 rs10902158 SNP) genotype and IVF outcome (Pilot study).

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Study question: Is there an association between PKP3 rs10902158 (G>A) polymorphisms and the risk of implantation failure after ICSI treatment?

Summary answer: The presence of the allele A of the PKP3 SNP rs10902158 is associated with a reduced clinical pregnancy outcome in the patients undergoing ICSI.

What is known already: Plakophilin-3 (PKP3) belongs to the Plakophilin family. It is described as beta- catenin and *armadillo* protein of the desmosomal plaque, which is synthesized in simple and stratified epithelia, present in cytoplasm, membrane and nucleus. The importance of cell-cell junction proteins in placentation and implantation biology is known. Although many studies indicate that alterations of intercellular adhesion may be associated with implantation failure, no study has investigated if the dysregulation or genetic polymorphisms of Plakophilins are associated with Implantation Failure.

Study design, size, duration: A total of 97 women, who underwent ICSI treatment for male factor infertility, were prospectively recruited in this cross-sectional study from 1 October 2017 to 1 April 2018, at Assisted Reproduction Unit at Orient Hospital, Syria.

Participants/materials, setting, methods: Genomic DNA was prepared from peripheral blood samples in order to analyze the polymorphism (rs10902158) at the PKP3 gene by PCR-RFLP. The Results were presented as a genotype (GG, GA, and AA), and their relationship to IVF outcome was analyzed.

Main results and the role of chance: The patients were divided into two groups according to clinical pregnancy: the pregnant group included 51 patients (53%) and the non-pregnant group included 46 patients (47%). The clinical pregnancy outcome was significantly different between genotypes, which was 0%, 45.8% and 58.8% in the patients having the genotype AA, GA and GG respectively (p-value = 0.03).

Limitations, reasons for caution: We could not do the genetic analysis for participants' parents. More studies are needed for both parents to demonstrate this hypothesis and to elucidate the precise molecular mechanism of PKP3 in folliculogenesis and embryo development.

Wider implications of the findings: The present study is the first pilot study, which indicates that the presence of the allele A of the *PKP3* SNP rs10902158 is

associated with a reduced clinical pregnancy outcome in the patients undergoing ICSI treatment. This variant may also constitute a novel predictor biomarker for implantation failure.
Trial registration number: none

P-546 Efficacy of whole genome amplification (WGA) and nested PCR methods for single cell analysis in preimplantation genetic diagnosis (PGD) for monogenic diseases

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Study question: Is whole WGA more efficient and reliable than nested PCR for single cell analysis in PGD for monogenic diseases?
Summary answer: The nested PCR technique may be more sensitive and specific method compared to WGA in single cell analysis.
What is known already: Both WGA and nested PCR technique are frequently used methods for genetic analysis in PGD. However, the data regarding the efficacy of different PGD molecular techniques for detecting gene mutations are still lacking. Allele dropout (ADO) remains the one of the most significant problem in PCR-based PGD since it can lead to misdiagnosis.
Study design, size, duration: A total of 222 lymphocytes from 7 couples with monogenic disease were allocated to WGA and nested PCR method. The efficacy of amplification rate and ADO rate for causative genes and short tandem repeat (STR) markers was compared.
Participants/materials, setting, methods: Single lymphocytes obtained from seven individuals who had heterozygous mutations of the causative genes (neurofibromatosis, gangliosidosis, familial adenomatous polyposis coli) and informative STR markers (Charcot-Marie-Tooth disease, Duchenne muscular dystrophy) were used. Using WGA method and nested PCR, the amplification rate and ADO rate were evaluated. The number of lymphocytes used in the WGA method was one to five lymphocytes, while only single lymphocyte was used in the nested PCR method.
Main results and the role of chance: For all experimental groups using mutant loci and markers, overall mean amplification rate in WGA and nested PCR were 94.3% and 99.3% and ADO rates were 22.7% and 4.6%, respectively. For six mutated loci, amplification rate were 97.9% of WGA and 98.7% of nested PCR, and ADO rates for these loci were 23.4% and 2.7%, respectively. In the analysis of informative STR markers in two genetic disorders, the ADO rate of WGA method was higher than direct nested PCR method (21.1% vs. 6.6%). Although WGA method is recommended in various genetic disorders, especially for simultaneous detection of two or more loci, it showed high ADO rate in this study. It is assumed that DNA fragmentation of the genome by restriction enzyme during WGA may cause the degradation of binding site of primers, resulting in higher ADO rate and non-specific products.
Limitations, reasons for caution: We need further study regarding techniques.
Wider implications of the findings: Our results suggest that nested PCR method, a less expensive without using commercial kits is more reliable method in the single cell analysis for PGD as compared to WGA method.
Trial registration number: None

P-547 Metaphase II (MII) oocytes obtained at different time points in the same patient undergoing Assisted Reproduction – Aneuploidy rate of resulting embryos

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Study question: Is there a difference in aneuploidy rates in embryos resulting from fresh versus frozen/thawed oocytes for the same patient?
Summary answer: In fresh and frozen/thawed oocytes sets, analysis of the day of biopsy and age showed no significance in aneuploidy rate between the same patient.
What is known already: Oocyte cryopreservation is now an established technology with a wide range of indications. Increasing evidence on the efficiency of IVF using vitrified oocytes has suggested that it could achieve similar outcomes to IVF using fresh eggs with survival rates of over 84%. Moreover, aneuploidy rates in embryos from frozen/thawed oocytes used in egg donor programs has shown no significant increase in chromosomal error types when compared to fresh oocyte donation. As the number of oocyte cryopreservation cycles increases there is a real need to monitor outcomes of this technique with regard to the health of live-offspring.
Study design, size, duration: We performed a retrospective data analysis to determine aneuploidy rates in 679 embryos as a result of fresh and frozen thawed oocytes in patients undergoing assisted reproduction (ART) between, 2016–2018. Patients who had previous oocytes vitrified due to either a high response to gonadotropins, or no sperm at time of oocyte pick up (OPU), returned for a fresh OPU where they also consented to the thawing of their stored oocytes.
Participants/materials, setting, methods: Embryo aneuploidy was detected using NGS HR. Aneuploidy rates were compared between both sets of fresh and frozen/thawed oocytes, for day 3, day 5 biopsy and age, ($\leq$ 36 years and $\geq$ 37 years) at the time of fresh OPU. Chromosomal error types, monosomy, trisomy and complex abnormals (more than 2 error types) were also determined within both fresh and frozen oocytes. Significant difference was determined when P= $<$0.05.
Main results and the role of chance: The average age of patients studied was 37 years old (23 to 45 years). Time between vitrification and the thaw of cryopreserved oocytes averaged 5 months (1 - 48 months). Between fresh and frozen oocytes embryo aneuploidy rates from biopsies on day 3 was 68% versus 70% respectively. On day 5 biopsy, 43% of fresh oocytes versus 61% of vitrified oocytes were aneuploid. In patients $\leq$ 36 years old, the rate of aneuploidy was 50% versus 56%. In $\geq$ 37 years, 78% of fresh versus 75% for frozen/thawed oocytes were aneuploid. No significance was found for all sets of data p= $>$0.05. Aneuploidy rate was raised for fresh (50% v 78%) and frozen/thawed oocytes (56% v 75%) between both age groups. Monosomy, trisomy and complex abnormal showed no significance for both biopsy day and age groups, p=$>$0.05. However, it was observed that there was a decrease in complex abnormalities between day 3 and day 5 biopsy due to a possible correction factor in extended embryo development.
Limitations, reasons for caution: The frozen/thawed oocytes were not recruited from the same fresh cycle, therefore the impact of different stimulation protocols on oocyte quality should be viewed with caution. Sample size is small for day 5 biopsied embryos compared to day 3 biopsy due to a restriction on embryo cryopreservation in the UAE.
Wider implications of the findings: This study aids health professionals in counseling patients when no sperm has been collected on the day of OPU, oocyte banking and oncology patients showing no increase in aneuploid embryos . It adds to the existing published data on the prevalence of aneuploidy in embryos from fresh and frozen/thawed oocytes.
Trial registration number: not applicable

P-548 Detection of embryonic monogenetic defects and chromosomal aneuploidies by whole genome sequencing

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Study question: To evaluate the performance of whole genome sequencing (WGS) combining linkage information in pedigree for the detection of embryonic monogenetic defects and chromosomal aneuploidies.
Summary answer: WGS with pedigree member of 15X depth and embryo of 4X depth can accurately detect embryonic monogenetic defects and aneuploidies.
What is known already: Comprehensive chromosomal screening (CCS) based on WGS has been used for the detection of embryonic chromosomal copy number variants with the widely clinical application of preimplantation

genetic testing for aneuploidy (PGT-A). Preimplantation genetic testing for monogenic defects (PGT-M) using target sequencing has been applied clinically as well.

Study design, size, duration: We performed WGS to detect embryonic monogenic defects and aneuploidies simultaneously in 7 families that had undergone *in vitro*fertilization (IVF) with totally 36 embryos. Seven families who had undergone IVF with risk of monogenetic defects in the genes of *COL1A1*, *IDUA*, *DMD*, *EXT1*, *IL2RG*, *ITGB4*and *FLG*respectively participated in this study. Each participant was recruited with informed written consent. The WGS results were validated by SNP-array karyomapping.

Participants/materials, setting, methods: There were 7, 6, 7, 5, 1, 4 and 6 embryos obtained for each family respectively. Blood from each pedigree member were used for averagely 15X depth WGS with MGISEQ-2000, and 3-10 cells were biopsied from each blastocyst and multiple displacement amplified (MDA) and used for averagely 10.5X WGS. We constructed the haplotype for the target gene, and confirmed genetic status of pathogenic mutation according to the haplotype in the target gene of each embryo.

Main results and the role of chance: For monogenetic defects, totally 16 embryos were confirmed to be heterozygous mutation, 3 embryos to be homozygous mutation, and 17 embryos to be normal; all the results were validated by karyomapping and were 100% concordant. For aneuploidies, totally 17 embryos were detected to be with aneuploidies or chromosomal copy number variants, 19 embryos were to be normal, all the results were 100% concordant with karyomapping. Combining the results of monogenetic defects and aneuploidies, there were 1, 1, 4, 1, 1, 0 and 0 embryos for each family respectively with both normal chromosomal karyomap and normal genetic status of target gene. We performed data volume gradient test and find that embryonic data as low as 4X depth WGS can accurately detect monogenetic defects and aneuploidies. The allele drop-out (ADO) rate was averagely 19.39% for embryo 10.5X WGS, and averagely 36.3% for embryo 4X WGS.

Limitations, reasons for caution: Limitation may be due to the size of the sample. More families with monogenetic defects who had undergone IVF need to be recruited and more embryos need to be sequenced. Performance of our method such as specificity and sensitivity can be evaluated in a large-sample study.

Wider implications of the findings: Our results demonstrated the reliability of WGS for the detection of embryonic monogenetic defects and chromosomal aneuploidies. Moreover, WGS provide the extendibility of our testing to detect embryonic complex mutations, de novo mutations, translocations, mitochondrial DNA variations etc.

Trial registration number: None

P-549 Preventing the recurrence risk by NGS-based PGT-M for monogenic disease resulting from mosaic germline mutation

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Study question: How to prevent the recurrent risk of monogenic disease resulting from mosaic germline mutation by NGS-based PGT-M technology?

Summary answer: The recurrence risk of monogenic disease with germline mosaicism could be prevented by mutation detection combined with haplotype analysis by NGS-based PGT-M.

What is known already: Mosaicism is due to post-zygotic de novo mutation, resulting in two or more cell populations with or without presenting distinct clinical phenotypes in a single individual. The identification of mosaic mutation could not only explain the phenotype heterogeneity and the cause of de novo dominant genetic disorder in some families, but also provide a basis for genetic counseling when mosaic mutation was found in a parent. Individuals are increasing to be discovered to carry mosaic harmful mutations by NGS-based deep sequencing. However, it is a still challenge how to prevent the recurrent risk when a parent carries a mosaic mutation.

Study design, size, duration: This is a retrospective study for four couples seeking for PGT between August 2017 and December 2018. Three couples were healthy. Couple 1 and Couple 2 delivered babies with adrenoleukodystrophy and Fanconi anemia, respectively. Couple 3 had two consecutive pregnancies with fetus skeletal malformations. In Couple 4 the female partner was affected neurofibroma. PGT-M was carried out after the causing genes were determined, including *ABCD1*, *FANCB*, *COL1A2*, and *NF1*, respectively.

Participants/materials, setting, methods: The four recruited couples underwent PGT-M in our hospital. Mosaic mutations were indicated by pedigree analysis or contradictory PGT results and were finally confirmed by deep sequencing (from 1142X to 5129X). After informative STR and/or SNP haplotypes were constructed, ICSI-PGT treatment was provided. Mutation detection combining linkage analysis was performed on biopsied blastocyst trophectoderm cells and polar bodies. The embryos without affected the related disease were considered to transfer to the uterus.

Main results and the role of chance: PGT-M successfully underwent for 4 families in which wild haplotype, mutant haplotype and mutant haplotype with no mutation were all distinguished. The results were analyzed according to the same evaluation standard as Couple 1. In Couple 1, 9 biopsied blastocysts were detected. Five inheriting wild maternal chromosome were considered for preferential transfer. Two male embryos inheriting risk chromosome but no mutation were also considered for transfer. Two female embryos carrying risk chromosome but no mutation were not considered for transfer for ADO could not be precluded. A wild-type embryo was transferred, resulting in a normal pregnancy by prenatal diagnosis, and delivering a healthy boy.

Other 3 couples have been finished PGT-M and were awaiting embryo transfer. 8, 5, and 3 blastocysts were biopsied in Couple 2-4 respectively, and the blastocysts considering for preferential transfer were 3, 2 and 1 respectively. For Couple 4, polar bodies were detected to construct haplotype analysis.

An interesting finding is that mutation detection and haplotype analysis could indicate germline mosaicism. The haplotypes of 2 embryos in Couple 2 and polar bodies in Couple 4 were contradictory to the mutation detection, which indicated maternal mosaicisms for both couples and was confirmed by deeping sequencing.

Limitations, reasons for caution: Male germline mosaicism was not included in this study. Only one couple had clinical outcome and other couples are awaiting transplantation.

Wider implications of the findings: Polar body analysis could be used to confirm mosaicism during PGT-M. The possibility of germline mosaicism should be considered when the proband carried de-novo mutation or asymptomatic couple delivered an affected offspring. During PGT-M, comprehensive analysis including mutation detection and haplotype analysis is necessary.

Trial registration number: Not applicable

P-550 Utilization of Magnetic-activated Cell Sorting after the failure of a first Pre-implantation Genetic Testing cycle improves the outcome of assisted reproduction treatments.

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Study question: Can MACS (Magnetic-activated Cell Sorting) reduce aneuploid and increase euploid rate in Pre-implantation Genetic Testing cycles for AMA (Advanced Maternal Age) after a failed attempt?

Summary answer: Our study highlighted a decrease in aneuploid blastocysts rate using MACS in infertile patients undergoing a Pre-implantation Genetic Testing cycle after a failed attempt.

What is known already: Many authors have demonstrated a strict correlation between the presence of apoptotic markers on spermatozoa and the failure of assisted reproduction treatments. An early indicator of apoptosis is the externalization of phosphatidylserine (PS) residues, a phospholipid-binding protein, on the sperm cytoplasmic membrane. The MACS system consists in a column selection method: apoptotic speramatozoa bind to magnetically-labeled Annexin-V at the PS residues and are retained in the column whereas non apoptotic sperm can flow through it. It has been showed that the use of MACS allows improving sperm quality, fertilization and cleavage rates, pregnancy and reducing miscarriage rates.

Study design, size, duration: From January 2015 to November 2018, 13 couples were enrolled in the study. All couples had a previous PGT (Pre-implantation Genetic Testing) cycle in which no euploid blastocysts were obtained; in some cases mosaic diploid-euploid blastocysts were obtained, which gave no pregnancy after a frozen embryo-transfer. All the couples underwent a second PGT cycle attempt in which sperms were treated with MACS in order to select non-apoptotic spermatozoa.

Participants/materials, setting, methods: Patients with severe oligoasthenoteratozoospermia were excluded. The MACS protocol was performed as follows: the semen sample was analyzed (WHO 2010), washed with buffered medium; the pellet was removed and a swim-up performed. The retrieved spermatozoa were washed with a binder buffer (Miltenyi Biotec), centrifuged and the supernatant discarded. The pellet was covered with Annexin-V and re-suspended. After a 15' incubation at room temperature, the sample was eluted through the column and collected to perform ICSI.

Main results and the role of chance: Female and male mean age±SD were 40.5±3.7 and 43.1±4.5, respectively, on the first PGT cycle (No-MACS Group=NMG). Female and male mean age±SD were 40.9±3.7 and 43.5±4.7, respectively, on the second cycle (MACS Group=MG). Respectively in NMG and MG, the injected oocytes were 114 and 118; fertilized oocytes were 79 (69.3%) and 71 (60.2%) (NS); blastocysts formation rates were 53.2% (42/79) and 53.5% (38/71) (NS). In NMG, 22 (52.3%) blastocysts formed on day-5, 17 (40.6%) on day-6 and 3 (7.1%) on day-7. In MG, 19 (50.0%) blastocysts formed on day-5, 15 (39.5%) on day-6 and 4 (10.5%) on day-7 (NS). In the NMG and MG respectively, no euploid blastocysts and 4 (10.5%) euploid blastocysts were obtained. In NMG, 3 mosaic diploid-euploid blastocysts were formed (7.1%) whereas in the MG, there were 4 (10.5%) (NS). Aneuploid blastocysts were 37 (88.2%) in NMG and 27 (71.1%) in MG ($p<0.05$). In NMG, 3 frozen embryo transfer of 3 mosaic blastocysts were performed, giving 2 biochemical pregnancies (66.7%). In MG, 8 frozen embryo transfer of 8 blastocysts (4 euploid and 4 diploid-euploid mosaics) were performed obtaining 2 clinical pregnancies (25.0%) with 2 babies born and 2 biochemical pregnancies (25.0%) (NS).

Limitations, reasons for caution: The sample size is small and the project is still ongoing to enlarge the number of couples enrolled. Study of aneuploidies with a possible male origin (chromosomes 9,13,15,16,17,18,21,22,X,Y) and analysis of clinical results needs a larger number of patients and biopsied blastocysts in order to perform a robust statistic analysis.

Wider implications of the findings: MACS is useful to select non apoptotic sperms; although fertilization, cleavage and blastocyst rates are not improved, aneuploid blastocyst rate statistically decreases with the use of MACS. By selecting spermatozoa free from PS residues, MACS probably selects spermatozoa with a better DNA packaging, thus affecting the embryo ploidy.

Trial registration number: not applicable

P-552 Contributions of new genomic analyzes (array-CGH and next generation sequencing (NGS)) in the identification of rare causes of human ovarian infertility.

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Study question: Identify rare genetic causes of human ovarian infertility using new genomic exploration technologies.

Summary answer: A genetic origin was identified for 40% of the cohort: 6 patients and variants of interest requiring further studies in 4 patients.

What is known already: Between 30 and 40% of the etiologies of infertilities are ovarian origin. Among them, 1% of women under 40 have premature ovarian failure (POF), many causes as been indentified including genetic, autoimmune, infectious, or iatrogenic. But the majority remains to be elucidated. Genetic causes of POF account for approximately 20–25% of patients and can be isolated (nonsyndromic), or as one component of a pleiotropic genetic syndrome. Chromosomal abnormalities could explain 10–15% of POF cases. Array-CGH and a recent approach using next generation sequencing (NGS) have led to new causatives being identified and candidates' genes being proposed.

Study design, size, duration: We carried out a cohort study in the departments of reproductive medicine and biology, cytogenetics and molecular genetics of the University Hospital of Amiens from January 2016 to December 2018.

We include 25 patients under 36 years of age with ovarian infertility (premature ovarian failure, or folliculogenesis blockage) without identified etiology after standard genetic assessment (karyotype and search for premature X fragile).

Participants/materials, setting, methods: For each patient:

-Search for small chromosomal variants (deletion/duplication) by array-CGH of 180K oligonucleotide type (Agilent technology) giving an average resolution of 60 kb.

-In the absence of chromosome variant, search for gene variants by NGS (high throughput sequencing) type "clinical exome" (panel of about 5000 genes involved in human pathology).

-Confirmation of identified variants and familial investigation by karyotype and FISH, semi-quantitative PCR or Sanger sequencing according to the nature of the variant identified.

Main results and the role of chance: The array-CGH identified a CNV of interest for infertility for 6 patients. One of the 5 CNVs identified is a recurrent abnormality described known to be responsible for infertility (deletion 15q25.2). Another CNV has already been described once as implicated in female infertility (5q13.2 duplication). Chromosomal reworking of X undetected karyotype was also found in another patient. Finally, three other CNVs of interest corresponding to undescribed microremanyments include potentially candidate genes in female infertility.

The analysis of 4 patients, including two sisters, in clinical exome allowed to identify variants of interest in each case. A truncating mutation in the homozygous state was identified in the FIGLA gene, a gene previously implicated in premature ovarian failure, in two sisters. Two heterozygous missense variants in the MLH3 gene were identified in another patient with meiotic blockade after ovarian stimulation. Finally, a pathogenic mutation in the homozygous state has been identified in the gene ZP1, a gene previously implicated in the syndrome of empty cumulo-oocyte complexes, in a patient with this rare pathology.

Limitations, reasons for caution: The main limitation of our study is the small number of patient.

Wider implications of the findings: There are many causes of infertility and infertility is often multifactorial. Genetic etiologies is estimated that about 50% of infertilities, isolated genetic component or associated with other factors. Combining a chromosome approach and a gene approach could identify the molecular substratum of unexplained female infertility.

Trial registration number: not applicable

P-553 FMR1-CGG-repeat length can induce AKT/mTOR signaling in human granulosa cells from IVF patients

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Study question: Does variable *FMR1*-gene-expression due to different CGG-Repeat-Lengths (RL) affect gene(s)-expression of mTOR-signaling-pathway functioning in human granulosa cells (GC) and interfere with the ovarian stimulation process applied for women undergoing IVF/ICSI?

Summary answer: Women with homozygous-low-CGG-RL (<26) display increased *AKT*, *mTOR* and *TSC2*-expression although *S6K*-expression remains stable suggesting involvement of *FMR1* in modulating mTOR-signal-pathway-activation during folliculogenesis in GC.

What is known already: *FMR1*-gene is a major-regulator in folliculogenesis; it contains a highly variable CGG triplet-block in its promoter-region and RL between 54-200 are associated in ~20% with premature-ovarian-insufficiency/failure. The protein FMRP is highly expressed in women´s GC

and known to control translation of a number of cellular transcripts involved in RISC-(RNA-induced-silencing-complex)-function.

The AKT/mTOR-signaling-pathway is a major-regulator for cellular-proliferation and differentiation involved in primordial-follicle-activation for follicular-pool-maintenance. Ovarian-reserve and -response to hormone stimulation impacts substantially women´s success during IVF/ICSI-treatment. Elevated *FMR1*-gene-expression in GCs of women with poor-response suggests the variable CGG RL as causative agent for this impairment probably due to mTOR-signal-pathway interference.

Study design, size, duration: 269 women undergoing controlled ovarian hyperstimulation for IVF/ICSI-treatment were prospectively enrolled. This study was approved by the local ethical committee and conducted according to the principles of the Declaration of Helsinki.

212 of the patients could be divided according to their ovarian response into either normal (n:150) or poor (n:62) responders. Poor response was defined according to Bologna-criteria.

Participants/materials, setting, methods: DNA was extracted from peripheral blood.*FMR1*-CGG-RL was determined using ABI 3100/3130x1-sequencer. A RL of 26-34 was considered as "normal", <26 as "low" and >34 as "high". Patientswere than subdivided into six genotypes (high/high; high/low; low/low; low/norm; high/norm, norm/norm) according to their CGG-repeat length on both alleles.

mRNA was extracted from GCs after follicular aspiration; quantitative gene-expression of *mTOR, AK* and*S6K*was determined using specific TaqMan-Assays. Statistical analyses with SPSS; significance set $p \leq 0.05$.

Main results and the role of chance: Expression levels of *AKT, mTOR* and *TSC2* were clearly variable between the six *FMR1*-genotypes; statistically most significant for *AKT* (p=0.045) and *mTOR* (p=0.08). Post-hoc analysis demonstrated that this difference was mainly due to samples carrying a homozygous low/low CGG genotype displaying significantly elevated gene-expression levels compared to the other CGG genotypes.

After subdivision the collective for ovarian response this effect of the CGG low/low-genotypes was even more prominent in patients with a poor response to ovarian stimulation (e.g. *AKT* p=0.035).

Most interestingly, *S6K*-expression levels between the different CGG genotypes were rather stable. Our data therefore suggest interference of *FMR1*/FMRP expression with AKT/mTOR signal pathway but not downstream because *S6K*-expression looks stable (internal control).

A clear correlation of *FMR1*-genotypes with mTOR-signaling can be assumed from these results. Homozygous low CGG triplet alleles putatively lead to lower FMR-protein levels causing lower RNA binding capacities in the RISC reducing cellular translation efficiency resulting in poor ovarian response.

Limitations, reasons for caution: Future experiments evaluating the RNA-protein interactions between FMRP and transcripts of mTOR-pathway are needed to understand how such RNP guided RISC interactions operate at the molecular level. Further *FMR1/mTOR*genes expression analyses on larger sample sizes are required to substantiate given data.

Wider implications of the findings: Our data can help to understand how *FMR1*/FMRP expression and expression of genes in the mTOR-signal-pathway functionally interact in the granulosa cells of the female germline and offer novel diagnostic points for optimization of infertility treatment for women with impaired folliculogenesis and/or poor ovarian reserve.

Trial registration number: not needed

P-554 Novel mutations in PLCζ: Expanding mutational and phenotypic spectrum associated with male Infertility

Abstract withdrawn by the authors

P-555 Can gene expression profiling help characterize human spermatogenesis in infertile men?

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Study question: Can gene products isolated from infertile men shed light on seminiferous tubule function and gamete competence?

Summary answer: Loss of DNA repair and apoptotic gene expression provides insight into the likelihood of assisted reproductive technology (ART) success.

What is known already: Evaluating human semen characteristics, even when using the most stringent criteria, has limited predictive value in terms of spermatozoa performance, even when used for ART. Moreover, standard semen analysis is inadequate for predicting reproductive outcome in men with unexplained infertility. As a result, attempts have been made to detect ploidy, DNA integrity, and the maturational status of the spermatozoon, as well as to conduct highly detailed morphologic assessments. Epigenetic assessment by profiling RNA transcripts in the sperm cell has been proposed as an alternative method to screen the male partner for infertility and predict pregnancy outcome.

Study design, size, duration: During the past year, we have analyzed the expression of genes involved in meiotic spermatogenesis in relation to reproductive outcome. RNA sequencing was carried out on the specimens of 13 consenting men.

By sequencing the genome, we compared the gene expression of ejaculated specimens from 5 infertile couples who were able to conceive with those of 8 fertile couples acting as controls.

Participants/materials, setting, methods: Ejaculated spermatozoa were used to isolate total RNA using a commercially available spin column kit. The RNA samples were then made into paired-end libraries. Pilot paired-end 76bp RNA-Seq using an Illumina platform (NextSeq 500) was performed and expanded to 60M reads. Expression values were calculated in fragments per kilobase of transcript per million fragments mapped reads (FPKM) and normalized read counts.

Main results and the role of chance: The infertile group (N=5) had a mean age of 37.6±3. The average semen parameters were: a concentration of 45.3±15 x10^6/mL, with 45.2±14% motility and 2.7±2% morphology. These men underwent 5 ICSI cycles with their female partners, resulting in a fertilization rate of 71.4% (30/42), with no pregnancies.

The control couples (n=8) had a mean age of 32.8±4 with an average sperm concentration of 42.4±13 x10^6/mL, with 44.2±11% motility and 2.9±2% morphology. These men underwent 8 ICSI cycles with their female partners, resulting in a fertilization rate of 79.3% (23/29) and all delivered.

A total of 86 genes were differentially expressed ($P<0.001$) between the infertile and control cohorts. Of them, 24 genes were overexpressed and 62 were under-expressed. Specifically, DNA repair genes (*APLF*, *CYB5R4*, *ERCC4*, and *TNRFSF21*) and apoptotic modulating genes (*MORC1*, *PIWIL1*, and *ZFAND6*) were remarkably under-expressed ($P<0.001$) in the study cohort. *MORC1* and *PIWIL1* were also significantly under-expressed in NOA men ($P<0.0001$).

Limitations, reasons for caution: This study profiles men with various stages of spermatogenesis and must be confirmed in a larger cohort. While the contribution of the female partner cannot be excluded, gene expression profiling of infertile men may serve as an assay to measure seminiferous tubule function and reproductive potential of the male gamete.

Wider implications of the findings: Deep sequencing of sperm RNA is a reliable and reproducible technique that may aid in the diagnosis and screening of infertile men. Epigenetic analysis demonstrated that DNA repair and apoptosis genes are linked to seminiferous tubule function, indicating that their involvement in meiosis and apoptosis is typical of germ cells.

Trial registration number: N/A

P-556 Assessing male gamete production and the impact of spermatozoa from azoospermic men on embryonic development by genome profiling

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Study question: Can NGS of DNA isolated from surgically retrieved specimens identify the genes involved in gamete production and the impact of spermatozoa on embryonic development?

Summary answer: Men with secretory azoospermia have a mutated set of genes responsible for producing spermatozoa that compromises meiotic function and impairs embryonic development.

What is known already: Azoospermia accounts for about 15% of male factor infertility cases. Although azoospermia is rarely caused by pre-testicular factors, the most common forms are testicular and post-testicular. The

post-testicular type is due to obstruction, congenital or acquired, but the more complicated type is secretory azoospermia, where scattered functional germinal epithelium strive to support the germline meiotic process in the generation of spermatozoa.

Study design, size, duration: Specimens were provided by consenting men who were being treated at our center for infertility. NGS assessment was carried out on the surgically retrieved spermatozoa from 15 azoospermic men, who were characterized as obstructive (OA) post-vasectomy or non-obstructive (NOA) according to their spermatogenetic profile. Since specific gene mutations may affect ICSI clinical outcome, they were assessed and compared between the two etiologies.

Participants/materials, setting, methods: DNA was extracted and amplified from at least 500 spermatozoa (DNA concentration, 395±217 ng/ul; quality, 1.7±0.1 nm). NGS was performed, and gene mutations, duplications, and deletions were detected using CLC Genomics 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. Gene mutation profiles of the OA and NOA men, in relation to their ability to generate a pregnancy, were assessed.

Main results and the role of chance: Of the 15 couples (paternal age, 41.2±6yrs; maternal age, 39.5±4yrs), 9 OA men underwent surgical sperm retrieval with a concentration of $2.0\pm3\times10^6$/ml and 0.5±1% motility. Six NOA men yielded spermatozoa with a concentration of $0.03\pm0.1\times10^6$/ml and 0.3±1% motility. NGS assessment showed no significant differences in sperm aneuploidy between the groups.

OA patients underwent 9 ICSI cycles, resulting in a pregnancy and delivery rate of 66.7% (6/9). OA men shared mutations of the SPINK14 and NXF2B genes, involved in serine protease inhibition and mRNA export from the nucleus to the cytoplasm, respectively. Interestingly, in all OA patients unable to generate a pregnancy, only the PRB1 gene was found to be duplicated.

NOA patients treated in 6 ICSI cycles, with a pregnancy and delivery rate of 50% (3/6), had shared mutations in 129 genes ($P<0.0001$). Clustering of these genes demonstrated that they were most involved in mRNA transcription (n=27), spermatogenesis (n=16), DNA repair (n=15), centrosomal function (n=14), and apoptosis (n=12).

When this assessment was performed in the NOA group, the fertile cohort had 1 gene duplicated (MPIG6B), related to organ development, while the infertile cohort had 2 deleted (MBD5 and RBFOX2) and 3 duplicated (CSRNP3, ARL4C, FRMPD1) that support early embryonic development.

Limitations, reasons for caution: This study contains a limited number of observations, which should be compared to azoospermic men with complete spermatogenic arrest or germ cell aplasia. Gene clustering and its function require further investigated in order to define the etiology of male infertility and predict reproductive outcome.

Wider implications of the findings: Screening men for gene mutations can potentially help characterize the spermatogenic function of their germinal epithelium. This study may help to identify patients who would benefit from testicular sampling. Moreover, assessment of the genome and epigenome will shed light on the etiology of this severe form of infertility.

Trial registration number: N/A

P-557 Interpreting the spermatogenic profile of NOA men through transcriptome analysis

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Study question: Can transcriptome analysis be used to characterize the spermatogenic function of NOA men?

Summary answer: Transcriptome analysis can be used to characterize the spermatogenic function of NOA men by predicting whether spermatozoa can be retrieved via testicular biopsy.

What is known already: In men with non-obstructive azoospermia (NOA) who undergo micro-testicular sperm extraction (TESE), about 40-60% of procedures fail to yield spermatozoa. The histological variations reported range from hypospermatogenesis, to spermatogenic arrest, to Sertoli-cell-only syndrome.

During spermatogenesis, precursor diploid stem cells are needed to form haploid sperm cells. Within this developmental process, various molecular mechanisms are involved, including signal transduction, DNA repair, DNA packaging, and chromosome condensation and silencing. These are required for steroidogenesis, spermatogenesis, and spermiogenesis, respectively. In the event that any of these mechanisms is compromised, spermatogenic arrest could occur, leading to the failure of spermatozoa retrieval after testicular biopsy.

Study design, size, duration: During the past year, we have assessed the expression of genes in relation to spermatogenesis and reproductive outcome. RNA sequencing (RNA-Seq) was performed on the specimens of 6 consenting men. Four non-obstructive azoospermic men where no sperm was found (NOA-) and one non-obstructive azoospermic man with recovered spermatozoa (NOA+) were compared to one obstructive azoospermia (OA) control.

Participants/materials, setting, methods: RNA was isolated from testicular biopsy tissues from 5 NOA and 1 OA men using a spin column commercial kit. No Y microdeletions were detected for any patients. Several embryologists extensively searched (71 ± 10 minutes) samples not demonstrating spermatozoa to confirm a failed TESE attempt. RNA isolates were sequenced by Illumina HiSeq at 2x150bp configuration per lane with ~64M reads per sample. Genes with $P<0.001$ and an absolute log2fold change >1 were considered significant.

Main results and the role of chance: This study included 6 consenting men: 1 NOA+, 4 NOA-, and 1 OA control. The NOA+ man (female age, 38 yr; male age, 45 yr) had a sperm concentration of 1.2×10^6/mL with 0.05% motility. The fertilization rate was 66.7% (8/12), and a clinical pregnancy was achieved. No spermatozoa were found in the 4 NOA- groups (male age, 42.5±6 yrs). The OA control (female age, 38 yr; male age, 39 yr) had a concentration of 21×10^6/mL with 48% motility. This couple also had a clinical pregnancy. A total of 8 genes were significantly under-expressed in all NOA men, regardless of whether spermatozoa were present (*FAM57B*, *GGN*, *OPLAH*, *OXCT2*, *MEX3D*, *WFDC3*, *TSKS*, *FAR2P1*), and are mainly involved in spermatogenesis and DNA double-strand repair.

A total of 20 genes were significantly under-expressed exclusively in the NOA- groups, which failed to yield spermatozoa compared to the OA control.

Among those genes, 6 were related to steroidogenesis (*AKAP4*, *DKKL1*, *PGK2*, *SLC26A8*, *ELFN2*, *POM121L12*), 4 were associated with spermatogenesis (*ACSBG2*, *CATSPERD*, *CATSPERG*, *ODF1*, *BOD1L2*), 7 were linked to spermiogenesis (*PRMI*, *PRM2*, *TNP1*, *H1FNT*, *DYDC1*, *HIST3H3*, *HIST1H2AA*), 1 involved cell-cell adhesion (*CRISP2*), and 1 was associated with negative regulation fat cell differentiation (*FAM95B1*).

Limitations, reasons for caution: Gene expression profiles of men failing to yield spermatozoa must be confirmed by a larger cohort. While the contribution of the female partner cannot be excluded, gene expression profiling of infertile men may serve as an assay to assess seminiferous tubule function and the reproductive competence of the male gamete.

Wider implications of the findings: Deep sequencing of sperm RNA is a reliable and reproducible technique that may aid in the diagnosis and screening of infertile men. Epigenetic analysis showed that DNA repair and spermatogenic genes are linked to seminiferous tubule function, suggesting their involvement in meiosis and apoptosis, typical of germ cells.

Trial registration number: N/A

P-558 A therapeutic approach for couples with compromised sperm DNA integrity and a history of aneuploid embryos

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Study question: Does selecting the highest progressively motile spermatozoa with optimal genomic integrity enhance the likelihood of generating euploid embryos?

Summary answer: Microfluidic sperm selection (MFSS) identified spermatozoa with the highest chromatin integrity, capable of generating euploid embryos in couples with a history of persistent implantation failure.

What is known already: Genomic impairment of the male gamete can hinder embryo cleavage and implantation. Dysfunction of the male genital tract increases both single-strand (ss) and double-strand (ds) DNA nicks and breaks that can inhibit the developmental competence of embryos.

In particular, ds DNA breaks present in the spermatozoa of fertile donors, at a concentration as high as 40%, may contribute to embryo aneuploidy with consequent implantation impairment.

Study design, size, duration: From October 2016 through January 2019, nine consenting couples with a history of embryo aneuploidy and/or recurring implantation failure and a male partner with high sperm chromatin fragmentation (SCF) in their ejaculate underwent a new ICSI cycle in which semen specimens were processed by density gradient centrifugation (DGC) and MFSS.

Participants/materials, setting, methods: SCF was measured by TUNEL on raw semen specimens as well as after DGC and MFSS. ICSI with pre-implantation genetic testing for aneuploidy (PGT-A) was carried out with spermatozoa selected by the two different methods. Fertilization and clinical pregnancy outcomes were assessed and compared between the two sperm selection methods. Embryo implantation and pregnancies were recorded after replacement of thawed euploid blastocysts.

Main results and the role of chance: A total of 9 men of average age 43±12 years had a mean sperm concentration of 55.1±47 x 10^6/mL, 30±14.7 motility, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 36.2±48 and 11±17 x 10^6/mL, with 59±41% and 97±4% motility, respectively ($P<0.0001$).

The raw sample sperm morphology improved from 2.4±1% to 4.0±1% after MFSS, while after DGC it was 3±1%. The average SCF decreased from 31% in raw samples to 21% following DGC, and became 1.4% after MFSS processing ($P<0.0001$).

These couples (female partners, 37±5 years) underwent 12 cycles with DGC-selected spermatozoa and achieved a fertilization rate of 78% (75/96), which generated 30% (12/40) morphologically good-quality embryos. PGT-A did not show any euploid embryos for transfer. Subsequently, the couples underwent ICSI cycles with MFSS, showing a fertilization rate of 73% (76/104), with 41% (14/34) good-quality embryos.

In this group, 24% (8/33) of euploid embryos were identified and cryopreserved.

Four couples received a thawed single euploid blastocyst and all 4 became pregnant ($P<0.0001$), resulting in a clinical pregnancy rate per cycle of 44% (4/9) and 100% per transfer.

Limitations, reasons for caution: This study represents a preliminary experiment on a small number of subjects.

While the oocyte contribution to aneuploidy cannot be discounted, MFSS was able to yield the highest progressive motile spermatozoa with optimal genomic integrity capable of enhancing the chances of generating euploid embryos.

Wider implications of the findings: The occasional presence of ds DNA in the male gamete has been considered responsible for contributing to embryo aneuploidy. MFSS of highly motile and genetically competent male gametes may enhance the chances of obtaining a euploid conceptus for transfer.

Trial registration number: N/A

POSTER VIEWING
REPRODUCTIVE ENDOCRINOLOGY

P-559 Prospective study of the association between in-utero exposures to circulating maternal phthalate metabolites and timing of menarche

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Study question: Does *in-utero* exposure to circulating maternal phthalate metabolites influence the timing of menarche?

Summary answer: Metabolites in the middle tertiles of high molecular weight (MW) phthalate MCMHP and low MW phthalate MEP were associated with later and earlier menarche respectively.

What is known already: Phthalates are ubiquitous environmental chemicals suspected to be endocrine disruptors, potentially impacting reproductive development. Animal studies, and limited human data, suggest that antenatal exposure influences pubertal timing. Understanding the influence of antenatal phthalate exposures on reproductive development is important as they are ubiquitous and present within cosmetics. Menarchal age is a sentinel event for reproductive function and long-term health. Preliminary data from a large, population based pregnancy cohort suggested that antenatal phthalate exposures influenced menarchal age. We aimed to measure the association between maternal phthalate metabolites (as a marker of exposure) and timing of menarche across the entire birth cohort.

Study design, size, duration: The Western Australian Pregnancy Cohort (Raine) Study is a longitudinal study of children born in 1989-1991 (1413 female infants born). Maternal serum was collected at 18 and 34 weeks of gestation, pooled and assayed to determine concentrations of 32 phthalate metabolites of 15 phthalate diesters. Phthalate metabolite concentrations were available for up to 516 mothers, BMI was measured at 8 years (n=324). Prospective recording of menarchal timing was available (n= 231).

Participants/materials, setting, methods: Maternal serum was stored at -80^{0}C. Aliquots from both samples were combined as an estimate of antenatal exposure.

Phthalate metabolites were measured by isotope diluted LC-MS/MS. Metabolites of five phthalate di-esters were detected above the limit of detection, and were included in the analysis: mono-ethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), Mono-(2-carboxymethyl-hexyl) phthalate (MCMHP), mono-iso-nonyl phthalate (MiNP), mono-(carboxy-iso-octyl) phthalate (MCiOP).

Timing of menarche was prospectively recorded.

Main results and the role of chance: Demographic characteristics of adolescents with maternal phthalate metabolite exposure and menarchal age data available, were similar to cohort members with missing data, apart from having a higher family income. Prospective data on age at menarche was available for n=231 girls (median age 12.9 years [IQR 12.0-13.6].

All models were adjusted for maternal education, smoking during pregnancy; expected birthweight ratio (observed birth weight over median birth weight appropriate for maternal height), sex, nulliparity and gestational age; and body mass index (BMI) at the 8 year follow up.

Maternal concentration of middle tertile MCMHP was associated with later menarche (adjusted hazard ratio [aHR] 0.690, 95% confidence intervals [CI] 0.499-0.953, p=0.025), (concentrations 1.04 - 1.73 ng/ml), when compared with lowest tertile MCMHP. There was no association between the highest tertile MCMHP and age at menarche, (aHR 0.759, 95% CI 0.548-1.052, p=0.098). Middle tertile MEP, (concentration 1.19 - 4.65 ng/ml), was associated with earlier menarche (aHR 1.45, 95% CI 1.033-2.037, p=0.032) compared with lowest tertile MEP, although there were no association of the highest tertile MEP with menarchal age. (aHR 1.019, 95% CI 0.740-1.403, p=0.907).

There were no associations of menarchal age with maternal phthalate metabolite concentrations when the metabolites were categorised as 'detectable' or 'non-detectable'.

Limitations, reasons for caution:

This study is limited by the sample size, prolonged storage of maternal serum samples, and multiple comparisons. The associations do not prove causation and we cannot account for postnatal environmental exposures that may influence age at menarche, and no dose response relationships between phthalates exposure and menarchal age were noted.

Wider implications of the findings: Irrespective of proven causation, the study findings are important, as the potential association of MEP exposure with an earlier menarche is consistent with the literature. Further it is important to

note that it is believed that some phthalates influence body fat distribution which is well known to modulate menarchal age.
Trial registration number: not applicable

P-560 Ovarian reserve and oocyte maturation rates are not correlated with the expression of aging markers in human cumulus cells

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Study question: Does the expression of somatic aging markers in human cumulus cells correlate with age, ovarian reserve and oocyte maturation rates?
Summary answer: While *TXNIP* and *CLU* were positively correlated with age, none of the aging markers tested were correlated with ovarian reserve or oocyte maturation rates.
What is known already: A distinct gene expression signature has been associated with ageing in several somatic tissues, including brain, muscle, liver and kidney. Reproductive aging in women is associated with a decrease in both ovarian reserve and oocyte developmental competence. The acquisition of developmental competence relies on interactions of the oocytes with the surrounding somatic cumulus cells (CC). We therefore hypothesize that CC from follicles of aged women might also show the characteristic somatic aging gene expression signature, affecting the process of oocyte maturation and possibly altering acquisition of oocyte developmental competence.
Study design, size, duration: This basic research study involved 82 CC samples from patients undergoing IVF recruited from March to October 2018. The mean woman age was 32 years old (SD=8.0, range 18-45), the mean ovarian reserve measured by antral follicle count (AFC) was 18.6 (SD=10.63, range 2-47) and the mean maturation, defined as the number of MII divided by the number of cumulus oocyte complexes at OPU, was 72.2% (range 20%-100%).
Participants/materials, setting, methods: For each participant, CC samples were pooled after oocyte denudation. Total RNA was extracted using a phenol-free method, reverse transcribed to cDNA using random hexamers and expression of 11 markers of aging was performed by qPCR (in triplicate) and normalized against *TBP*. Expression levels of aging markers were then plotted against woman age, ovarian reserve and oocyte maturation rates. Non-parametric Spearman's correlation were used to determine the relationship between variables.
Main results and the role of chance: As expected, our results showed lower ovarian reserve and oocyte maturation rates with increasing woman age (r_s=-0.42, p=0.00008) and (r_s=-0.3, p=0.0078), respectively. Six of the 11 genes analyzed (*LYZ, TXNIP, CLU, FABP3, TGFBR3 and ANXA5*) were expressed in human CCs. Woman age showed a moderate positive correlation with *CLU* (r_s=0.23, p=0.0362), a secreted chaperone involved in cell death and tumor progression and *TXNIP* (r_s=0.36, p=0.0007), a tumor suppressor gene involved in redox regulation and recently described as a non-invasive biomarker of oocyte quality in the corona cells of human oocytes. The remaining aging markers did not correlate with age, ovarian reserve or oocyte maturation rates (r_s<0.1 and p>0.05).
Limitations, reasons for caution: CC analyzed in this study are obtained after controlled ovarian stimulation. Thus, the CC transcriptome obtained might differ from CC obtained from non-stimulated follicles.
Wider implications of the findings: The general lack of correlation between the signature observed in CCs from women of different ages and that previously found in aged human tissues suggests that these ovaries were not prematurely aged, but rather at the end of their reproductive life.
Trial registration number: not applicable

P-561 impact of melatonin and resveratrol on embryo quality in poor prognosis IVF patients

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Study question: Can melatonin, resveratrol, or a combination of the two improve embryo quality in poor prognosis IVF patients? How long is the appropriate administration period?
Summary answer: More than two weeks administration of melatonin, resveratrol, or a combination of the two significantly improved embryo quality in poor prognosis IVF patients.
What is known already: Oxidative stress is well known as a factor of aging-related deterioration of oocyte quality. Recently, limited evidence has suggested that antioxidants improve fertility in animal models and ART cycles. In assisted reproductive technology (ART), melatonin has been reported to improve embryo quality. Meanwhile, resveratrol acts not only as an antioxidant but also as an activator of mammalian sirtuin-1 (SIRT1). SIRT1 behaves as a protector from many aging-related diseases. However, there have been no clinical studies that evaluated the benefit of resveratrol for embryo quality. Furthermore, the appropriate period of administration has not yet been determined.
Study design, size, duration: This retrospective study was conducted at a private clinic from October 2010 to September 2018. A total of 257 patients who had failed to conceive in an ART cycle were included and divided into three groups: melatonin administration (MLT group), resveratrol administration (RSV group), and melatonin and resveratrol co-administration (MLT/RSV group). These were divided by administration period as follows: less than two weeks (2w), two to three weeks (3w), and 4 weeks or more (4w).
Participants/materials, setting, methods: ycle numbers were 161 in the MLT group (aged 39.8±3.6 years), 35 in the RSV group (aged 40.0±3.5 years), and 136 in the MLT/RSV group (aged 40.6±3.2 years). We designated the previous ART cycle of each patient as control. Development of each embryo was compared with that of its control. Patients took 2 mg/day melatonin and/or 250 mg/day resveratrol before and/or during controlled ovarian stimulation in an IVF cycle.
Main results and the role of chance: In the MLT, RSV, and MLT/RSV groups, AMH levels were 1.5±1.6ng/ml, 1.2±2.4ng/ml, and 1.3±1.4ng/ml, respectively. The number of collected oocytes in these groups were 5.2±4.7, 4.1±3.7, and 4.6±3.3, respectively. In the MLT group, 2w administration significantly improved fertilization rate [69.5% (383/551) vs. 57.1% (343/551), p<0.01] and day3 good-embryo rate [30.3% (109/360) vs. 16.1% (51/316), p<0.01] compared with control. Furthermore, 2w, 3w, and 4w administration significantly improved good-blastocyst rate [12.3% (65/528), 7.1% (3/42), 8.5% (6/71) vs. 0.2% (1/557), respectively, p<0.01]. In the RSV group, 2w administration significantly improved fertilization rate [72.0% (18/25) vs. 45.1% (46/102), p<0.05] and 2w and 4w administration significantly improved good-blastocyst rate [18.2% (4/22), 15.6% (7/45) vs. 1.3% (1/80), respectively, p<0.01]. In the MLT/RST group, 2w, 3w, and 4w administration significantly improved good-blastocyst rate [15.3% (24/157), 22.7% (20/88), 14.5% (25/172) vs. 0.7% (3/402), respectively, p<0.01] compared with control. In the MLT and MLT/RSV groups, clinical pregnancy rate was significantly higher than control. In the RSV group, clinical pregnancy rate tended to be higher, although the difference was not statistically significant. There were no notable side effects during or after MLT and/or RSV administration.
Limitations, reasons for caution: An age bias may exist among the administration period. Advanced age patients tended to be included in 4 weeks or more. A larger scale, more extensive clinical study, such as a well-designed double blind randomized placebo-controlled study, is needed to recommend the use of melatonin and/or resveratrol in clinical practice.
Wider implications of the findings: This study is the first clinical trial to demonstrate the efficacy of resveratrol in poor prognosis IVF patients. Although administration of melatonin or resveratrol alone gave improved embryo quality, the combination of melatonin and resveratrol having synergistic effects on embryo quality, might be more favorable for poor prognosis IVF patients.
Trial registration number: N/A

P-562 Optimal metabolic and hormonal modulation induced by the combination of acetyl-L-carnitine, L-carnitine, L-arginine and N-acetyl cysteine in overweight/obese PCOS patients – A pilot study

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Study question: Can the metabolic and endocrine impairments seen in overweight/obese polycystic ovary syndrome (PCOS) patients be ameliorated by supplementation with specific metabolic and nutrient compounds.
Summary answer: The test combination was greatly effective in overweight/obese PCOS patients with particular efficiency in those with hyperinsulinemia as tested with the oral glucose tolerance test.
What is known already: PCOS is a frequent disease characterized by several endocrine impairments and frequent metabolic abnormality, that is a compensatory hyperinsulinemia. The role of obesity and exercise has been suggested in this disorder. We have examined the role of supplementation with compounds that may modulate the metabolism and thereby also the endocrine parameters in this disorder.
Study design, size, duration: A total of 30 overweight/obese patients were evaluated. Patients were administered the combination of the compounds per os for at least 12 weeks. The test combination included acetyl-L-carnitine (ALC) 250 mg, L-carnitine (500 mg), L-arginine (500 mg) and N-acetylcysteine (NAC) (50 mg).
Participants/materials, setting, methods: All patients were evaluated before and after a 12-week treatment interval for LH, FSH, estradiol (E2), progesterone (P), androstenedione (AS), insulin, plasma levels, lipid profile, oral glucose tolerance test (OGTT) for glucose, insulin and c-peptide response.
Main results and the role of chance: The integrative treatment decreased AS and insulin as well as LDL and HOMA index in all the group of PCOS. Also, insulin and c-peptide response to OGTT were significantly reduced. Considering patients according to the insulin response to OGTT the combination of integrators was more effective in PCOS subjects with hyperinsulinemia (77% of the patients) on both metabolic and hormonal profiles. In addition, the insulin response to OGTT was greatly reduced.
Limitations, reasons for caution: This is a small pilot study and more data are needed from a larger study.
Wider implications of the findings: This study suggests that in overweight/obese PCOS patients there is possibility to ameliorate metabolic and endocrine parameters with supplementation of specific metabolism modulating compounds.
Trial registration number: PXW-001B

P-563 Poor ovarian response in ART as a predictor for pregnancy complications

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Study question: Is there a possible association between poor ovarian response in artificial reproductive technology (ART) and pregnancy complications?
Summary answer: Ovarian poor response in ART is an independent risk factor for gestational diabetes mellitus (GDM) and intrauterine growth restriction (IUGR).
What is known already: The term "poor responder" refers to patients with diminished ovarian reserve. Approximately 10% of women seeking fertility treatment fit this definition; very often, older patients presenting with low ovarian reserve.

It is generally accepted that obstetric and perinatal complications (GDM, preeclampsia, IUGR) are more common in older women. Whether this is due to the general aging process or due to the aging of the ovary and the ovum, is not known. The results of studies that investigated this issue were inconclusive.
Study design, size, duration: Retrospective, case-control study, conducted at a tertiary, university-affiliated IVF center, between 2011 and 2017. Patients who conceived and delivered after ART treatment were analyzed. 75 Poor responders (3 or fewer oocytes retrieved) after stimulation with gonadotropins (study group) were matched by the same day as the ovum pick-up (OPU) to 75 normo-responders (4 or more oocytes retrieved).
Participants/materials, setting, methods: A total of 150 patients were included: 75 poor responders were compared to 75 normo-responders. Main outcome measures were the incidence of preeclampsia (PET), gestational diabetes mellitus (GDM) and neonatal birth weight.
Main results and the role of chance: There were no significant differences in maternal age, gravity, parity, maternal BMI, gestational age at delivery, mode of delivery and Apgar score between groups. The use of intracytoplasmic sperm injection (ICSI) was similar between groups (P=0.08). Poor responders had higher incidence of gestational diabetes mellitus (GDM) (27% compared to 6.8%, P=0.001) and higher incidence of intrauterine growth restriction (IUGR) (13.5% compared to 4.1%, P=0.04) than did normo responders. Although poor responder patients experienced a higher incidence of preeclampsia (8.1% compared to 5.4%), this did not achieve statistical significance (P=0.74). Poor responders with GDM were of similar age (34.6+5.6 vs. 34.4+5.1, P=0.8) and BMI (27.8+6 vs. 24.8+5.4, P=0.06) as poor responders without GDM. However, normo-responders who had GDM were older (34.6+3.7 vs. 32.4+5.8, P=0.01) and had higher BMI (29.5+0.6 vs. 22.9+4.7, P=0.008) than normo-responders without GDM.
Limitations, reasons for caution: This study is retrospective and the presence of residual unknown bias cannot be excluded.
Wider implications of the findings: Poor responders had higher incidence of GDM and IUGR compared to women with normal ovarian response. These findings were not due to older maternal age or higher maternal BMI. This may have a wider implication on the mother and the fetus, and appropriate counseling should be considered.
Trial registration number: Not applicable.

P-564 Dual trigger of final oocyte maturation in overweight women improves oocyte maturity rates during GnRH antagonist cycles in women of advanced age.

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Study question: Does dual trigger with GnRH agonist and human chorionic gonadotropin improve IVF outcomes according to body-mass index (BMI) of women compared with hCG-only trigger?
Summary answer: Dual trigger in women ≥35 years old improves oocyte maturity rate of overweight Korean women (BMI ≥ 23 kg/m^2).
What is known already: Dual trigger is a feasible alternative to hCG in GnRH antagonist cycles.
Study design, size, duration: This is a retrospective cohort study including 235 infertile couples with 262 completed IVF/ICSI-ET cycles between January 2017 and September 2018 in single Korean IVF unit.
Participants/materials, setting, methods: Patients were categorized in four groups: young (<35 years), normal weighted women (BMI < 23 kg/m^2) (G1 = 77 cycles), young, overweighted women (BMI ≥ 23 kg/m^2) (G2 = 52 cycles), old (≥35 years), normal weighted women (G3 = 88 cycles), old, overweighted women (G4 = 45 cycles). In each group, IVF outcome measures were compared between dual triggering and hCG-only triggering. The primary outcome measure was the oocyte maturation rate.
Main results and the role of chance: Oocyte maturation rate in dual triggering was significantly higher than that in hCG-only triggering, only in old, overweighted women group (81.9%, 70.1%, respectively, P =0.03). Clinical pregnancy rate in dual triggering also had a higher tendency than that in hCG-only triggering, only in same group (52.9%, 28.6%, respectively, P =0.05). There were no significant differences in other groups, even in overall.
Limitations, reasons for caution: Because of a limitation of retrospective study, our present data should be confirmed by a prospective study. Although many investigators have revealed dual triggering would be helpful whether normal or poor responders, our data did not confirm the effect of dual trigger in overall.
Wider implications of the findings: This is the first report studying the effect of dual trigger according to the BMI. Our study provides the preference dual trigger for final oocyte maturation to hCG-only trigger when women is old and overweighted.

Trial registration number: N/A

P-565 HCG-induced AREG stimulates aromatase expression in human granulosa-lutein cells: a mechanism for E2 production in the luteal phase

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Study question: Does amphiregulin (AREG), the most abundant and important epidermal growth factor receptor (EGFR) ligand in the follicular fluid, regulate aromatase expression in human granulosa-lutein (hGL) cells?
Summary answer: AREG mediates the hCG-induced up-regulation of aromatase expression and estradiol (E2) production in hGL cells.
What is known already: AREG expression is rapidly induced by human chorionic gonadotropin (hCG) in hGL cells and mediates physiological functions of luteinizing hormone (LH)/hCG in the ovary. EGFR protein is expressed not only in preovulatory follicles but also throughout the luteal phase of menstrual cycle. After LH surge, human corpus luteum secretes high levels of E2 which regulates various luteal cell functions. Aromatase is an enzyme responsible for a key step in the biosynthesis of E2. However, whether AREG regulates aromatase expression and E2 production in hGL cells remains unexplored.
Study design, size, duration: This is an experimental study which was performed over a 1-year period. *In vitro* investigations were directed towards examining the role of AREG in regulation of aromatase expression and E2 production in primary hGL cells.
Participants/materials, setting, methods: Primary hGL cells were obtained from women undergoing *in vitro* fertilization (IVF) treatment in an academic research center. Aromatase mRNA and protein levels were examined after exposure of hGL cells to recombinant human AREG or hCG. The EGFR tyrosine kinase inhibitor AG1478, PI3K inhibitor LY294002 and small interfering RNAs (siRNAs) targeting EGFR and AREG were used to verify the specificity of the effects and to investigate the underlying molecular mechanism.
Main results and the role of chance: Treatment of hGL cells with AREG stimulated aromatase expression and E2 production. Using pharmacological inhibitor and specific siRNA, we revealed that AREG stimulated aromatase expression and E2 production via EGFR-mediated activation of the AKT signaling pathway. In addition, inhibition of EGFR activity and AREG knockdown attenuated hCG-induced up-regulation of aromatase expression and E2 production. Follicular fluid and serum were collected from 65 infertile women during IVF treatment.Importantly, the protein levels of AREG in the follicular fluid were positively correlated with the serum E2 levels after 2 days of oocyte pick-up in the IVF patients.
Limitations, reasons for caution: The *in vitro* setting of the study is a limitation which may not reflect the real intra-ovarian microenvironment. Clinical data were obtained from a small sample size.
Wider implications of the findings: Our results provide the first evidence that hCG-induced AREG contributes to the aromatase expression and E2 production in the luteal phase of menstrual cycle. Better understanding of the hormonal regulation of female reproductive function may help for developing new strategies for the treatment of clinical infertility.
Trial registration number: Not applicable.

P-566 Luteal after follicular-phase-stimulation in the same ovarian cycle is a promising strategy to increase the chance of delivery in poor responder women fulfilling Bologna Criteria

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Study question: Is Luteal Phase-Stimulation (LPS) after Follicular-Phase-Stimulation (FPS) in the same ovarian cycle (Duostim) an efficient strategy for poor responder women fulfilling the Bologna Criteria?
Summary answer: LPS performed after FPS in women fulfilling the Bologna criteria increased the overall live-birth-rate (LBR) per intention-to-treat (ITT) from 7% (FPS-only) to 15% (DuoStim)
What is known already: Three theories of follicle recruitment were postulated to picture the dynamism of folliculogenesis: 'continuous recruitment', 'single recruitment' and 'wave' theories. The advances in IVF such as blastocyst culture, aneuploidy-testing and vitrification encouraged clinicians to maximize the exploitation of the ovarian reserve via tailored stimulation protocols especially in poor-responder women. In this regard, larger cohorts of oocytes might be collected from LPS during DuoStim protocols. These oocytes showed similar developmental, chromosomal and reproductive competence as paired FPS-derived ones. Therefore, DuoStim protocol might be an intriguing option to obtain more competent blastocysts in a single ovarian cycle also in poor-responder women
Study design, size, duration: This paired observational study was performed in a private IVF clinic between January 2015 and January 2018. One hundred consecutive poor-responder women fulfilling the Bologna Criteria underwent DuoStim combined with preimplantation-genetic-testing-of-aneuploidies (PGT-A). All patients included satisfied $\geq$ 2 of the following characteristics: maternal age $\geq$ 40yr and/or $\leq$ 3 oocytes previously retrieved after conventional stimulation and/or reduced ovarian reserve (assessed as astral-follicle-count <5–7 follicles or AMH <0.5–1.1ng/ml). Couples with severe male factors were excluded.
Participants/materials, setting, methods: FPS and LPS were performed with recombinant-gonadotrophins in an antagonist protocol with the same daily dose. Final oocyte maturation was induced by agonist trigger to reduce the time of luteolysis. LPS was started 5 days after the first retrieval. All oocytes underwent ICSI, blastocyst culture, trophectoderm biopsy and vitrification. The primary outcome was LBR per ITT. Only single euploid blastocyst transfers were performed in an artificial cycle. All embryological outcomes per stimulation were also monitored.
Main results and the role of chance: One hundred patients started FPS. Mean maternal age was 42.1±1.4yr; they already went through 0.7±0.9 IVF cycles with $\leq$3 oocytes collected; their AFC was 3.8±1.2 and their AMH was 0.56±0.3 ng/ml. Ninety-one patients completed DuoStim, since 5 did not respond to FPS and 4 to LPS, respectively. Nevertheless, all the patients were included in the analysis. The total number of oocytes after FPS and LPS were 237 (mean per stimulation: 2.4±1.5) and 309 (3.1±2.2; $p<0.01$), respectively. The total number of blastocysts after FPS and LPS were 70 (0.7±0.8) and 107 (1.1±1.1; $p<0.01$), respectively. The mean blastulation rate per inseminated oocyte was 30.7%±32.8% per FPS and 36.2%±33.5% per LPS (p=NS). The number of euploid blastocysts after FPS and LPS were 14 (0.1±0.4) and 21 (0.2±0.5; p=NS), respectively. The mean euploid blastocyst rate per MII was 4.8%±12.7% per FPS and 6.6%±16.2% per LPS (p=NS). The rate of patients obtaining $\geq$1 blastocyst increased from 53% after FPS to 82% with the contribution of LPS. Similarly, the rate of patients obtaining $\geq$1 euploid blastocyst increased from 14% after FPS to 31% with the contribution of LPS. Lastly, the LBR per ITT increased from 7% after FPS to 15% after Duostim.
Limitations, reasons for caution: Single center retrospective study. No cost-effective and time to pregnancy analyses were performed. Lastly, more data are required to confirm the safety of LPS in terms of obstetrical and peri-/post-natal outcomes.
Wider implications of the findings: These data highlight that LPS in a DuoStim protocol is promising for the treatment of a thorny population of patients like poor responder women fulfilling the Bologna-Criteria.No ideal protocol has been outlined yet to manage these women.Therefore,these data encourage future trials to assess the consistency and reproduciblity of this strategy
Trial registration number: none

P-567 To explore the mechanisms of Insulin Resistance in Polycystic Ovarian Syndrome by analysis of their Body Composition before and after the lifestyle intervention

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Study question: By comparing the changes of body composition in PCOS patients before and after lifestyle intervention,we wanted to know the the mechanism of IR in PCOS patients .
Summary answer: We found that lifestyle intervention improved insulin resistance by reducing body fat in obese PCOS patients or increasing skeletal muscles in non-obese PCOS patients
What is known already: Studies have shown that insulin resistance is key in the pathogenesis of PCOS.Improving the IR of PCOS patients is an important part of PCOS treatment.Lifestyle intervention can significantly reduce visceral fat content, improve insulin sensitivity, reduce androgen levels, and restore spontaneous ovulation; therefore, lifestyle intervention is the first-line treatment for PCOS patients.
Study design, size, duration: We used a prospective self-control study.89 PCOS patients were divided into two groups according to BMI: overweight and obesity group (n=46,BMI $\geqq$ 24) and non-obesity group (n=43,BMI < 24). Individualized diet and exercise intervention programs were formulated based on the results of body composition measurement(according to the results of their body fat rate, standard proportion of skeletal muscle weight) and group management. Lifestyle Intervention was for 12 Weeks.
Participants/materials, setting, methods: Before and after lifestyle intervention,all patients were tested for body composition by Inbody270 body composition analyzer.Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone (TT) were measured by immunofluorescence chemiluminescence assay. Oral glucose tolerance test (OGTT) and insulin release test of 75 g glucose load were performed by glucose oxidase method. Blood glucose and insulin levels were measured at 0.5, 1, 2 and 3 hours after fasting and glucose administration.
Main results and the role of chance: After 12 weeks of treatment: 1.Body fat percentage of both groups significantly decreased ($p<0.01$), while only skeletal muscle mass percentage increased in the non-obese group ($p<0.01$); 2. HOMA-IR and insulin area under curve (AUC) in both groups significantly decreased ($p<0.01$). Intervention improved glucose tolerance in four diabetic patients (2 cases of obese group, 2 cases of non-obese group) . 3. The decrease of HOMA-IR in obese group was positively correlated with the decrease of body fat percentage in the obese group (r=0.368,p=0.021); while, positively correlated with the increase of skeletal muscle mass percentage in the non-obese group (r=-0.512,p=0.001). 4. The recoveries of spontaneous ovulation in obese group and non-obese group were (28/46) 60.9% and (31/43) 72.1%, respectively. The recovery of ovulation was positively correlated with the decrease of body fat percentage in the obese group (r=0.343,p=0.003); while, positively correlated with the increase of skeletal muscle mass percent in the non-obese group (r=0.506,p=0.001).
Limitations, reasons for caution: Our study was designed to be self-controlled, lacking randomized grouping and control groups. We will further expand the sample size and use randomized controlled trials to further validate the above conclusions.
Wider implications of the findings: We found that the mechanism of insulin resistance in PCOS is related to its body composition, that is, the insulin resistance in obese PCOS patients is related to the increase of body fat, and the insulin resistance in non-obese PCOS patients is related to the decrease of skeletal muscle
Trial registration number: ChiCTR-OOC17011175

P-568 Vitamin D3 regulates mitochondrial biogenesis and function in granulosa cells through mitogen activated protein kinase-extracellular signa- regulated kinase pathway in polycystic ovary syndrome mouse model

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Study question: Could vitamin D3 regulates mitochondrial biogenesis and function in granulosa cells through mitogen activated protein kinase-extracellular signal-regulated kinase (MAPK-ERK1/2) pathway in a mouse model of polycystic ovary syndrome?
Summary answer: Vitamin D3 could improve mitochondrial biogenesis and function through MAPK pathway in granulosa cells of PCOS mice that may improve follicular development and oocyte quality
What is known already: Since oocyte quality, as one of the most crucial determinant in fertility output, requires undisturbed mitochondrial biogenesis and is easily affected by low mitochondrial membrane potential, increased oxidative stress and apoptosis, application of a suitable treatment preventing these problems may increase fertility and pregnancy rate. Various treatments, mainly vitamin D3, have been administered to lessen features of PCOS. Vitamin D can affect the function of MAPK-ERK pathway through phosphorylation of tyrosin kinase receptors to regulate mitochondrial biogenesis
Study design, size, duration:
Granulosa cells isolated from PCOS were cultured in six groups: (1) granulosa cells treated with vitamin D3 (100 nM for 24 hours)
(2) granulosa cells treated with MAPK activator (10 μM for 4 hours)
(3) granulosa cells treated with MAPK inhibitor (10 μM for 4 hours)
(4) granulosa cells treated with vitamin D3 and MAPK inhibitor
(5) granulosa cells treated with MAPK inhibitor and MAPK activator
(6) non-treated granulosa cells
Participants/materials, setting, methods: PCOS model was triggered by dehydroepiandrosterone to prepubertal female BALB/C mice for 20 days (N=20). To approve the effect of hormone, Seasame oil was injected to mice. Sex hormones by ELISA and ovaries by H&E staining were assessed. Granulosa cells were identified using FSHR and CD45 and cultured. Genes expression was compared using RT-PCR. MAPK-ERK1/2 expression was investigated by western blotting. ROS levels were determined using 2', 7'-dichlorofluorescin diacetate. mtMP was determined by JC1 staining
Main results and the role of chance: We observed Corpus luteum in control group indicating ovulation. Numbers of pre-antral and antral follicles, atretic follicles, and degenerated granulosa cells were high in the PCOS group, and there was no corpus luteum due to the ovulation disturbance. High amounts of estradiol, progesterone, LH, and FSH serum levels were observed in the PCOS group in comparison to control and vehicle groups. We detected a significantly increased ratio of LH/FSH in the PCOS group. Mitochondrial biogenesis, function and anti-apoptotic genes including PGC1-a, NRF1, SOD1, GPX, UCP1, catalase and BCL-2 were downregulated in granulosa cells of PCOS mice when compared to normal mice, but treatment with vitamin D3 and MAPK activator increased mRNA expression levels of these genes. Vitamin D3 and MAPK activator also decreased ROS levels.The mtMP was lowered in PCOS cells, while treatment of PCOS granulosa cells with vitamin D3 promoted an increased mtMP. Like vitamin D3, MAPK activator could enhance the mtMP. Western blot analysis revealed that treatment of granulosa cells with vitamin D3 (100 nM) intensified phosphorylation of MAPK-ERK1/2 after 24 hours. MAPK activator also increased phosphorylation of MAPK but MAPK pathway was not activate in the other groups
Limitations, reasons for caution: In this study, all experiments were performed on animal. So, we encountered with some difficulties in keeping animals in an appropriate condition
Wider implications of the findings: Considering that the quality of oocyte determines the success of fertility and mitochondria is major determinant of oocyte quality, vitamin D3 can be prescribed to improve the mitochondrial function and biogenesis of granulosic cells and find a way to improve fertility in PCOS patients
Trial registration number: non-clinical trials

P-569 Live birth rate is higher in primary subfertile women undergoing ICSI with high normal compared to low normal range TSH levels

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Study question: Are TSH levels within the normal range in women not using thyroid hormone substitution associated with live birth rate in IVF/ ICSI?
Summary answer: Live birth rate is higher in primary subfertile women with high compared to low normal TSH levels undergoing ICSI.
What is known already: If and how TSH levels are associated with live birth rate in subfertile women undergoing IVF/ ICSI is a matter of debate. Studies show contradictory results concerning live birth rate, pregnancy rate, pregnancy loss and embryo quality, comparing women with high normal TSH levels (2.5-4.5 mIU/L) to women with low normal TSH levels (0.3-2.49 mIU/L). Subclinical hypothyroidism and/ or thyroid autoimmunity are hypothesized as a pathologic mechanism. Because of the conflicting results, the relevance of TSH levels and thyroid function might count for subgroups, possibly meeting benefit from special attention or treatment.
Study design, size, duration: For that reason we analyzed a large database on TSH levels and pregnancy outcomes in a retrospective cohort study in women starting IVF (n=949) or ICSI (n=752) between the first of January 2008 and the first of March 2012 with follow up till 2014. Pregnancy outcomes of 4451 cycles were compared, till first ongoing pregnancy, with patient as unit of analysis. Primary and secondary subfertility were used to define subgroups.
Participants/materials, setting, methods: Women aged 22-45 years with TSH 0.3-4.5 mIU/L not using thyroid hormone substitution were included in a single center in an Iodine-sufficient area. Logistic regression was used with (the natural logarithm of) TSH as continuous predictor, a quadratic term was added in a second model to account for a non-linear relationship. Chi-square tests and logistic regression were used to compare two groups of patients based on TSH levels on aforementioned outcomes while adjusting for confounders.
Main results and the role of chance: In primary subfertile women undergoing ICSI (n=455) the odds ratio for live birth rate is higher for high TSH, with the natural logarithm of TSH used as a continuous variable (OR 1.66, p=0.02) or TSH as a continuous variable (OR 1.37, p=0.01). An added quadratic term to TSH to look for possible u-shaped/ non-linear associations shows no difference (OR 1.00, p=0.99). Comparison of two groups based on TSH level (0.3-2.49 mIU/l vs 2.50-4.5 mIU/L) corrected for age, BMI, smoking, use of alcohol and diminished ovarian reserve shows also a significantly higher odds ratio for live birth rate in primary subfertile women undergoing ICSI with high normal TSH values (n=111) compared to women with low normal TSH values (n=344; OR 2.38, p=0.001). For clinical pregnancy and ongoing pregnancy rates odds ratios were also significantly higher, where no difference was seen in rate of pregnancy loss. The higher odds for live birth rate with high normal TSH is not seen in primary subfertile women undergoing IVF, (OR 1.02, p=0.94) nor in secondary subfertile women undergoing IVF (OR 1.14, p=0.63) or ICSI (OR 0.72, p=0.30). The role of chance is little because of consistency in linear and categorical outcomes and small p-values.
Limitations, reasons for caution: The retrospective character of the study with unknown levels of free thyroxine and of thyroid peroxidase or thyroglobulin antibodies limit the understanding of the results.
Wider implications of the findings: Our findings suggest that TSH levels count in primary subfertile women undergoing ICSI. Using (higher) TSH as a marker for thyroid autoimmunity might disclose ICSI as a treatment for a female factor hindering natural fertilization. In future studies measurement of TPO- and TG-antibodies are warranted to further explore these observations.
Trial registration number: n.a.

P-570 Derivation of reference intervals (RIs) for Anti-Müllerian hormone (AMH), specific for Russian population, using automated Access AMH assay

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Study question: Each laboratory is expected to establish its own RIs, but most laboratories use RIs provided by assay manufacturers which may not match to the Russian
Summary answer: Age-specific Russian RIs for AMH in women and men were derived and compared to the published literature for other populations and by the assay manufacturer.
What is known already: The age-specific RIs were established by the assay manufacturer for an American population. Another investigation for derivation of age-specific RIs for AMH for women and men was performed by Woloszynek R. et al. for a Brazilian population.
Study design, size, duration: Following CLSI EP 28AC, 436 women and 24 men were recruited; women were further grouped by age. Pregnant women, women with polycystic ovary syndrome and women undergoing ovarian surgery were excluded. Antral follicle counts (AFCs) were defined as sum of follicles 2–10 mm in diameter in both ovaries. The women were divided into groups with non-detectable, low (<10) and normal (11-20) AFCs by transvaginal ultrasound (TVUS). The duration of the study was 1 year.
Participants/materials, setting, methods: 460 volunteers aged 18-60 years were recruited, AMH was measured on the Access 2 analyzer (Beckman Coulter, Inc.) at CIR Laboratories on peripheral blood collected without regard to menstrual cycle day. AFCs were assessed in women on days 1–4 of their menstrual cycles using TVUS (Medison Ultrasound Systems). A non-parametric analysis of RIs was carried out using Stata 11 statistical analysis software.
Main results and the role of chance: In the Russian population, women aged 26-30 and 31-35 years had median and upper limit (UL) values for AMH that were significantly higher than those provided by the manufacturer (median 3.38 vs. 2.27, 2.85 vs. 1.88; UL 11.03 vs. 7.37, 11.61 vs. 7.35, respectively). In comparison to a Brazilian study (Woloszynek, 2015), the median and UL in the Russian population were lower for women aged 18-30 years group (median 3.35 vs. 3.7, respectively). In women aged 41-45, >46 and men, RIs were consistent with those provided by the manufacturer. Significant negative correlation between AMH and age was shown in women over 35 (rp=-0.46). Correlation between AMH concentration and AFC in both ovaries was demonstrated (rp=0.64; 0.69), with significant differences noted between AMH levels in groups of the same age but with different AFCs (low vs. normal AFC, 1.15 vs. 2.6, respectively).
Limitations, reasons for caution: No limitations found.
Wider implications of the findings: This study establishes Russian population-specific RIs for AMH using Beckman Coulter's Access AMH assay. These results reinforce the importance of obtaining population-specific reference intervals. Correlation among age, AMH concentration and antral follicle count (AFC) was estimated.
Trial registration number: N/A

P-571 Real world evidence : long term live birth rate in a French fertility care unit, a retrospective cohort study of the patient's journey

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Study question: Can we easily evaluate the cumulative live birth chances for a couple beginning medically assisted reproduction in our fertility care unit? What about the patients who drop out?
Summary answer: Administrative and medical data show that half of the couples who reach our unit leaves it with a baby after medically assisted reproduction (MAR).
What is known already: The pregnancy rate by attempt is no longer an appropriate method to estimate a couple's real chances of having a baby. Indeed,

improvement in reproductive technology and the promotion of single embryo transfers lead to increase the number of frozen-thawed embryo transfers. The cumulative live birth rate (CLBR) is a better indicator. Unfortunately this crucial information is difficult to obtain and time consuming. Only few studies try to evaluate it with different approaches. Every fertility centre should have the tools to make is own evaluation and give to their patients precise and true information about the overall medical journey.

Study design, size, duration: It is a retrospective cohort study. All the couples who had their first attempt of IVF, ICSI or IUI between January 2012 and December 2015 were selected. They were followed at least 2 years until delivery or drop out.

Participants/materials, setting, methods: We analysed data extracted from the billing department software completed with medical data extracted from a medical software specialized in MAR management. 1475 patient's journeys were studied, divided into three groups: "IUI alone" (group 1), "first attempt embryo transfer" (group 2)" and "mixed attempts" (group 3), corresponding to patients who began with IUI and then needed ART after IUI failure. Then we analyzed the journey of the patients with delivery success and without delivery.

Main results and the role of chance: The women were 33.8 years old in average. 56% began their journey with IVF, 44% with IUI but 46.7% of them needed to continue with IVF after IUI failure.

In total 50.3% had a baby after MAR. These patients are 33.2 years old in average. The live-birth rate (LBR) is respectively 56.3%, 49% and 46.8% in the three groups. The LBR is better for the younger women : 60% after 4 trials for women before 38 and less than 45% for women older than 38. For all groups there is a plateau after four attempts.

The pregnancy leading to the delivery is fast and detected in the first two attempts for the groups 1 and 2. It is interesting to see that the impact of age is minor in terms of number of attempts when we focus on the group of patients with delivery success.

The patients for which ART failed are 34.4 years old in average with a statistically significant difference with the success group . We notice that they drop out soon : only 10% pursue the 4 oocyte retrievals possibly reimbursed by the healthcare system. After one year of treatment failure 56% of the couples have stopped MAR.

Limitations, reasons for caution: The estimation of the CLBR doesn't take into account spontaneous pregnancies nor success after moving to another centre.

The study period is limited and some patients could deliver after this period.

The reasons why the patient stopped the treatments are not known (medical or personal decision?).

Wider implications of the findings: This study is a real word evidence of the patient's course in our unit and gives a valid estimation of MAR cumulative success rate. The characteristics of the different groups of patients could be further analyzed and provide us huge information to improve our decision-making and our patients counselling.

Trial registration number: not applicable

P-572 Convenience and efficacy of treatments for endometrial preparation prior to frozen embryo transfer (FET): a randomised-controlled trial comparing stimulated cycle (SC) versus natural cycle (NC)

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Study question: Is ovarian stimulation with recombinant FSH (rec-FSH) more convenient than natural cycle for endometrial preparation prior to FET in terms of number of visits reduced?

Summary answer: Endometrial preparation with recombinant FSH significantly reduces the number of visits needed to schedule frozen embryo transfer compared to natural cycle.

What is known already: Frozen embryo transfer has to be carried out at a time when the endometrium is receptive, defined as "implantation window". Hormone replacement therapy, stimulated cycle (SC), or close monitoring of a natural cycle (NC) can be performed to prepare the endometrium before FET. So far, neither option has shown superiority in terms of live birth rates. Although natural cycle may appear more physiological, it might also be more burdensome for patients and centers by requiring more monitoring and reducing flexibility. To date, no study has compared the convenience of stimulated cycle versus natural cycle for frozen embryo transfer.

Study design, size, duration: Our prospective open-labeled randomised controlled study was lead in a public IVF center from May 2015 to October 2017. We calculated that 48 patients per group were required to demonstrate a decrease of 1 visit using stimulated cycle compared to natural cycle (two-sided alpha-error of 0.05 and 90% power). Sample size was increased by 30% to consider transfer cancellations and patients lost to follow-up, representing a total of 124 patients (62 patients per group).

Participants/materials, setting, methods: Women aged 20 to 38yo undergoing their first or second FET (day 2/3 embryos) were randomised to either NC (monitoring by ultrasound and hormonal measurements from day 12), or to SC (75 IU/day rec-FSH from day 6 to 11 and HCG triggering when leading follicle > 17 mm). Number of visits (ultrasounds and blood tests), quality of life (FertiQol score), HCG positive rates, ongoing pregnancy rates, spontaneous miscarriage rates, and live birth rates were compared.

Main results and the role of chance: 124 women were selected, of which 5 were excluded. Our final population study was composed of 119 patients (NC group= 59; SC group=60). Mean age was of 32.9(+3.7) years old. Baseline characteristics were comparable between the two groups. The number of visits (primary outcome) was significantly lower in the SC group compared to the NC group (3.7+0.9 *vs.* 4.5+1.0, respectively, $p<0.0001$). Furthermore, the SC group was significantly associated to a lower number of blood tests (2.7+0.8 *vs.* 3.5+1.0, respectively, $p<0.0001$), and to a lower number of ultrasounds performed (1.2+0.4 *vs.* 1.5+0.6, respectively, $p<0.05$). Both the number of FET during "non-opening" hours (22.6% *vs.* 27.5%, respectively, $p=0.32$) and cancellation rates (8.6% *vs.* 12.3%, respectively, $p=0.52$) were lower for patients in the SC group compared to NC, without reaching significant difference. Concerning pregnancies, HCG-positive rates were higher but not significantly different in SC compared to NC patients (29.1% *vs.* 23.1%, respectively, $p=0.47$). No difference concerning life birth rates was observed between the two groups (20.0% for SC *vs.* 23.1% for NC, respectively, $p=0.69$). Quality of life as defined by the FertiQol score was not different between the two groups ($p>0.05$ for every item).

Limitations, reasons for caution: Potential biases are limited by the strict monitoring of cycles. The advantage of SC versus NC on pregnancy and cancellation rates has to be confirmed on larger effectives. Comparing the costs of rec-FSH to the cost and constraints of more visits engendered by the natural cycle protocol is also required.

Wider implications of the findings: Treatment burden is a major issue for infertile patients. Natural cycle appears as a good option for women reluctant to have injections, but patients have to be informed of the increased monitoring. FSH stimulation enables to reduce the number of visits, and offers more flexibility for patients and IVF centers.

Trial registration number: N° EUDRACT: 2015-A00088-41
ClinicalTrials.gov ID: NCT02834117

P-573 Fertility treatment in women with primary ovarian insufficiency who reject foreign oocytes

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Study question: The aim of the study was to explore the resumption of ovarian activity and the proper fertility treatment in women with POI who reject foreign oocytes.

Summary answer: Timed intercourse or IVF/IVM combined HRT may be the proper alternative to those women with POI who reject foreign oocyte.

What is known already: POI is a disorder associated with women infertility, It affects 1-2% of women younger than 40 years of age and 0.1% of women younger than 30 years of age. the mechanism is not clear. POI women especially those in late stage with FSH>40IU/ml represents a major challenge in reproductive medicine and brings a great dilemma to patients and their caregivers.
Study design, size, duration: retrospective study, 255 women with POI between July 2013 to December 2017 received HRT (Climen, Schering, Berlin, Germany) every month. If the resumption of ovarian activity was observed, timed intercourse or IVF/IVM was performed.
Participants/materials, setting, methods: 255 women with POI between July 2013 to December 2017 received HRT (Climen, Schering, Berlin, Germany) every month. If the resumption of ovarian activity was observed, timed intercourse or IVF/IVM was performed.
Main results and the role of chance: A total of 206 POI women with consistent HRT were followed up, 46 patients presented with resumption of ovarian activities, Among them 1/46 women got pregnant spontaneously, 4/25 women became pregnant with timed intercourse, 6/20 women became pregnant with IVF/IVM treatment
Limitations, reasons for caution:
small sample in IVF/IVM, And the retrospective study nature that these POI women without strict enrollment and standard intervention, Everyone's lifestyle and habits vary greatly.
Wider implications of the findings: The resumption of ovarian function is temporary and intermittent, it should be always taken into consideration, catching the intermittent chance and taking appropriate intervention measures such as timed intercourse and IVF/IVM could improve the pregnancy rate to those who reject foreign oocytes
Trial registration number: ChiCTR-IPR-17010945

P-574 mitochondrial dysfunction induced by high estradiol concentrations in endometrial epithelial cells

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Study question: Are there changes of mitochondrial function in high estradiol (E_2)-stimulated endometrial epithelial cells (EECs)?
Summary answer: High E_2 concentrations increase reactive oxygen species (ROS) production in EECs that subsequently induce mitochondrial dysfunction.
What is known already: A supraphysiological E_2 concentration after ovarian stimulation is known to result in lower embryo implantation rates in *in vitro* fertilization (IVF). EECs apoptosis occurs after the stimulation with high E_2 concentrations, and mitochondria play important roles in cell apoptosis.
Study design, size, duration: *In vivo* and *in vitro* experiments were done to examine mitochondrial functions in EECs stimulated with different E_2 concentrations.
Participants/materials, setting, methods: Isolated human EECs (hEECs) were purified from 6 women in the follicular phase, and were *in vitro* cultured with different E_2 concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M), in which 10^{-7} M is supraphysiologically high. Eight-week-old female ICR mouse endometrium was obtained 5.5 days after the injection of 1.25 IU or 20 IU equine chorionic gonadotropin (eCG).
Main results and the role of chance: *In vivo* and *in vitro* experiments showed decreased mitochondrial DNA contents and ATP formation after EECs were stimulated with supraphysiologically high E_2 concentrations than those stimulated with a physiologic E_2 concentration. Less prominent immunofluorescence mitochondrial staining, fewer mitochondria number under electron microscopy, lower JC-1 aggregate/monomer ratio, and more ROS production were found after EECs were stimulated with supraphysiologically high E_2 concentrations. The high E_2-induced ROS production was reduced when EECs were pretreated with N-acetyl-cysteine (NAC), but remained unchanged after the pretreatment with coenzyme Q10 (mitoQ).
Limitations, reasons for caution: Since mitochondria contribute to many processes central to cellular function and dysfunction, it is difficult to investigate all the mitochondrial functions in this study.
Wider implications of the findings: The high E_2-induced mitochondrial dysfunction in EECs is probably a temporary rather than a permanent effect. The mitochondrial function might return to a normal level when the high E_2 effect is gone.
Trial registration number: N/A

P-575 Association of BRCA mutations with serum anti-Müllerian hormone level in young breast cancer patients

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Study question: What is the relationship between *BRCA* mutation and serum anti-Müllerian hormone (AMH) level in young breast cancer patients?
Summary answer: Young breast cancer patients with *BRCA* mutation have significantly lower AMH value, which is indicative of decreased ovarian reserve, compared to BRCA-negative patients.
What is known already: Several studies have demonstrated significantly decreased serum AMH level in *BRCA* mutation carriers. Moreover, in breast cancer patients who underwent ovarian stimulation for fertility-preservation, there was a higher rate of poor ovarian response in *BRCA*-mutation carriers compared to non-carriers. However, some studies have found no difference in serum AMH level according to *BRCA* mutation status, and therefore, the association between *BRCA* mutation status and decreased ovarian reserve is not conclusive. In addition, only a few studies have shown a significant association between *BRCA* mutation and decreased ovarian reserve in young breast cancer patients.
Study design, size, duration: A cross-sectional study. Between December 2011 and May 2018, 316 young breast cancer patients were included in this study, and 264 were *BRCA*-negative and 52 were *BRCA*-positive (27 *BRCA1*-positive and 25 *BRCA2*-positive).
Participants/materials, setting, methods: This study included pre-menopausal patients $\leq$ 40 years of age who were diagnosed with breast cancer and had a known baseline status regarding *BRCA* mutation and serum AMH level. The serum level of AMH was compared according to presence of a *BRCA* mutation, and linear and logistic regression analyses were performed to evaluate the association between *BRCA* mutation and serum AMH level.
Main results and the role of chance: The median age was 34 years. No differences were found in the clinical characteristics between the *BRCA*-positive and *BRCA*-negative groups. Patients with any *BRCA* mutation had a significantly lower median AMH than those without a mutation (2.60 vs. 3.85 ng/mL, P=0.004). Both serum AMH levels of the *BRCA1* (2.56 ng/mL, P=0.001) and *BRCA2* groups (2.64 ng/mL, P=0.036) were significantly lower than that of BRCA-negative group, but no difference was found between the *BRCA1* and *BRCA2* groups. In linear regression analysis, serum AMH level was still significantly lower in the *BRCA*-positive group than in the BRCA-negative group (P=0.039) after adjusting for age and body mass index. When evaluating the association between risk of poor ovarian response and *BRCA* mutation status by the logistic regression model, 35 (13.3%) and 9 (17.3%) patients had AMH level less than 1.2 ng/mL in the *BRCA*-negative and *BRCA*-positive groups, respectively, presenting no statistical difference. After adjusting for age and body mass index, there was no increased likelihood of poor ovarian response in the *BRCA*-positive group. In addition, no differences were found between the *BRCA1*- and *BRCA2*-positive groups in either the linear or logistic regression analysis.
Limitations, reasons for caution: This was a cross-sectional study in one center. In addition, not all the potential confounders affecting serum AMH level were considered for analysis. Moreover, although we measured AMH and estimated poor ovarian response, long-term fertility outcomes were not assessed in the present study.
Wider implications of the findings: Considering that it is currently recommended for *BRCA*-mutation carriers to undergo a risk-reducing salpingo-oophorectomy after childbearing and that various anti-cancer therapies are necessary in young breast cancer patients, our finding can support that fertility preservation should be considered more aggressively in young breast cancer patients with *BRCA* mutation.

Trial registration number: Not appicable

P-576 The combination of daily vaginal progesterone administration and intramuscular progesterone every-third day secures ongoing pregnancy rates in artificial frozen-embryo transfer cycles

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Study question: Does intramuscular progesterone every third day (imP4-suppl) secure serum progesterone levels and enhance ongoing pregnancy rates (OPR) compared to vaginal progesterone-only (VP4-only) in artificial frozen embryo transfer (FET) cycles?

Summary answer: Although imP4-suppl and VP4-only arms have comparable OPRs, 10% of patients in the VP4-only arm have lower progesterone levels and significantly lower OPRs.

What is known already: Despite the worldwide common use of the hormone replacement therapy (HRT) cycle for FET, the optimal route and dosing of progesterone is uncertain. A recent randomized controlled trial reported lower OPRs with VP4-only when compared to daily imP4 or imP4-suppl (Devine at al-2018). Although significant inter- and intra-personal variation exists, there is paucity of data as regards serum progesterone levels and monitoring in HRT cycles. Moreover, a threshold and ceiling level of serum progesterone has not been clearly defined.

Study design, size, duration: Observational cohort study from October 2017-November 2018, having OPR as primary outcome measure. A total of 302 patients undergoing Day 5/6 vitrified FET in an HRT cycle, were included. From October-February 2018 VP4-only was used for luteal support (n=111). From March 2018 and onwards imP4-suppl every third day was added to the vaginal support (n=191). In all cycles, a blood sample was drawn and cryo-preserved immediately before transfer on the 6th day of progesterone administration.

Participants/materials, setting, methods: In the VP4-only arm vaginal progesterone gel (Crinone 8%, Merck) bid was administered. In the imP4-suppl arm, starting from the first day of vaginal progesterone administration, 50 mg im progesterone (Progynex, Farmako) was administered every third day. The blood sample was drawn 3-4 hours after the morning administration of vaginal gel. The impact of serum progesterone percentiles on the OPR, was retrospectively assessed. Logistic regression analysis was performed to delineate the covariates affecting OPR.

Main results and the role of chance: Female age, body-mass index and the number of previous attempts were comparable between the two arms. Although the mean number of embryos transferred was significantly higher in the imP4-suppl arm (1.36±0.48 vs 1.23±0.43, p=0.018), the OPRs in the imP4-suppl and VP4-only arms were comparable (51.3% and 48.7%, respectively, p=0.372). The pregnancy loss rates were also comparable between the two arms. The impact of serum progesterone on OPR was assessed by percentiles (<10%, 10-49%, 50-75%, >75%), taking the 50-75% as reference subgroup, in both the imP4-suppl and VP4-only arms. In the VP4-only arm (n=111), 11 patients were in the <10% percentile (<7.95 ng/ml), having significantly lower OPR (18.2% vs 64.3%; OR=0.12 (95%CI 0.02-0.69), p=0.01). There was a trend for a ceiling effect with lower OPR in the >75% subgroup (>16.10 ng/ml; 48.1% vs 64.3%; OR=0.52 (95%CI, 0.18-1.52), p=0.228). In the imP4-suppl group, no differences were seen as regards OPRs in percentiles. Similar to the VP4-only arm, there was a trend for a ceiling effect with lower OPR in the >75% percentile (>28.60 ng/ml; 48.9% vs 66.7%; OR=0.48 (95%CI, 0.21-1.10), p=0.080). Logistic regression analysis showed that only female age and BMI, whereas mode of supplementation and serum progesterone did not predict OPR.

Limitations, reasons for caution: Although baseline demographic features were comparable between the two arms, the retrospective study design is a limitation. More embryos were transferred in the imP4-suppl arm.

Wider implications of the findings: Overall, imP4-suppl and VP4-only groups had comparable OPRs. However, 10% of patients in the VP4-only group had low progesterone levels and significantly lower OPRs. In contrast, imP4-suppl secured the OPRs in all progesterone percentiles, suggesting the addition of imP4-suppl every third day to vaginal support in HRT cycles.

Trial registration number: Not available.

P-577 The ovarian sensitivity index (OSI) is in infertile patients predictive of live birth chances after IVF

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Study question: Does the ovarian sensitivity index (OSI) predict embryo quality, pregnancy and live birth in patients undergoing FSH/hMG stimulation for in vitro fertilization (IVF)?

Summary answer: The ovarian sensitivity index is predictive of pregnancy and live birth in women undergoing FSH/hMG stimulation for IVF.

What is known already: The OSI was previously reported to reflect gonadotropin requirements among high, normal and poor responders and to predict pregnancy potential in younger patients undergoing ovarian stimulation with FSH.

Study design, size, duration: Retrospective cohort study that included 1934 fresh IVF cycles in 1282 women undergoing IVF with FSH/ hMG stimulation between January 2010 and December 2016. OSI [n oocytes x1000/total gonadotropin dosage], based on ROC curve analyses of randomly selected development sample comprising a third of cycles, was grouped into two classes.

Participants/materials, setting, methods: Remaining cycles comprised a validation group. ROC curves were also used to compare predictive values of OSI to baseline FSH and AMH. Logistic regression models evaluated effect of high (>0.83) and low OSI (≤ 0.83) on pregnancies and live births in validation group. Models were adjusted for female age, baseline FSH, AMH, oocyte yield and gonadotropin dose.

Main results and the role of chance: Women were 39.3±5.3 years old and had AMH levels of 0.6 (95% CI 0.56 to 0.65) ng/mL. They received 5,375 ± 2,412 IU of gonadotropins and produced 5.2 (95% CI 5.0 to 5.4) oocytes. Pregnancy and live birth rates for all were 18.7% and 14.0 %, respectively. Cycles with higher OSI (less gonadotropin requirement per oocyte retrieved) produced significantly more high-quality embryos than cycles with low OSI (3.6 ± 3.7 vs. 1.1 ± 1.1, P=0.001) and demonstrated higher pregnancy (24.2 % vs. 9.7 %) and live birth rates (18.2 % vs. 6.2%) than their counterparts (P=0.001 and P=0.001, respectively). After adjustments for age, baseline AMH and FSH, total gonadotropin dosage and oocyte yield, OSI > 0.83 was associated with greater odds of pregnancy (OSI: OR 2.12, 95% CI 1.30-3.45, 0.003) and live birth: OSI: OR 1.91, 95% CI 1.07 to 3.41, p=0.028.

Limitations, reasons for caution: Here presented results may not be applicable to women with other ovarian stimulations.

Wider implications of the findings: OSI's predictive capacity for determining embryo quality, pregnancy and live births, may add in counseling patients about their pregnancy potential and live birth chances to other currently available means.

Trial registration number: n/a

P-578 Good clinical results of natural cycle IVF: A 10 year record in our clinic

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Study question: We examined the clinical outcome of natural cycle IVF where the burden on patients is extremely low.

Summary answer: A high pregnancy and production rate was obtained from natural cycle IVF.

What is known already: Although multiple oocytes can be collected after stimulation cycle IVF, there is a risk of OHSS, which can cause health risks to the patient and also impose a large financial burden. In Japan, some patients require natural cycle IVF with its attendant lower physical risk and financial burden. However, there are very few reports on the clinical outcome of long-term natural cycle IVF.
Study design, size, duration: We examined the clinical outcome of long-term natural cycle IVF in a retrospective study of 1768 cycles in which natural cycle IVF was performed during the period 2008–2017. The patients included in the study were under 37 years of age.
Participants/materials, setting, methods: GnRHa was administered nasally when the dominant follicle was 17 mm or more in diameter in a natural cycle; 34 hours later, the oocyte was collected. The retrieved oocyte was used for IVF or ICSI. Following successful fertilization of the oocyte, the resultant embryo was cultured for 2 or 5 days; at the end of the culture period, single fresh embryo transfer (early embryo or blastocyst) was performed.
Main results and the role of chance: One or more mature oocytes were obtained in 1412/1768 cycles (79.9%). In 172 cycles, immature or degenerate oocytes were obtained; oocytes could not be obtained in 184 cycles. A fertilization rate of 85.8% (1693/1974) was achieved: IVF, 85.3% (1012/1186); ICSI, 86.4% (681/788). The pregnancy rate after single fresh embryo transfer was 44.3% (467/1054), and the production rate was 83.1% (388/467).
Limitations, reasons for caution: The data in this study includes cases in which multiple mature oocytes were obtained when oocytes were taken from small follicles and not only from the dominant follicle (327 cases). When multiple embryos were obtained, one of the best-grade embryos was transferred.
Wider implications of the findings: Natural cycle IVF has been shown to provide a high pregnancy rate and a high production rate while minimizing the OHSS risk and reducing the financial burden on patients.
Trial registration number: None.

P-579 The relationship between the factual ovarian volume and serum hormone levels including the levels of the AMH, LH/FSH ratio, and total testosterone

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Study question: Is there a relationship between ovarian volume equivalent to the weight and serum hormone levels including the AMH level, LH/FSH hormone ratio or total testosterone?
Summary answer: Serum AMH, LH/FSH ratio, and testosterone were significantly positively associated with ovarian volume.
What is known already: Ovarian volume is used to distinguish between normal women and women with polycystic ovary syndrome (PCOS) with a cutoff of around 10 ml. Some studies have reported that the AMH and the LH/FSH ratio are significantly related to the ovarian volume estimated with transvaginal ultrasound. The standard technique for measuring the ovarian volume in women is transvaginal ultrasonography and ovarian volumes are calculated using prolate ellipsoid formula (width×depth×length×0.52) . However, it has been reported that ovarian volume measured by ultrasound was at least 27% smaller as compared to the volume obtained by weight.
Study design, size, duration: This study is a retrospective cohort study. The study population included female patients older than 15years (range: 15.0-43.1 years) with diagnosis requiring fertility preservation and was referred for ovarian tissue cryopreservation (OTC) at the Laboratory of Reproductive Biology in Copenhagen, Denmark from August 1999 to December 2018. This research targeted 264 patients who have had a checkup serum hormone levels including the AMH, LH, FSH, or testosterone before OTC.
Participants/materials, setting, methods: 264 patients were enrolled in this retrospective study. The ovarian volume of one ovary excised from each patient for OTC was recorded by weighing the tissue. Ovarian weights were measured using a precision analytical weight – we have previously shown that 1 g of ovarian tissue corresponds to 1ml of tissue. We retrospectively collected these data from the patients' medical records. We evaluated the relation with ovarian volumes and AMH values, LH/FSH level, and testosterone.
Main results and the role of chance: All data are presented as the mean ± SD. The patients' average age was 28.2±6.1 years. In our study, the average AMH level was 19.1±17.2 pmol/L, LH level was 7.41±7.48 IU/L, FSH level was 6.5±11.5 IU/L, LH/FSH ratio was 1.47±1.23, total testosterone level was 0.78±0.56 nmol/L, and the ovarian volume by weight was 7.17±3.73 ml (range: 2.1-20.8 ml) . The ovarian volume was statistically significant positive associated to AMH levels ($p<0.001$) . Also ovarian volume was statistically significant positively associated with increasing the LH/FSH ratio and total testosterone (both $p<0.001$) .
Limitations, reasons for caution: In our study, ovarian volume was measured at a random day during the menstrual cycle due to prioritizing immediate initiation of cancer therapy. Ovarian volume has been shown to be associated to the day of the menstrual cycle and possess the biggest volume on cycle day 19.
Wider implications of the findings: Serum AMH, LH/FSH ratio, and testosterone show a continuous positive association to ovarian volume and does not suggest that marked differences occur when the volume increase beyond 10 ml, which has been suggested as a point to distinguish between women with normal and PCOS.
Trial registration number: not applicable

P-580 Transfer of frozen-thawed embryos in or out of window of implantation yields similar live birth rates

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Study question: Does transfer of frozen-thawed embryos in relation to the proposed window of implantation (WOI) affect live birth rates (LBR)?
Summary answer: Frozen-thawed single blastocyst transfer performed in and out-of-WOI yields similar LBR.
What is known already: The WOI, the time when the endometrium is most able to support trophoblast-endometrial interactions, is proposed to remain open between cycle days (CD) 19-23 of an ideal 28-day natural cycle. It is not clear whether it is important that frozen-thawed embryo transfer (FTET) in an artificially prepared cycle coincides with this relatively short period of time.
Study design, size, duration: A retrospective cohort study was conducted in the American Hospital (Koc University affiliated private hospital) between October 2015 and October 2017. All FTET cycles with a single blastocyst transfer using artificial endometrial preparation w/o GnRH agonist pretreatment were included. Only grade 1 and grade 2 blastocysts had been cryopreserved. Women >43 years of age, treatment cycles with incomplete data and women who were lost to follow-up were excluded.
Participants/materials, setting, methods: All women received oral E treatment starting on day 2 of a spontaneous menstrual bleeding. The duration of E administration varied from 8 to 19 days due to concerns regarding ET scheduling. A total of 498 FTET cycles was analyzed according to the timing of ET that was performed in (CD-19-23, n=256) or out (CD<19 or >23, n=242) of WOI. The primary outcome measure was LBR.
Main results and the role of chance: The timing of ET varied from CD-15 to CD-26 according to the changing duration of E administration. Among a total of 498 frozen thawed single blastocyst transfers, 51.5% (in-WOI) and 48.5% (out-of-WOI), LBR was not significantly affected (OR=0.85, 95%CI [0.6-1.2, p=0.36]). On logistic regression analysis, GnRH pretreatment, E duration, total dose of E used were not associated with the analyzed outcomes.
Limitations, reasons for caution: Retrospective design of the study may have inevitably resulted in bias that may affect the conclusions.
Wider implications of the findings: According to the results of this retrospective study FTET in or out of the proposed natural WOI leads to similar LBR questioning the need for a rigid pretransfer hormone administration period.
Trial registration number: Not applicable

P-581 Comparison of the outcome between conventional mild stimulation and progestin-primed ovarian stimulation in women of advanced age: a retrospective study

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Study question: Does progestin-primed ovarian stimulation (PPOS) result in a better embryonic and clinical outcome compared to mild stimulation in women aged ≥ 40 years old?
Summary answer: Higher percentage of top-quality embryos on Day 3 and comparable clinical pregnancy rate was obtained in PPOS protocol than in mild stimulation protocol.
What is known already: Women with advanced age, especially above 40 years old, is a rapidly growing population in IVF patients since universal two-child policy took effect in 2015 in China. Along with the increase of maternal age, oocyte quantities and qualities decline. PPOS protocol has become a feasible and efficient way to obtain good embryos and better clinical outcomes in normal and poor ovarian responders when compared with GnRH agonist/antagonist protocol and natural cycle. No significant differences are found in neonatal outcomes and congenital malformations risks between PPOS and conventional GnRH agonist short protocol.
Study design, size, duration: This was a retrospective study that included 169 women aged ≥40 years and underwent IVF/ICSI and subsequent frozen embryo transfer (FET) cycle between April 2016 and January 2019 in the reproductive medicine centre of Zhongshan hospital in Shanghai.
Participants/materials, setting, methods: Patients who were above 40 years old at the time point of ovarian stimulation were included. In mild stimulation protocol, daily clomiphene citrate (100 mg/d) and HMG (150-225 IU/d) were used. In PPOS protocol, patients received dydrogesterone (DYG, 20 mg/d) with HMG or recombinant FSH (150-225 IU/d) daily. Freeze-all strategy was employed, and FET cycle was followed. The primary outcome was top-quality embryo rate on day 3, and the second outcome was clinical pregnancy rate.
Main results and the role of chance: Baseline characteristics of patients was similar in mild stimulation group (122 cycles) and PPOS group (47 cycles). The mean values (mean±SD) of age (43.32±2.49 vs 43.15±2.56), body mass index (22.63±2.68 vs 21.95±2.63 kg/m^2), AFC (8.23±3.17 vs 7.09±3.29), basal FSH (8.70±2.51 vs 7.91±2.21 mIU/ml) and LH (4.58±1.60 vs 4.68±1.98 mIU/ml) level and duration of infertility (5.78±4.49 vs 6.33±5.95) were comparable. No significant differences were found in the number of retrieved oocytes (3.57±2.77 vs 3.72±2.76) and mature oocytes (3.08±2.39 vs 2.87±2.20). Meanwhile, the fertilization rate (79.05% vs 77.36%) and cleavage rate (87.25% vs 90.06%) was similar too. Of interest, the rate of top-quality embryos was significantly higher (33.29% vs 50.08%, p=0.015) in PPOS group, with an increasing trend of viable embryo rate (61.16% vs 73.55%) as well. Although the duration of stimulation was comparable, a greater amount of gonadotropin was used in PPOS protocol (1518.14±547.25 IU vs 2061.17±1254.63 IU, p<0.05). During the following FET cycle, 77 cycles was completed, in which 59 cycles was from mild stimulation group and 18 cycles was from PPOS group. The data showed no significant difference in the clinical pregnancy rate (mild stimulation group 16.9% vs PPOS group 16.7%, p= 1.000).
Limitations, reasons for caution: This is a retrospective study and the sample size is small, which is a drawback for the interpretation of the current results. Besides, the data is lack of blastocyst formation rate due to the fact that a proportion of top-quality embryos is vitrified on Day 3.
Wider implications of the findings: This study provides evidence of the efficiency of PPOS in women above 40 years old. Although clinical pregnancy rate hasn't been improved yet, PPOS can be used when these patients are trying to accumulate transferrable embryos in a short period, but randomized clinical trials are required to confirm these findings.
Trial registration number: Not clinical trial.

P-582 Cumulin and FSH cooperate to regulate inhibin B and activin B production by human granulosa-lutein cells in vitro

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Study question: Does the GDF9:BMP15 heterodimer termed cumulin regulate granulosa cell inhibin and activin production and does this require cooperation with FSH?
Summary answer: Cumulin, but not the homodimers GDF9 or BMP15, exerts paracrine control of FSH-induced regulation of inhibin B and activin B.
What is known already: There is genetic evidence that the oocyte-secreted factors bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) constitute key regulators of folliculogenesis, ovulation rate and fecundity in mammals. BMP15 and GDF9 interact functionally and it is hypothesised that this interaction may be mediated by formation of a GDF9:BMP15 heterodimer, termed cumulin. BMP15 and GDF9 regulate folliculogenesis by acting as paracrine growth factors in the follicle and are known to regulate inhibin expression, although the participation of cumulin in control of the inhibin-activin system is unknown.
Study design, size, duration: To assess the effects of cumulin versus GDF9 and BMP15, we bioengineered and purified wild-type non-covalent cumulin, as well as various covalent dimers of cumulin, GDF9 and BMP15, containing an introduced inter-subunit disulphide bridge. To assess the effect of the pro-domain, we engineered mature cumulin (without the pro-domain).
Participants/materials, setting, methods: Human granulosa-lutein (hGL) cells from IVF patients were cultured for 5 days, then treated for 24h ± FSH with various forms of recombinant cumulin (native and cysteine mutants, and with-/without the pro-domains), and the disulphide-subunit bridged forms of GDF9 or BMP15. Messenger RNA expression of the subunits of inhibins/activins (*INHA*, *INHBA*, *INHBB*), and secretion of inhibin A, inhibin B, and activin B protein by immunoassay into media were measured (n=3-7 biological replicates per experiment).
Main results and the role of chance: Mature and pro-forms of cumulin dose-dependently stimulated (P<0.05) *INHBB* mRNA expression (encoding the inhibin βB subunit of inhibin B or activin B), but did not alter *INHBA* (encoding the βA subunit of inhibin A). Correspondingly, cumulin stimulated ≥5-fold secretion of inhibin B and activin B (P<0.05), but did not alter inhibin A. In contrast to cumulin, GDF9 or BMP15 exhibited no significant effect on inhibin B or activin B expression (P≥0.05). Furthermore, cumulin, but not GDF9 or BMP15, interacted synergistically (two-way ANOVA interaction, P<0.05) with FSH to increase *INHBB* mRNA and inhibin B expression. FSH markedly stimulated (≥16-fold) *INHA* expression, which encodes the α subunit of inhibin A/B, and suppressed activin B (P<0.05). Mature and pro-forms of cumulin ± FSH stimulated comparable effects on *INHBB, INHBA*, inhibin B, activin B and inhibin A (P≥0.05), suggesting that the pro-domains of cumulin has a minimal role in its actions on granulosa cells.
Limitations, reasons for caution: In vitro study using granulosa-lutein cells from women undergoing stimulation for IVF.
Wider implications of the findings: Together these data demonstrate that the GDF9:BMP15 heterodimer cumulin exerts control of FSH-induced regulation of inhibin B and activin B, which may contribute to oocyte-secreted factor regulation of folliculogenesis and fecundity in women.
Trial registration number: not applicable

P-583 Relationship between diminished ovarian reserve and matrix metalloproteinase-9 (MMP9) p.Gln279Arg polymorphism

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Study question: Are there any MMP9 gene polymorphisms associated with diminished ovarian reserve (DOR)?

Summary answer: MMP9p.Glutamine(Gln)279Arginine(Arg)(rs17576) polymorphism is associated with diminished ovarian reserve. The presence of the Gln amino acid in homozygosis increases the chance of women presenting with DOR.

What is known already: Ovarian reserve refers to the woman's reproductive potential based on number and quality of eggs. DOR is the loss of normal reproductive potential in the ovaries due to a lower count of the remaining eggs. Normal aging causes most cases of DOR but environmental, medical treatments (as radiotherapy) and genetic factors can influence DOR as well. MMP9 has been suggested to be involved in different stages in female reproduction, such as menstrual cycle, ovulation, implantation, parturition and involution of the mammary glands after lactation but little is known about its influence on ovary functions.

Study design, size, duration: All of the recruited women (n=144) met the following inclusion criteria: age ≤37years, normal karyotype, ultrasonographic evidence of the two ovaries and no surgery on ovaries, endometriosis, hydrosalpinx, infections or endocrine problems. The women were divided into two groups according to Anti-Müllerian hormone/AMH (ng/ml) levels and antral follicle count/AFC (2-9 mm), evaluated during the early follicular phase:

Diminished ovarian reserve (DOR/n=85): AMH<1+AFC<9

Normal ovarian reserve (NOR/=59): AMH≥2+AFC≥15

Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from DOR and NOR groups. DNA was sequenced on MiSeq(Illumina) to search for single nucleotide polymorphisms (SNPs) in the MMP9 gene. SNPs were identified using TruSeq Custom Amplicon (TSCA) Panel (DesignStudio Illumina). This design was performed in order to sequencing the exons, 3' and 5' untranslated regions (UTRs) and some intronic regions. SNPs found by Next-Generation Sequencing (NGS) were analysed to find a possible association with DOR.

Main results and the role of chance: The MMP9 p.Gln279Arg polymorphism was identified. The genotyping results showed an association between MMP9 Gln/Gln and women presenting with DOR (table1).

Logistic regression (table 2) showed that patients with MMP9 Gln/Gln genotype had 3.0-fold increase in the chance of being included in the DOR group.

Table 1 Distribution of genotype/allele frequency in diminished and normal ovarian reserve groups.

MMP9 p.Gln279Arg	Diminished ovarian reserve	Normal ovarian reserve	*P*
Genotypes			
Gln/Gln	70.6%	44.1%	0.005
Gln/Arg	21.2%	44.1%	
Arg/Arg	8.2%	11.8%	
Alleles			
Gln	81.2%	66.1%	0.006
Arg	18.8%	33.9%	

Limitations, reasons for caution: Additional validation of the SNP analysed would be important to provide more information about the relationship of this polymorphism and ovarian reserve, once sample size was limited despite recruiting all eligible participants during the study period. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings: The SNP identified could provide an additional tool to test ovarian reserve, helping in the individualization of ovarian stimulation protocols. To the best of our knowledge this is the first study relating this SNP and ovarian reserve.

Trial registration number: Not applicable. The local ethics committee authorized the study. This study was supported by Merck Grant for Fertility Innovation (GFI-2014-16).

Table 2 Logistic regression. Genotype X chance of diminished ovarian reserve.

MMP9 Genotypes	Odds Ratio	95% Confidence Interval	*P*
Gln/Gln	3.02	1.44 – 6.47	0.002
Gln/Arg	0.32	0.15 – 0.71	0.004
Arg/Arg	0.77	0.26 – 2.26	0.64

P-584 Cumulus cell telomere length is associated with vitamin D3's main catabolite 24,25-dihydroxyvitamin D3 concentration in serum and follicular fluid: An ovarian ageing explanation.

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Study question: Is Vitamin D status associated with telomere length (TL) in cumulus cells (CC) and therefore implicated in ovarian ageing?

Summary answer: 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) concentrations in serum (S) and follicular fluid (FF) positively correlates with TL in CC of mature follicles in women of reproductive age.

What is known already: The vitamin D hypothesis of ageing has been recently proposed and involves the process of telomere shortening. Progressive shortening of telomeres has been associated with reproductive ageing and genomic instability during early development. $24,25(OH)_2D$, the main catabolite of 25-hydroxyvitamin D (25(OH)D), has been suggested as a systemic indicator of vitamin D status. We previously reported a high correlation between this metabolite and $25(OH)D_3$ concentrations in S and FF. Furthermore, after analyzing each metabolite separately, the strongest correlation between S and FF concentrations was observed for $24,25(OH)_2D_3$, suggesting $24,25(OH)_2D_3$ is an accurate indicator of ovarian vitamin D status.

Study design, size, duration: 35 egg donors were included during four months in this prospective, non-interventional cohort study. Following controlled ovarian stimulation using an antagonist protocol and standard doses of subcutaneous FSH, oocytes retrieval was performed 36 hours after a bolus of GnRH agonist. S samples and pooled FF from mature follicles were collected during the pick-up for $24,25(OH)_2D_3$ measurement via liquid chromatography-tandem mass spectrometry (LC-MS/MS). CC for cumulus cells telomere length (CCTL) measurement were obtained after oocyte stripping.

Participants/materials, setting, methods: Genomic DNA of CC from the 35 subjects was isolated. For relative CCTL, average delta cycle threshold (dCt) was obtained using a SYBR green quantitative real-time PCR protocol. A Taqman assay for the multicopy gene Alu was used for normalization of measurements. $24,25(OH)_2D_3$ concentrations were measured via LC-MS/MS using a UPLC-TQ-S Xevo Waters system with a Waters Acquity BEH C18 (1,7µm 2,1 x100mm) column. Pearson correlation analysis between $24,25(OH)_2D_3$ concentrations and relative CCTL was used.

Main results and the role of chance: Mean donor's age was 25.43±4.56 years. All the cases were included and studied during autumn and winter months.

None of them had taken vitamin D oral supplements during the last six months before sampling.

Mean value for $24,25(OH)_2D_3$ concentrations in S was 15.616±10.997 nmol/l. A previous report in a large British healthy population identified a serum concentration of $24,25(OH)_2D$>4.2 nmol/L as a diagnostic cut-off for 25(OH)D replete status. According to this, there was no vitamin D deficiency in our studied population of 35 healthy egg donors.

Mean value for $24,25(OH)_2D_3$ concentrations in FF was 11.263±6.0859 nmol/l, showing a high correlation with S values (R2=0.768, P-value=1.121e-7). Wilcoxon paired test showed significantly lower concentrations in pooled FF (P-value=0.004).

CCTL (Average dCt) mean value was 7.184 ± 0.477. Average dCt for CCTL was significantly correlated with $24,25(OH)_2D_3$ concentrations in S and FF (R2=-0.349, P-value=0.043 and R2=-0.365, P value=0.031, respectively). Since Ct values are inversely proportional to the amount of nucleic acids amplified, a higher dCt value indicates a shorter TL. Therefore, the obtained significant inverse correlation means that, the higher the vitamin D concentrations in S and FF, the longer the telomeres in the cumulus cells.

Limitations, reasons for caution: Our real-time PCR protocol measures relative telomere length, which allows for comparisons between samples, but does not provide an absolute length of the telomeres. It might not be sensitive enough to detect slight differences. Further, although a correlation exists, there are no known mechanisms by which vitamin D alters TL.

Wider implications of the findings: This is the first reported association between CCTL and the concentrations in S and FF of the first catabolite of vitamin D_3, $24,25(OH)_2D_3$. These results suggest that ovarian ageing could be associated with this accurate indicator of vitamin D status at systemic and reproductive level.

Trial registration number: Not applicable

P-585 Inhibition of GnRH-mediated intracellular signaling in vitro differs according to type of antagonist

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Study question: Do Cetrorelix, Ganirelix and Teverelix have similar potency to antagonize GnRH?

Summary answer: 10 nM Cetrorelix is more potent than Ganirelix and Teverelix in inhibiting GnRH-mediated cAMP and intracellular Ca^{2+} increase *in vitro*.

What is known already: Cetrorelix, Ganirelix and Teverelix are GnRH antagonists that have decapeptidic structures that differ by only one and two amino acids. Cetrorelix and Ganirelix are used during ovarian stimulation as part of treatment with assisted reproduction technologies for preventing premature luteinizing hormone (LH) surges and inhibit LH production within about 3–12 hours. Although some reports have suggested that GnRH antagonists have different potencies at the cellular level, potentially leading to differences in outcomes, these differences are not well understood.

Study design, size, duration: This was an *in-vitro* model study of different doses of Cetrorelix, Ganirelix and Teverelix (ranging from pm to µM) for evaluating the inhibition of GnRH-induced short-term (0–30 minutes) and long-term (0–24 h) responses. Between three and six treated samples were compared, and GnRH antagonist-untreated samples served as controls.

Participants/materials, setting, methods: GnRH receptor I (GnRHR)-transfected HEK293 cells were used. Transfected and untransfected human neuroblastoma SH-SY5Y and the LβT2 mouse pituitary cell lines were also studied. Inhibition of GnRH-induced intracellular cAMP and Ca^{2+} increase, and pCREB, pERK1/2 and β-catenin activation were evaluated by bioluminescence resonance energy transfer (BRET), western blotting and immunostaining. Inhibition of 12-h *Lhb* gene expression was evaluated by real-time PCR.

Main results and the role of chance: In GnRHR-transfected HEK293 cells, 10 nM Cetrorelix inhibited GnRH-induced intracellular Ca^{2+} increase, whereas similar inhibition was seen with Ganirelix and Teverelix at a ten-fold higher concentration (100 nM [Mann-Whitney's U-test; $p<0.05$; n=6]). These findings were confirmed in GnRHR-transfected SH-SY5Y cells, where the 3 x 50% effective concentrations of GnRH antagonists were 10-fold higher than in HEK293 cells, probably due to differences in receptor expression at the cell surface between the two transfected cell lines. Untransfected HEK293 and SH-SY5Y cells did not reveal any detectable response to GnRH. Cetrorelix had higher efficacy than Ganirelix or Teverelix, resulting in an inhibitory concentration (IC_{50}) of 1.56±2.49 nM, which was lower than the inhibitory concentrations of Ganirelix (IC_{50}=16.60±3.76) or Teverelix (IC_{50}=62.80±3.77) (Mann-Whitney's U-test; $p<0.05$; n=5). In spite of the aforementioned differences between drugs in inhibiting short-term GnRH-mediated cell response, the antagonists depleted pCREB, pERK1/2 and β-catenin activation at similar concentrations (range 100 nM–1.0 µM; one-way ANOVA; $p\geq0.05$; n=3). All the antagonists tested inhibited GnRH-induced *Lhb* expression in LβT2 cells, suggesting that they may efficiently target luteinizing hormone production.

Limitations, reasons for caution: This was an *in-vitro* study and the differences in potency/efficacy seen for the three antagonists might not translate to *in-vivo* effects, as the metabolic clearance and pharmacokinetics may differ between the antagonists, especially due to different formulations and solubility.

Wider implications of the findings: The GnRH antagonists Cetrorelix, Ganirelix and Teverelix were different with respect to *in-vitro* activity for inducing intracellular Ca^{2+} increase. This may lead to different short-term regulation in the target cells, which could modulate the *in-vivo* outcomes.

Trial registration number: not applicable

P-586 Growth differential factor 9 suppresses expression of STAR, CYP17A1 and LHCGR in cultured human theca cells

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Study question: What's the effect of growth differential factor 9 (GDF9) on production of androgen in human theca cells?

Summary answer: GDF9 can suppress expression of *STAR*, *CYP17A1*, *LHCGR* in cultured human theca cells; ALK5 and Smad4 may mediate suppression of *CYP17A1* by GDF9.

What is known already: GDF9, one of the oocyte-derived factors, has a critical impact on folliculogenesis. It has been shown that GDF9 can inhibit *CYP17A1* expression and production of androgen in cultured bovine theca cells and mouse theca-interstitial cells.

Study design, size, duration: This is a control versus treatment study. Each experiment was triplicated.

Participants/materials, setting, methods: Human theca cells were collected from discarded ovarian tissue after surgery and pathological examination and cultured in vitro. After the first 48 hours of culture, cells were treated with GDF9, GDF9 with SB525334 (an ALK5 inhibitor) or Kartogenin (an agonist of Smad4) at various doses. The medium was then collected to determine the concentration of androstenedione by chemiluminescent immunoassay. Cellular RNA was collected and extracted for quantification by real-time quantitative fluorescence PCR (RT-PCR).

Main results and the role of chance: GDF9 suppressed expression of *CYP17A1* and inhibited androstenedione production in human theca cells at a dose-dependent manner. At the dosage of 800ng/ml, GDF9 significantly suppressed *STAR* mRNA by 61% (p=0.014), *CYP17A1* mRNA by 54% (p=0.016)and *LHCGR* mRNA by 80% (p=0.02) and did not affect expression of *HSD3B* and *CYP11A1* in human theca cells. SB525334 antagonized the suppression of *CYP17A1* ($p<0.001$) by GDF9 while its antagonistic effect on suppression of *STAR* and *LHCGR* by GDF9 did not show statistically significance. Kartogenin significantly suppressed expression of *STAR* mRNA by 46% (p=0.010)and *CYP17A1* mRNA by 54% (p=0.042)and did not affect expression of *LHCGR, HSD3B* and *CYP11A1* in human theca cells.

Limitations, reasons for caution: This is an in vitro experimental study mainly focused on the association of GDF9 and GDF9 signaling pathway. In vivo

study and other possible signaling pathway should be taken into consideration for further research.

Wider implications of the findings: According to our results, dysregulation of GDF9 will result in an increased expression of *STAR*, *CYP17A1* and *LHCGR* genes in human theca cells, subsequently increasing the sensitivity to LH and androgen production in theca cells and finally forming hyperandrogenism phenotype in PCOS.

Trial registration number: N/A

P-587 Prevalence of psychological distress in polycystic ovarian syndrome (PCOS) infertile patients and non PCOS infertile controls and their relationship with clinical-biochemical parameters of the syndrome.

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Study question: Are there increased levels of psychological distress in PCOS infertile patients in comparison to infertile controls and its association with clinical-biochemical features of the syndrome.

Summary answer: PCOS is associated with increased levels of psychological distress when compared to non PCOS infertile controls. Screening and management should be considered during routine assessment.

What is known already: Polycystic ovarian syndrome is the commonest endocrine disease affecting young women. Forty percent of infertile women are diagnosed with PCOS as a cause of infertility. Apart from infertility, PCOS has huge long term metabolic consequences that affects patient's quality of life (QOL). Coexisting mental health disorders have also been shown to impair patient's QOL. However it is difficult to say if they are particularly attributable to some clinical - biochemical features of PCOS per se. The emotional and financial strain of infertility treatment might also contribute to increased psychological distress in such patients.

Study design, size, duration: A single centre cross sectional study was carried out at a tertiary care infertility centre in India from 1st January 2018 through 31st December 2018. Two hundred and thirty eight infertile patients consented to participate in the study. One hundred and twenty PCOS infertile patients were matched to one hundred and eighteen non PCOS infertile controls.

Participants/materials, setting, methods: For assessing levels of anxiety and depression, Hamilton's Rating Scales (HAM-A, and HAM-D) were used. Schoff Questionnaire for eating disorders was used. Fertility and Quality of Life Questionnaire (FertiQoL) was used to index the quality of life. Hirsutism score and body mass index (BMI) were determined. Primary outcomes were the incidence of psychological disorders and their association with BMI and hyperandrogenism.

Main results and the role of chance: The baseline prevalence of depression in PCOS patients was 37.5% and in non PCOS infertile controls was 25.42% (p=0.04), baseline prevalence of anxiety in PCOS patients was 41.67% and in non PCOS infertile controls was 29.66% (p=0.05); both were statistically significant. The prevalence of eating disorders in POCS patients was 0.02% and in non PCOS infertile controls was 0.01%; the difference was not statistically significant (p=0.57). The HAM-A scores in PCOS and non- PCOS infertile controls (14.45±8.24 vs. 11.75±8.39; p= 0.012) and HAM-D scores (14.25±8.06 vs. 11.45±7.05; p=0.004) in PCOS and non-PCOS infertile controls; the difference was statistically significant. There was no difference in scores of Core FertiQoL for both the groups. Both groups showed comparable reduced quality of life. BMI and hirsutism score was positively correlated with HAM-A and HAM-D scores.

Limitations, reasons for caution: Although it is a prospective study, it has limitation of small sample size.

Wider implications of the findings: PCOS is a complex disorder associated with alarming levels of psychological distress which is much greater when compared to non PCOS infertile controls. Counsellor should routinely evaluate all infertile patients, especially PCOS from a mental health perspective. Counselling in addition to pharmacotherapy would help improve their quality of life.

Trial registration number: MCDH/2018/80

P-588 What is the optimal duration of progesterone supplementation prior to frozen-warmed blastocyst transfer in a hormone replacement therapy cycle?

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Study question: Is there a difference in live birth rate (LBR) between 6 and 7 days of progesterone (P) administration, prior to frozen-warmed embryo transfer (FET)?

Summary answer: LBRs are not significantly different when 6 or 7 days of P are administered. Six days of P yields higher clinical pregnancy and miscarriage rates.

What is known already: Thanks to recent developments, embryo cryopreservation has become a first-line procedure. Concerning the optimal duration of P administration prior to FET no consensus is yet defined. Current evidence showed no significant difference in clinical pregnancy rates between 5 and 7 days of P administration preceding blastocyst transfer. Although some studies presumed that endometrial receptivity could be achieved after very short P exposure, other studies showed a higher risk of pregnancy loss if the endometrium was insufficiently decidualized. These findings could be explained by an embryo-endometrium asynchrony potentially caused by transferring an embryo in a not yet sufficiently P primed endometrium.

Study design, size, duration: We performed a retrospective cohort study in a tertiary university-based referral hospital from December 2015 until December 2017. A total of 984 patients, undergoing a FET in a hormone replacement therapy cycle (HRT) were included. Day 5 and day 6 vitrified blastocysts were warmed and transferred the same day. Patients were included only once with the first FET.

Participants/materials, setting, methods: The primary outcome was live birth defined as a live born delivery after 24 weeks. LBRs were compared between 6 and 7 days of P supplementation. Multivariable regression analysis was used to account for confounding variables, specifically: age at vitrification, BMI, indication for treatment, parity, smoking habits, outcome of the fresh IVF/ICSI cycle, applied type of in vitro technique, embryo quality, endometrial thickness, duration of estradiol supplementation preceding FET and single or double embryo transfer.

Main results and the role of chance: The median age at vitrification was 31 years (6 days) and 32 years (7 days), respectively (p=0.31). Preimplantation genetic testing was performed significantly more in the day 6 group (5.5% versus 1.6% for respectively 6 and 7 days; p=0.001). Ovulation disorders and polycystic ovarian syndrome were also more common in the day 6 group (23.2% for 6 days and 17.0% for 7 days, p=0.018). Double embryo transfer was performed more frequently in the day 7 group (9.0% versus 13.2%, p=0.036).

Pregnancy outcomes after univariable analysis showed significantly higher positive hCG rates and clinical pregnancy rates after 6 days of P supplementation compared to 7 days (60.1% versus 53.6%, p=0.041 and 55.7% versus 48.4%, p=0.023).

LBRs showed similar results in both treatment arms (36.9% for 6 days and 37.2% for 7 days, respectively, p=0.91), even after adjusting for confounding factors (aOR 1.05, 95% CI 0.78-1.40, p=0.75). Biochemical pregnancy rates were comparable between both groups (4.4% for 6 days and 5.2% for 7 days, p=0.55), but miscarriage rates were significantly higher in the 6 days group compared to the 7 days group (27.2% versus 19.5%, respectively, p=0.034).

Limitations, reasons for caution: Although this study adjusts for multiple potential confounding factors and includes patients only once with the first FET, the results remains limited by the retrospective nature of the study and its potentially associated bias and should be interpreted with caution. Confirmation of the findings in a prospective setting is necessary.

Wider implications of the findings: Six or 7 days of P administration does not significantly affect LBRs. However, we argue that 6 days could be insufficient to induce adequate endometrial secretory changes, showing significantly higher clinical pregnancy and miscarriage rates in this group. Further research is needed to reach consensus regarding the ideal P duration.
Trial registration number: not applicable

P-589 High pregnancy rate in women with diminished ovarian reserve (DOR) after attempted drugfree in vitro activation of ovarian follicles

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Study question: Can ovarian biopsying per se and/or autotransplantation of cortical tissue fragments activate and increase the number of recruitable follicles for IVF/ICSI in women with DOR?
Summary answer: Neither ovarian biopsying nor autotransplantation of the cortical tissue fragments increased the number of recruitable follicles after ovarian stimulation for IVF/ICSI after 10 weeks follow-up.
What is known already: Infertile women with DOR constitute a group of patients with poor reproductive outcome mainly due to the low number of mature oocytes available after ovarian stimulation for IVF/ICSI. Recent studies have shown, that *in vitro activation* of residual follicles by both chemical drug treatment and ovarian tissue fragmentation followed by autotransplantation resulted in return of menstrual cycles and pregnancies in a fraction of women with premature ovarian insufficiency.
Study design, size, duration: A prospective clinical cohort study including 20 women with DOR treated at the Fertility clinic at Rigshospitalet, Denmark during April 2016 - December 2017. Follow-up of non-pregnant women ended in September 2018 and were on average 280 days (range 118-408). Women who conceived are followed until delivery.
Participants/materials, setting, methods: Infertile, menstruating women aged 30-39 years with IVF/ICSI indication and repeated AMH measurements < 5 pmol/L (0.7 ng/ml) were included. By laparoscopy four biopsies were taken from one ovary randomized between the left or right ovary. The other ovary served as control. Cortical tissue was fragmented and autotransplanted to peritoneum. Recordings of hormones, antral follicle count (AFC) and assessment of ectopic follicle growth were done weekly. After 10 weeks ovarian stimulation for IVF/ICSI was initiated.
Main results and the role of chance: In term of our primary out-come, no difference was observed in the number of mature follicles in the biopsied ovary versus the control ovary after ovarian stimulation (1.0 versus 0.7 follicles, p=0.35). In only three women, growth of four follicles were detected at the graft site 24-268 days after the procedure. One ectopic oocyte was retrieved and fertilized, but embryonic development failed. Overall AMH levels did not change significantly (p=0.2). Mean AFC increased by 0.14 (95%CI: 0.06;0.21) per week (p<0.005) and the biopsied ovary had on average 0.6 (95%CI: 0.36;0.88) follicles less than the control ovary (p = 0.01). Serum levels of androstenedione and testosterone increased significantly by 0.63 nmol/L (95%CI: 0.21;1.04) and 0.11 nmol/L (95%CI: 0.01;0.21) one week after the procedure, respectively. Testosterone increased continuously by 0.0095 nmol/L (95%CI: 0.0002;0.0188) per week (p=0.045). In 7 of the 20 women mean AMH increased from 2.08 pmol/L (range 1.74-2.34) to 3.94 pmol/L (range 3.66-4.29) from week 1-4 to week 5-8. Clinical pregnancies were obtained in 12 of the 20 (60%) women, either naturally (n=3) or after a total of 55 IVF/ICSI/insemination treatments (n=9). We expect a cumulated live birth rate per started IVF/ICSI cycle of 18.4% (including two ongoing pregnancies).
Limitations, reasons for caution: Limitations of the study were the low number of included women and the lack of a control group. Moreover, 45% of the women had no male partner at inclusion. However, these women had an average of 6.5 (range 4-9) unsuccessful medically assisted reproduction treatments with donor sperm prior to inclusion.
Wider implications of the findings: These findings suggest that biopsying and autotransplanting of ovarian fragments do not augment the number of recruitable follicles for IVF/ICSI after 10 weeks. However, a high pregnancy rate and that some women had an AMH increase after 5-8 weeks warrant a larger study on the possible effects of biopsying alone.
Trial registration number: NCT02792569

P-590 Effect of serum vitamin D level on the cumulative live birth rate of the first in vitro fertilization cycle

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Study question: Is serum vitamin D level associated with the cumulative live birth rate (CLBR) of the first in vitro fertilization (IVF) cycle?
Summary answer: Serum vitamin D level is not associated with the CLBR in women undergoing the first IVF cycle.
What is known already: Vitamin D receptors are present in various tissue in the female reproductive tract. Studies on the association between serum vitamin D level and clinical outcome after IVF showed conflicting results. Data from meta-analyses showed a lower live birth rate for women with vitamin D deficiency, the possible mechanism being mediated through the endometrium. The existing reports mainly focused on a single fresh or frozen embryo transfer cycle. There is no data on the effect of vitamin D on the CLBR in one IVF cycle.
Study design, size, duration: This is a retrospective cohort study including 1220 infertile women aged less than 43 years who underwent the first IVF cycle in a university reproductive medicine centre from January 2013 to December 2016 inclusive. Women using donor oocytes, modified natural cycles, in vitro maturation, pre-implantation genetic testing or those whose archived serum sample cannot be retrieved were excluded.
Participants/materials, setting, methods: All women underwent their first IVF cycle in the unit. Archived serum samples taken on the second day of menses before gonadotrophin stimulation and stored at -20°C until measurement were analyzed for 25(OH)D levels. The main outcome measure was the CLBR following the fresh embryo transfer and replacement of frozen embryos derived from the index stimulation cycle. Vitamin D deficiency/insufficiency was defined as serum 25(OH)D < 30ng/ml based on the Endocrine Society Clinical Practice guidelines.
Main results and the role of chance: Among the 1220 women, 5 had ongoing pregnancy, 78 women still had embryos in storage and 3 were lost to follow up. The median age was 36 (25^{th}-75^{th}percentile 34-38) years and the median serum 25(OH)D level was 18.2 (25^{th}-75^{th}percentile 14.7-22.0)ng/ml. 61.7% had deficient vitamin D levels (< 20ng/ml) and 95.7% had vitamin D in the deficient/ insufficient range (<30ng/ml). There was no significant difference in the median vitamin D levels in those who achieved a live birth and those who did not [18.1 (14.9-21.8)ng/ml vs 18.1(14.5-21.9)ng/ml, p=0.997]. When analyzing the result based on the Endocrine Society Clinical Practice guideline threshold of 30ng/ml, there was no significant difference in the CLBR in the vitamin D deficient/ insufficient group compared to the replete group (515/1088, 47.3% vs 21/46, 45.7% respectively, p=0.823). There was no difference in the clinical pregnancy rates in the fresh cycle in both groups (423/1168, 36.2% vs. 16/52, 30.7%, p=0.423) but a lower live birth rate per fresh transfer in the vitamin D replete group (327/891, 36.7% vs 11/34, 32.4%, p<0.001). Vitamin D did not independently affect the live birth rate per fresh transfer after controlling for age and antral follicle count.
Limitations, reasons for caution: Our study was retrospective in nature and the majority of the women in the study were vitamin D deficient/ insufficient. The recommended serum vitamin D levels were based on evidence

on bone health and there is no consensus on the appropriate cut-off for non-skeletal effects.

Wider implications of the findings: 95.7% of infertile women undergoing IVF in our study were vitamin D deficient/ insufficient. Future interventional studies on the effect of vitamin D on the live birth rate in infertile women undergoing IVF are needed to answer the question on whether preconceptual vitamin D supplement should be provided.

Trial registration number: HKUCTR-2361

P-591 Late-follicular phase progesterone rise does not affect embryo euploidy and cumulative live birth rates. An analysis of 1500 embryos following PGT-A

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Study question: Is late-follicular phase progesterone rise associated with a deleterious on embryo quality or embryo euploidy rates and cumulative live birth rates (CLBRs)?

Summary answer: Late-follicular progesterone rise does not have a deleterius effect on embryo quality and embryo euploidy rates nor on cumulative LBRs.

What is known already: Previous studies have shown that late follicular phase progesterone elevation may adversely affect pregnancy outcomes in IVF/ICSI cycles. Although the vast majority of the studies supported a potential detrimental effect of progesterone elevation on implantation rates, recent reports support a potential detrimental effect of progesterone elevation on embryo quality

Study design, size, duration: A retrospective analysis of all GnRH antagonist down-regulated ICSI cycles followed by (PGT-A) and deferred Frozen embryo transfer between 2016 and 2017 was performed. The sample was stratified according to the following P levels on the day of triggering: <1.50 and ≥1.50 ng/ml. The primary outcomes were embryo euploidy rates, embryo utilization rates and cumulative LBR

Participants/materials, setting, methods: Overall 1525 embryos were from 345 ICSI PGT-A cycles were treated in a university-affiliated hospital. Results were compared between patients with normal or elevated progesterone levels on the day of triggering, using multivariable regression analysis in order to account for potential confounding factors. Furthermore, mixed regression models were created using progesterone on the day of triggering as a continuous variable to evaluate its effect on embryo euploidy rates, embryo utilization rates, and cumulative LBRs.

Main results and the role of chance: Late follicular phase progesterone was associated with a higher number of oocytes retrieved, higher BMI, higher gonadotropin starting dose and late follicular estradiol levels.

Embryo euploidy rates were comparable between patients with normal (<1.50ng/ml) vs. high (≥1.50 ng/ml) late follicular phase serum progesterone levels 39.5% [36.4%-42.5%] vs. 38.7% [29.4%-48.0%].

Similarly, no differences were observed in embryo utilization rate 59.5% [56.9%-62.1%] vs. 58.0% [50.6%-65.4%] and clinical pregnancy 63.4% vs 68.3%, p=0.543.

Live birth rate in the first FET cycle 49.2% vs. 46.3%, p=0.733 and CLBR 40.4% vs. 50%, p=0.257. Cycles with elevated progesterone were cycles with higher number of embryos, normal (<1.50ng/ml) vs. high (≥1.50 ng/ml) 4.19±2.7 vs. 6.21±2.99 p<0.001 and borderline non-significant higher number of euploid embryos 1.66±1.69 vs. 2.34±2.27, p=0.082.

Multinomial regression analysis adjusted by age, number of oocytes retrieved and progesterone elevation (<1.50ng/ml vs. ≥1.50 ng/ml) demonstrated that progesterone rise (>1.50) does nor affect euploidy rates (OR 0.942 95% CI [0.671-1.321]). Similarly, a regression analysis failed to demonstrate any effect of progesterone levels on the number of euploid embryos following adjustments for age and number of oocytes retrieved whereas CLBRs were also not associated with progesterone elevation OR 0.91 (0.398-1.649).

Limitations, reasons for caution: This is a retrospective study and despite the use of regression models, residual confounding bias cannot be excluded. In addition, our study focused mainly on embryo quality and euploidy rates and was restricted only in PGT-A cycles followed by FET preventing us from evaluating progesterone elevation effect on fresh ET.

Wider implications of the findings: Our findings seriously question results from previous studies claiming a detrimental effect of progesterone elevation on embryo quality and progesterone elevation appears to have no effect on embryo euploidy and embryo utilization rates.

Trial registration number: NA

P-592 hCG improves steroidogenic activity and luteal function through the activation of JNK signaling pathway in human luteal granulosa cells

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Study question: What is the molecular mechanism of improvement of luteal function after hCG treatment in luteal granulosa cells?

Summary answer: hCG-induced up-regulation of steroidogenic enzymes and increased progesterone (P_4) production involves activation of JNK pathway in human luteal granulosa cells (HLGCs).

What is known already: hCG improves luteal function (luteal phase support) and increases P_4 production by binding to LH receptors expressed on the luteal granulosa cells in human. But the molecular mechanisms underlying these actions of hCG are not well-characterized.

Study design, size, duration: An in vitro translational research study conducted on human luteal granulosa cells obtained from IVF patients stimulated with GnRH antagonist (n=15) and agonist protocols triggered with recombinant hCG (15) or with a GnRH agonist (n=15).

Participants/materials, setting, methods: Human luteal granulosa cells were isolated from follicular aspirates during oocyte retrieval procedure. The expression of steroidogenic enzymes and LH receptor were validated by qRT-PCR and western blotting. E_2/P_4 productions were measured by ECLIA method. Endogenous JNK activity was measured using a commercially available kit. siRNA technology was used to interfere with JNK and LH receptor. Pharmacological JNK inhibitors were also used to interrupt the pathway.

Main results and the role of chance: hCG significantly increased the expression and activity of JNK along with up-regulated expression of steroidogenic enzymes (stAR, SCC, 3B-HSD, 17B-HSD, aromatase), LH receptor and VEGF in a dose-dependent manner in the HLGCs in all three types of stimulation protocols. As a result, in vitro E_2/P_4 production of the cells were increased in a dose-dependent manner. These effects were abolished when the cells were treated with hCG after down-regulation of LH receptor via siRNA. The inhibition of basal and hCG induced JNK activation via pharmacological inhibitors (AS601245/SP600125) or siRNA down-regulated the expression of steroidogenic enzymes and decreased in vitro hormone production. Promoter assay revealed that c-Jun binds to activator protein-1 (AP-1) motif in the promoter region of stAR and increases both stAR transcription and P_4 synthesis in the luteal granulosa cells and hCG enhanced this effect. Activin-A opposes hCG and reverses luteinization. Activin-A treatment significantly up-regulated the expression of FSH and suppressed LH receptors, steroidogenic enzymes and P_4 and E_2 production in a dose-dependent manner along with down-regulated expression and activity of JNK in the cells. These suppressive effects of activin-A on steroidogenesis and JNK pathway were reversed when its receptor was antagonized with a specific blocker.

Limitations, reasons for caution: Corpus luteum is composed of many different cell types and has as early, mid and late phases. Mural luteal graulosa cells is only one of those cell types and represent early stage of the phase.

Wider implications of the findings: Our findings could be important from the perspective of corpus luteum biology in human because we have for the

first time identified a role for JNK pathway in the regulation of steroid synthesis in human luteal granulosa cells.
Trial registration number: None

P-593 Can post-hoc progesterone supplementation rescue the clinical outcomes in the patients with low serum progesterone on the day of embryo transfer?

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Study question: Can the low serum progesterone (P) on the day of embryo transfer (ET) be rescued by post-hoc supplementation in patients with frozen embryo transfer?
Summary answer: With additional P supplements, 15.8% of patients with low P on ET day displayed low P on pregnancy test (PT) day and diminished clinical outcomes.
What is known already: Progesterone plays an important role in the embryo implantation. In a natural cycle, P is secreted by corpus luteum mediating endometrial receptivity and stability. Previous reports indicated that embryo implantation and ongoing pregnancy required exogenous P influence on the endometrium, leading to lower miscarriage rate and higher implantation rate in IVF patients.
Study design, size, duration: A retrospective cohort study went from January to November in 2018 (11 months). A total of 1057 cycles from 911 patients were involved in the cohort, consisting of 469 cycles from 420 auto-IVF patients and 588 cycles from 491 oocyte recipients.
Participants/materials, setting, methods: All the patients received hormonal replacement therapy (HRT), with mean endometrium thickness of 9.79 mm, mean age at 38.58 years (24-56), and mean body mass index (BMI) of 21.95 kg/m^2. The administration of exogenous P included intramuscular injection (25 mg/vial), oral (100 mg/tablet), or vaginal supplements (90 mg). Serum P was monitored on the two endpoints during transfer cycle: P on the day of ET, and P on the day of PT.
Main results and the role of chance: Thirty-eight patients (4.2%, 38/911) had serum P $\leq$ 10 ng/ml on the day of ET, and thus their post-hoc dose of exogenous P supplements was significantly higher than that in the patients with P $>$ 10 ng/ml (805.44 $\pm$ 35.3 mg/day versus 734.82 $\pm$ 5.21 mg/day, p=0.01). Then, no difference was observed in the serum P levels on the day of PT between the above two groups (34.42 $\pm$ 5.96 ng/ml versus 41.97 $\pm$ 1.51 ng/ml; p=0.34), and the following b-HCG(+) rate, clinical pregnancy rate (CPR), implantation rate (IR), and ongoing pregnancy rate (OPR) showed comparable performances. Thirty-two patients (84.2%, 32/38) with originally low P on the day of ET had acceptable P on the day of PT (39.55 $\pm$ 6.71 ng/ml), while 6 patients remained low P on the day of PT (15.8%, 6/38); OR (95% CI): 2.74 (1.10-6.81). When analyzed by the serum P on the day of PT, 62 patients had P $\leq$ 10 ng/ml (6.8%, 62/911), and their clinical outcomes were significantly compromised than that in the group with P $>$ 10 ng/ml.
Limitations, reasons for caution: Women with abnormal uterine state, thin endometrium thickness ($<$ 6 mm), or displaced window of implantation were not analyzed in the study.
Wider implications of the findings: Administration of additional dose of post-hoc P supplements according to the minimum threshold of serum P level on the day of ET may recover the clinical outcomes.
Trial registration number: Not applicable.

P-594 Prospective randomized trial comparing corifollitropin-alfa late start (day 4) vs. corifollitropin-alfa standard start (day 2) in expected poor, normal and high-responders undergoing IVF/ICSI

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Study question: Can CF-alfa late start administration obtain similar oocyte yield (primary outcome) and clinical pregnancy rate/ embryo transfer (cPR/ET) than CF-alfa standard day 2 administration?
Summary answer: In high or normal responders to controlled ovarian stimulation (COS), CF-alfa may be administered either on day 4 or day 2 obtaining comparable IVF outcome.
What is known already: It is known that acceptable IVF results may be obtained when FSH is started on day 4-5 of GnRH-antagonist cycle, with the advantage of reducing the number of injections. The quick rise of circulating FSH levels after CF-alfa administration makes it very fit for use in late start COS regimens. A previously published retrospective analysis showed that late-start CF-alfa is as effective as late-start daily recombinant FSH in obtaining oocytes. Moreover, a pilot prospective study comparing CF-alpha day 2 vs. day 4 start in a small cohort of IVF patients reported a similar outcome with the two regimens.
Study design, size, duration: RCT included 113 women undergoing IVF/ICSI for tubal or male infertility, aged 18-43, without polycystic ovary or history of severe ovarian hyperstimulation syndrome. According to biomarkers (anti-Mullerian hormone and antral follicle count), patients were subgrouped as follows: 43 patients with expected high responsiveness to COS (AFC$>$15, AMH$>$3.5 ng/ml), 31 patients with expected poor responsiveness (Bologna criteria for poor response, AMH$<$1.1 ng/ml, AFC$<$7), 39 patients with expected normal responsiveness (AFC 7-15, AMH 1.1-3.5 ng/ml).
Participants/materials, setting, methods: In all subgroups, patients were randomized (computer-driven randomization) into 2 treatment arms: a) CF-alfa 100-150 mcg on day 4 + GnRH-antagonist 0.25/d s.c from day 8 + (eventually) recFSH 150-300 IU/d from day 11; b) CF-alfa 100-150 mcg on day 2 + GnRH-antagonist 0.25/d s.c from day 6 + (eventually) recFSH 150-300 IU/d from day 9. IVF or ICSI were performed according to standard protocols of our IVF Unit.
Main results and the role of chance: Considering all patients, CF-alfa late start regimen obtained comparable oocyte yield and cPR/ET than standard day 2 administration (8.9$\pm$5.6 vs. 8.8$\pm$6.2 and 24.1% vs. 30.5% respectively, p=n.s.). The same was observed in both high and normal responders, in which oocyte yield and cPR/ET were comparable (12.8$\pm$5.1 vs. 12.8$\pm$6.1 and 44% vs. 34.5%, respectively, in high responders, p= n.s.; 6.7$\pm$4 vs. 7.3$\pm$5.2 and 16.7% vs. 26.3%, respectively, in normal responders, p=n.s.). Differently, in poor responders oocyte yield was similar (4.2$\pm$2.9 vs. 4.3$\pm$3.3), but cPR/ET was significantly lower with late start CF-alfa administration than with standard administration (zero vs. 27.2%; p=0.03), with 40% cancellation rate due to monofollicular response in the late start arm. The analysis of ROC curves showed that the threshold AMH level implying a high probability of cycle cancellation (specificity 90%) was $\leq$0.6 ng/ml for late start regimen, $\leq$0.2 ng/ml for standard regimen.
Limitations, reasons for caution: The power analysis was performed according to the primary outcome (number of retrieved oocytes), and therefore all differences between treatment arms for the secondary outcomes should be considered as purely indicative, and verified in further adequately powered trials.
Wider implications of the findings: CF-alfa allows flexibility in COS as it may be administered either on day 2 or 4 to patients with expected high or normal response to FSH. Late start administration seems to be unadvisable for expected poor responders with AMH $\leq$0.6 ng/ml due to the high risk of cycle cancellation.
Trial registration number: NCT03816670

P-595 Controlled ovarian stimulation (COS) for breast cancer patients: the effect of starting day of letrozole

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Study question: Is there any difference in the results of controlled ovarian stimulation (COS) for breast cancer patients depending on the starting day of letrozole?

Summary answer: When letrozole was started prior to gonadotropin, the duration of gonadotropin stimulation was shorter than when letrozole and gonadotropin were started on the same day.

What is known already: For breast cancer patients, COS with letrozole has been performed to prevent the elevation of serum estradiol levels. Initially a COS protocol was suggested in which letrozole was started at the early follicular phase and follicle-stimulating hormone (FSH) was added 2 days later. Since the random-start COS protocol for fertility preservation was introduced, letrozole and gonadotropin have usually been started on the same day of the menstrual cycle. There is no consensus on when to initiate letrozole and the effect of starting day of letrozole on COS outcome is not well known.

Study design, size, duration: In this retrospective study, data from 74 breast cancer patients who underwent total of 78 COS cycles at a single tertiary center were examined. Cycles for fertility preservation (n=65) and general in vitro fertilization (IVF) cycles (n=13) were all included. Cases for fertility preservation comprised of cycles for oocyte cryopreservation (n=43) and those for embryo cryopreservation (n=35).

Participants/materials, setting, methods: Subjects were classified into two groups, 'pre-start' group (n=21) in which letrozole was initiated one or two days before gonadotropin and 'co-start' group (n=57) where letrozole and gonadotropin were started from the same day. Clinical characteristics and outcome variables of COS were compared between two groups. Subgroup analysis was carried out for the cycles started at the follicular phase.

Main results and the role of chance: Baseline characteristics were not significantly different between two groups. In the 'pre-start' group, the duration of ovarian stimulation (days) was shorter and total dosage of gonadotropin (IU) was less than the 'co-start' group (7.0 ± 1.8 vs. 8.8 ± 1.9, $p<0.001$, 1826.8 ± 1053.3 vs. 2500.7 ± 1208, $p=0.013$, respectively). The number of total oocyte retrieved and mature oocyte, oocyte maturity rate were similar between two groups. For cases with embryo cryopreservation, total number of embryo and fertilization rate were also examined and showed no significant difference between 'pre-start' cases and 'co-start' ones. Among random-start cycles which were initiated at the luteal phase, letrozole and gonadotropin were started from the same day in all cases. Accordingly, subgroup analysis was performed only for the cycles initiated at the follicular phase. In subgroup analysis, the duration of stimulation (days) was still significantly shorter in the 'pre-start' cycles (n=21) than the 'co-start' ones (n=25) (7.0 ± 1.8 vs. 8.0 ± 1.6, $p=0.009$). Total dosage of gonadotropin (IU) was less and peak estradiol (E2) level was lower in the 'pre-start' subgroup than the 'co-start' subgroup, though not statistically significant.

Limitations, reasons for caution: Limitations of this study included the retrospective design and the small sample size. Another limitation is the heterogeneity of subjects, including both fertility preservation and conventional IVF cases, which makes it difficult to analyze the results and compare the pregnancy outcome. Further prospective studies with larger, homogenous cohorts are needed.

Wider implications of the findings: When performing COS in patients with breast cancer, administering letrozole earlier than gonadotropin may result in comparable outcomes with less dosage of gonadotropin and shorter injection period, which can be regarded as a cost-effective and more patient-friendly method.

Trial registration number: No external funding was used for this study. None of the authors has any potential

P-596 PPARG splicing variant inhibits cell proliferation,migration and apoptosis in granulosa cells of PCOS patients

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Study question: What's the effects of PPARG splicing variant on granulosa cells of PCOS patients?

Summary answer: PPARG splicing variant inhibits cell proliferation, migration and apoptosis in granulosa cells.

What is known already: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with hyperandrogenism, rare ovulation or anovulation, and ovarian polycystic changes. In recent years, the role of epigenetic modification abnormalities in PCOS has gradually become a hot spot in reproductive research. Peroxisome proliferator activated receptor gamma(PPARG) belongs to the nuclear hormone receptor superfamily and is implicated in a wide range of physiological processes. PPARG plays an irreplaceable physiological function in reproduction and metabolism. Quantities of studies have suggested that epigenetic modifications of PPARG regulate ovarian hormone synthesis/secretion, follicular development, insulin resistance, and lipid metabolism etc.

Study design, size, duration: 153 women with PCOS and 153 normal women who underwent in vitro fertilization-embryo transfer therapy due to tubal factors or male factors were recruited. The expression of PPARG and its splicing variant in granulosa cells in both groups were detected by PCR and analyzed with clinical data. The overexpressed KGN cell model stably transfected with PPARG and its splicing variant were established using lentivirus. Then we detected cell proliferation, migration and apoptosis through multiple experiments.

Participants/materials, setting, methods: Expression of normal PPARG and PPARG splicing variant(SV) in human granulosa cells was determined by RT-PCR. Then the over-expression of PPARG and PPARG SV were constructed in KGN cell. Cell proliferation, migration and apoptosis were measured by western blot, immunofluorescence, and wound healing assay, respectively. Western blot analysis was used to measure the protein expression of apoptosis-related proteins.

Main results and the role of chance: The expression of PPARG splicing variant was detected in both PCOS and control groups. The sequencing results showed that exon5 was deleted. The expression of PPARG was decreased in PCOS group and the expression of its splicing variant was increased compared to the control group . Clinical data showed that there were obvious menstrual disorders, higher androgen and estrogen level in PCOS group .Besides, the clinical phenotype of PCOS patients with PPARG splicing variant was more obvious. Upregulation of PPARG splicing variant inhibits cell proliferation, migration and cell apoptosis. While granulosa cells contribute a lot to the development of follicles, our results suggest that PPARG SV may dysregulate cell proliferation and apoptosis in granulosa cells, which could partially explain mechanisms of ovulation dysfunction in PCOS.

Limitations, reasons for caution: The oocytes present in the follicles are encapsulated in granulosa cells. This study found that PPARG SV in granulosa cells inhibited cell proliferation, migration and apoptosis. However, we still don't known the direct effects of PPARG SV on the oocytes.

Wider implications of the findings: The results suggest the epigenetic role of PPARG splicing variant in the pathogenesis and throw new light on the treatment of PCOS. Our findings will allow us to take the next step in the new field of PCOS pathogenesis.

Trial registration number: /

P-597 Pharmacological Treatment vs. Combination of Electroacupuncture and Medication in Improving Menstrual Cycle, Ovarian Volume, and Antral Follicle Count in PCOS Patients: Double-Blind Randomized Clinical Trial

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Study question: Does combination of electroacupuncture and pharmacological treatment improve menstrual cycle, ovarian volume, and antral follicle count in PCOS patients?

Summary answer: Combination of electroacupuncture with medical therapy has better efficacy in decreasing menstrual cycle length, ovarian volume, and antral follicle counts in PCOS patients

What is known already: Ovulatory dysfunction is one of the pivotal properties of PCOS. Acupuncture has been shown to improve ovulatory function in PCOS patients through several mechanisms, such as the reduction of free androgen, which in turn helps normalize the menstrual cycle. Acupuncture

exerts its effect on ovulatory function through the modulation of somatic and autonomic nervous systems, which modulate endocrine and metabolic functions.

Study design, size, duration: This double blind randomized clinical trial was performed on 44 subjects from 2017-2018. Subjects're randomly assigned to treatment (true electroacupuncture+medication) and control group (sham electroacupuncture+medication) in 1:1 ratio, using web-based computer random-number generator. Based on a power of 80%, a of 0.05(two sided) and the assumption of 10% drop out,22 subjects per group was needed to observe a 1.58 difference in menstrual cycle length. Randomization was carried out by a project manager that not involved.

Participants/materials, setting, methods: PCOS patients between 18-40 years old at Yasmin clinic were recruited in a consecutive manner. Each subject underwent medical interview to assess the menstrual cycle length and ultrasound examination to measure number of antral follicles and ovarian volume. Both procedures were performed for 12 session, 3 times a week. Points of Needles inserted were ZhongjiCV3, GuanyuanCV4, QihaiCV6, TianshuST25, ST28Shuidao, ST36Zusanli, SanyinjiaoSP6, and ChengsanBL57. Needles in treatment groups got stimulated electrically at 2 Hz frequency.

Main results and the role of chance: The study showed there' significant menstrual incidence during the treatment with combination of electroacupunture, absolute risk reduction 50% (95% CI, p=0.001). The mean cycle lenght in treatment group also shorter than before (ARR 0.5, 95% CI, p = 0.003) meanwhile in control group not. The initial mean ovarian volume in the treatment group was 11.52 ± 4.316 and control group was 12.03 ± 3.075. After 12 session, mean ovarian volume in treatment group decreased, 9.61 ± 3.939 otherwise its increase in control group 13.21±3.414. This difference was statistically significant. (ARR 0.68, 95% CI, p=0.002).There's no difference mean of antral follicle count before treatment, mean of treatment group was 14.50±2.365 and the control group was 14.86±1.833. After treatment, number of antral follicles in the control group was 14.18 ± 2.239 while the treatment group was decrease 11.50 ± 3.569. Combination of electroacupunture also decrease number of antral follicles (ARR 0.68; 95% CI, p=0.005). As the result, there are significant mean differences between ovarian volume in two groups before and after treatment (p=0,002); antral follicle count (p=0,005); menstrual incidence during the treatment (p=0,001); and shortened menstrual cycle (p=0,003). Electroacupuncture combined with medical treatment could improve PCOS patients' complaint and ovarian image.

Limitations, reasons for caution: There are some confounding factors that might affect number of antral follicles, ovarian volume,and menstrual cycle length which weren't assessed,such as AMH, inhibin B, FSH, LH, androgen, free testosterone,and lipid profiles.Longer follow-up times is needed to determine persistence of shortening menstrual cycle and antral follicles for 3 forward menstrual cycles.

Wider implications of the findings: The findings of this study implicate that electroacupuncture combination therapy might serve better treatment outcomes in respect to menstrual cycle length, ovarian volume, and number of antral follicles in PCOS patients.

Trial registration number: not applicable

P-598 Does oocyte accumulation optimize clinical outcomes in poor ovarian responders? A real-life non-randomized prospective study.

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Study question: Does oocyte accumulation offer reasonable chances of pregnancy in infertile poor ovarian responders (PORs)?

Summary answer: Oocyte accumulation in PORs appears as a successful strategy for both treating infertility as well as preserving female fertility potential through embryo cryopreservation.

What is known already: In Assisted Reproductive Technology (ART), chances of live birth are highly correlated to the number of retrieved oocytes. PORs are particularly affected by this issue, since recent studies have set a threshold of 3 oocytes as a predictive factor of live birth. Moreover, repeating ART cycles in such patients represents a high risk of repeated failure, associated to an elevated drop-out rate. Hence, the management of PORs remains quite challenging, and the strategy of oocyte accumulation might constitute an interesting option to optimize clinical outcomes in these patients. However, data on this topic are still scarce.

Study design, size, duration: This single-center, prospective study has been in progress since January 2014. To date, a total of 100 infertile couples have been enrolled in an oocyte accumulation program when female partner was diagnosed as POR according to Bologna criteria (2 out of the 3 following criteria: <4 oocytes in a previous cycle, abnormal markers of ovarian reserve, age >40 years) associated to any other infertility cause (male, female or both).

Participants/materials, setting, methods: Enrollment in the program required patients' written informed consent. Metaphase-2 (M2) oocytes were accumulated by vitrification during one or more controlled ovarian hyperstimulation cycle(s). Then, all vitrified oocytes were warmed and inseminated using ICSI simultaneously with fresh M2-oocytes retrieved during a last cycle of ovarian stimulation. Thirty-nine (n=39) couples underwent the whole procedure (n=101 oocyte-vitrification cycles, followed by oocyte warming +/- simultaneous fresh ICSI cycles). Biological and clinical outcomes of these cycles were analyzed.

Main results and the role of chance: Briefly, median patients' age was 36.0 years (28-42). Their median serum AMH and FSH levels as well as antral follicle count were 0.9 ng/mL (0.2-2.8), 8.9 IU/L (3.2-15), and 8 follicles (3-14), respectively. Each couple underwent an average of 2.3 previous IVF/ICSI attempts before entering our program of oocyte accumulation. During the oocyte accumulation period, a mean of 2.3 cycles/couple (in total, 101 cycles) were performed, leading to the vitrification of 6.4 M2-oocytes/couple. A total of 244 M2 oocytes were warmed (survival rate=87.7%), and 214 M2-oocytes were micro-injected simultaneously with 127 fresh M2-oocytes (9.0 M2-oocytes injected/couple). Number of oocytes retrieved, maturation, fertilization rates and embryo quality were comparable between oocyte accumulation and fresh cycles. So far, oocyte accumulation strategy resulted in 38 embryo transfers (ET) (mean number of transferred embryos=2.5): 15 ET arose only from warmed oocytes (39.5%), 10 only from fresh oocytes (26.3%) and 13 from both warmed and fresh oocytes (34.2%). Finally, clinical pregnancy rate (CPR) of 41.0%/cycle (16/39), and cumulative CPR of 43.6/cycle (17/39) were achieved. Furthermore, supernumerary embryos have been cryopreserved for 20 couples (51.3%).

Limitations, reasons for caution: This preliminary study lacks statistical power, and further investigations are required to confirm the efficiency of oocyte accumulation in the management of infertile PORs. Then, cost-effectiveness issues will have to be considered.

Wider implications of the findings: This study highlighted acceptable CPR for poor-prognosis patients. Thus, enrollment of infertile PORs in oocyte accumulation programs might optimize their success rates. Moreover, this strategy could offer an opportunity of fertility preservation for women whose ovarian reserve is inexorably decreasing and enhance their chances of achieving a subsequent pregnancy.

Trial registration number: Not applicable

P-599 Alpha-lactalbumin enhances the intestinal absorption of myo-inositol but not glucose in healthy volunteers

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Study question: Is alpha-lactalbumin specific in increasing the intestinal absorption of myo-inositol and/or glucose?

Summary answer: Alpha-lactalbumin enhances intestinal myo-inositol uptake but does not affect glucose absorption.

What is known already: Myo-inositol is a well-known insulin-sensitizing agent, successfully used in the therapy for Polycystic Ovary Syndrome (PCOS) and other disorders. It is known that alpha-lactalbumin can improve myo-inositol absorption in the gut, while no data are as of now available for sugars. In this preliminary study, we evaluated the specificity of alpha-lactalbumin

effect in increasing the intestinal absorption of myo-inositol in the presence of glucose, which shares structural similarities with myo-inositol and, under certain conditions, can compete with it for the same membrane transporter.

Study design, size, duration: The kinetics of intestinal absorption of myo-inositol and glucose in the absence or the presence of alpha-lactalbumin were analysed in male and female young healthy volunteers by measuring their plasma levels at increasing times, 0', 30', 60', 120', 180', 240, 300', after their oral administration. Observations were performed on the same subjects at a time interval of one week: the first one in the absence of alpha lactalbumin, the second one in its presence.

Participants/materials, setting, methods: Thirteen men and 17 women (18-35 years, normal BMI), divided in two groups, received orally 6 g myo-inositol (6 men, 9 women) or 75 g glucose (7 men, 8 women) in water. After 7 days, they received the same solutions with 150 mg alpha-lactalbumin. Myo-inositol and glucose plasma levels were respectively detected chromatographically and electrochemically, at increasing times after administration. Maximum plasma concentration, time to reach it and time-course curve were calculated.

Main results and the role of chance: All the healthy volunteers completed the trial without adverse events. Analysis of myo-inositol and glucose plasma concentrations after being administered alone or with alpha-lactalbumin, allowed to obtain the respective pharmacokinetic profiles. The overall shape of the pharmacokinetic curves for both compounds did not change when administered with alpha-lactalbumin. However, when alpha-lactalbumin was administered, myo-inositol maximum plasma concentration and time-course curve significantly increased ($p< 0.0001$), whereas no changes were observed for glucose. This finding suggests that alpha-lactalbumin enhances myo-inositol but not glucose uptake.

In conclusion, alpha-lactalbumin seems to have a specificity of action on the pharmacokinetic profile of myo-inositol after oral administration and this may have significant, positive implications in the management of PCOS patients that are resistant to intestinal absorption of the insulin-sensitizing agent.

Limitations, reasons for caution: This is a preliminary study with several limitations, since we tested only glucose and only one dose of alpha-lactalbumin. We are thus properly extending our observations.

Wider implications of the findings: We believe that this field of research may represent an advancement in the clinical approach of PCOS and thus deserves to be investigated in-depth.

Trial registration number: Not applicable

P-600 Assisted reproductive outcomes in chronic viral illness-infected women: HIV, HBV, HCV

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Study question: What outcomes can be expected in women undergoing IVF/ICSI depending on the type of chronic infection (HIV, HBV, HCV)?

Summary answer: HIV infected women have poorer cumulative delivery rate and a higher risk of perinatal complications compared to women with hepatitis B.

What is known already: Although fertility remains a priority for many individuals living with chronic viral illness, this topic is often unaddressed. Concerning HIV, there has been concern that the virus, and/or its treatment, may negatively impact female fertility and IVF/ICSI outcomes but studies are conflicting and of small size. In the same manner, to date, only few studies have focused on fertility outcomes after IVF/ICSI cycles in women infected by chronic hepatitis B or C. Thus, more data about IVF/ICSI outcomes in patients with chronic viral illness are required to provide accurate reproductive counseling to patients.

Study design, size, duration: This cohort study was conducted in a tertiary care university hospital between 01/2012 and 10/2018. All women with a chronic viral illness – HIV or HBV or HCV undergoing IVF/ICSI cycles were included. Women were followed until four ART cycles were completed until delivery or until discontinuation of treatment before completion of four cycles. Co-infected women were excluded.

Participants/materials, setting, methods: A total 235 women with a chronic viral illness were allocated to three groups according to the type of infection: 101 HIV - infected women, 114 HBV - infected women and 20 HCV - infected women. The main outcome measured was the cumulative delivery rate. Main secondary outcomes were the cancellation rate, obstetrical and neonatal outcomes. Statistical analysis was conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: At baseline, HIV- and HBV- infected women did not have the same characteristics: Women infected with HIV had significantly more often a previous history of pregnancy, a tubal cause of infertility and the ovarian reserve parameters were significantly lower (AMH and AFC). There were no significant differences concerning the women age, the smoking habits and the duration of infertility. The mean number of IVF/ICSI cycles was significantly higher in HIV- compared to HBV/HCV- infected women (2.01 ± 1.05 versus 1.67 ± 0.9 and 1.5 ± 0.8 respectively; $p=0.012$). The cumulative delivery rate was significantly lower in HIV- compared to HBV- infected women (23/101 (22.8%) versus 44/114(38.6%) respectively; p:0.036). The mean number of cancelled cycles were not significantly different among groups ($p=0.323$). Concerning obstetrical outcomes, the mean birth weight was lower in HIV- compared to HBV - infected women (2799 ± 588 versus 3200 ± 562; $p=0,038$). After multivariate analysis, the type of chronic viral illness did not have a significant effect on delivery chances but women age, the AMH level and the number of IVF/ICSI cycles performed were significantly associated with delivery chances.

Limitations, reasons for caution: No solid conclusion could be made for HCV given the small number of women included in this group.

Wider implications of the findings: This study confirms that HIV- infected women have poorer ART outcomes probably due to poorer ovarian reserve parameters. Those results seem essential to better inform infectiologists and chronic viral illness-infected women of their fertility and to not postpone fertility assessment.

Trial registration number: NA

P-601 Impact of elevated progesterone on cumulative live birth rates: an analysis of 956 freeze-only cycles

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Study question: Is late follicular elevated progesterone (LFEP) in the fresh cycle hindering cumulative live birth (CLBR) rates when a freeze only strategy is applied?

Summary answer: LFEP in the fresh cycle does not affect embryo utilization rate, nor CLBR of the frozen transfers in a freeze only approach.

What is known already: Ovarian stimulation promotes the production of progesterone (P) which has been demonstrated to have a deleterious effect on IVF outcomes. While there is robust evidence that this elevation produces impaired endometrial receptivity, the impact on EQ remains still a matter of debate. Previous studies have shown that LFEP is associated with hindered CLBR, nonetheless, clinical insight on the effect of progesterone on embryo quality in terms of cumulative live birth rates should be derived from freeze-all cycles where no fresh embryo transfer is performed in the presence of progesterone elevation, and the entire cohort of embryos is cryopreserved.

Study design, size, duration: This was a matched case-control, multicentre (three centres), retrospective analysis including all GnRH antagonist ICSI cycles where a freeze-only policy of embryos on day 3/5/6 of development was applied between 2012-2018. A total of 956 patients (478 cases with elevated

P and 478 matched controls with normal P values) were included in the analysis. Each patient was included only once.
Participants/materials, setting, methods: The sample was stratified according to the following P levels on the day of ovulation triggering: ≤1.5 ng/mL and >1.50 ng/mL. The matching of the controls was performed according per age ±1 year and number of oocytes retrieved ±10%. The main outcome was CLBR defined as a live-born delivery after 24 weeks
Main results and the role of chance: The baseline characteristics of the two groups were not significantly different. The estradiol levels on the day of trigger were significantly higher in the elevated P group. There was no significant difference in terms of number of fertilization rate between the two groups. The elevated P group had significantly more cleavage stage embryos frozen compared to the normal P group; while the total number of cryopreserved blastocyst stage embryos was the same. The CLBR did not differ between the two study groups, (29.1% and 28.0%, p=0.720, respectively), also when following confounder adjustment using multivariable logistic regression analysis (accounting for age at pick up, number of fertilized oocytes, progesterone levels, total number of cryopreserved embryos and top-quality embryos).
Limitations, reasons for caution: This is a multicentre observational study based on a retrospective data analysis. Better extrapolation of the results could be validated by performing a prospective analysis.
Wider implications of the findings: This is the first study demonstrating that LFEP in the fresh cycle does not hinder CLBR of the subsequent frozen cycles in a freeze only approach.
Trial registration number: N/A

P-602 Cumulus cell-bound bone morphogenetic protein-15 varies with maternal age and PCO/S in ICSI patients

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Study question: Is the oocyte-secreted factor bone morphogenetic protein-15 (BMP15) on cumulus cells (CCs) associated with infertile pathologies, age or embryology outcomes in women undergoing ICSI?
Summary answer: BMP15/CC DNA positively correlated with the number of oocytes retrieved and negatively correlated with maternal age in patients without polycystic ovaries or polycystic ovary syndrome.
What is known already: BMP15 is an oocyte-specific factor and is a central regulator of folliculogenesis, differentiation and function of CCs, and mammalian fecundity. BMP15 is captured by cumulus cells which are discarded prior to ICSI during infertility treatment. Measuring BMP15 protein levels from CCs could provide a novel, non-invasive diagnostic tool for assessing oocyte and embryo quality and for investigating reproductive pathologies of infertile patients. Thus far reliable quantification of BMP15 protein has not been reported.
Study design, size, duration: A novel BMP15 immunoassay was developed in-house, validated and applied to protein extracts from pooled CCs of individual patients. A total of 120 patients aged 29-47 years who underwent controlled ovarian stimulation between September 2017 and April 2018 were included in the study. PCO patients (antral follicle count ≥12 in unstimulated cycle) and PCOS patients were grouped into a PCO/S group. Maternal age, endometriosis (diagnosed laparoscopically), embryology outcomes and ovarian stimulation protocols were recorded.
Participants/materials, setting, methods: Pooled CCs from individual patients were collected post oocyte denudation, hyaluronidase was removed, and cell pellets stored at -80°C. Recombinant-BMP15 showed non-parallelism with native BMP15 on CCs hence an extract of granulosa cells was used as reference preparation for BMP15 quantification and assigned an arbitrary unitage. Total BMP15 was normalised to total CC DNA (BMP15/CC DNA) in the same CC extract (measure of cell count). Interactions between parameters were examined by multivariate linear regression analyses.
Main results and the role of chance: The BMP15 immunoassay was validated for specificity and reproducibility. Total BMP15 from pooled CCs of individual patients showed a tight correlation with total CC DNA (r=0.95, p<0.0001, n=120) compared to a moderate correlation with the number of oocytes retrieved (r=0.53, p<0.0001, n=120), necessitating normalisation of BMP15 to DNA (BMP15/CC DNA). BMP15 levels on CCs varied considerably between individual patients from undetectable to 19-fold above the baseline, suggesting that factors such as underlying clinical pathologies influence oocyte production of BMP15. In patients without PCO/S, BMP15/CC DNA positively correlated with the number of oocytes retrieved (r=0.26, p<0.05, n=84) and negatively correlated with maternal age (r=-0.30, p<0.01, n=84), suggesting that BMP15 may correlate with reproductive potential in non-PCO/S patients. By contrast, BMP15/CC DNA did not correlate with age (p>0.05) or the number of oocytes retrieved (p>0.05) in the PCO/S group, suggesting aberrant production of BMP15 in those patients. BMP15/CC DNA was not significantly different between patients with and without known history of endometriosis and was not affected by the type of ovarian stimulation protocol or trigger type. BMP15/CC DNA did not correlate with embryo developmental trajectory (%MII, %2PN, % day 3 embryos, % day 5 blastocysts, % euploid embryos).
Limitations, reasons for caution: BMP15/CC DNA was measured from pooled cumulus cells from all oocytes retrieved from individual patients. Investigation of BMP15 from individual oocytes is required to assess the relationship between BMP15 and oocyte quality in patients with differing infertility conditions. Further investigation of native forms of the ligand measured is necessary.
Wider implications of the findings: This is the first study reporting the development of an assay for quantification of oocyte-secreted BMP15 protein. This study opens the opportunity to measure BMP15 in ICSI patients, as a novel non-invasive diagnostic test of oocyte quality and for providing insight into the role of oocyte-secreted factors in infertility pathologies.
Trial registration number: N/A

P-603 BMI is not associated with oocyte recovery rate or maturation rate in high-risk for severe OHSS patients triggered with GnRH-agonist for final oocyte maturation

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Study question: Is body mass index associated with oocyte recovery rate or maturation rate in high-risk for severe OHSS patients triggered with GnRH-agonist for final oocyte maturation?
Summary answer: BMI is not associated with oocyte recovery rate or maturation rate in high-risk for severe OHSS patients triggered with GnRH-agonist for final oocyte maturation.
What is known already: Previous studies using human chorionic gonadotrophin (hCG) for triggering of final oocyte maturation have shown a negative effect of high body-mass index (BMI) on IVF outcomes. Overweight women face a lower likelihood of pregnancy and an increased risk of miscarriage after IVF. They also have reduced number of oocytes retrieved despite requiring higher gonadotrophin doses. No data, however, are currently available regarding the association between body mass index (BMI) oocyte recovery rate or maturation rate following triggering of final oocyte maturation with GnRH-agonist in women at high risk for severe OHSS.
Study design, size, duration: Prospective observational cohort study of 94 patients undergoing ovarian stimulation for intracytoplasmic sperm injection (ICSI) between January 2017 until July 2018, who received 0.2mg of triptorelin to trigger final oocyte maturation. All patients were at high risk for developing severe OHSS, defined by the presence of at least 18 follicles≥11mm in diameter.
Participants/materials, setting, methods: Ovarian stimulation was performed with recombinant follicle stimulating hormone (rec-FSH) and gonadotrophin releasing hormone (GnRH) antagonists. Final oocyte maturation was triggered using 0.2 mg of the GnRH agonist triptorelin. All resulting blastocysts were cryopreserved. Hormonal evaluation included assessment of FSH, LH, oestradiol and progesterone on the day of triggering, 8 hrs, 36 hrs

as well as on days 3, 5, 7, 10 following triptorelin administration. Statistical analysis was performed using generalized linear models.

Main results and the role of chance: A negative association was present between BMI and the number of oocytes retrieved(OR: 0.66, 95% CI 0.45 - 0.98). The association between BMI and the number of mature oocytes was not significant (OR: 0.74, 95% CI 0.53 – 1.04). In addition, no association was present between BMI and oocyte recovery rate in either bivariate analysis (OR:= 0.99, 95% CI 0.98 to 1.01, p=0.991) or multivariable analysis controlling for maternal age and AMH (OR: = 1, 95% CI 0.99 to 1.01, p=0.868). Moreover, no association was present between BMI and oocyte maturation rate in bivariate analysis (OR: = 0.99, 95% CI 0.99 to 1.01, p=0.993) or multivariable analysis controlling for maternal age and AMH (OR: = 0.99, 95% CI 0.99 to 1.01, p=0.868).

Limitations, reasons for caution: The results of the present study refer to the use of a standard dose of 0.2 mg triptorelin in a limited number of patients. Whether these results are also valid for different doses of triptorelin used for triggering final oocyte maturation cannot be deducted from this study.

Wider implications of the findings: The absence of an association between BMI and oocyte recovery rate or maturation rate suggests that BMI might not exert an effect on these variables in high-risk for severe OHSS patients triggered with GnRH-agonist for final oocyte maturation.

Trial registration number: Not applicable.

P-604 Intrauterine insemination (IUI) with or without letrozole for unexplained or mild male factor infertility: a randomized pilot study

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Study question: Is a randomized controlled trial (RCT) comparing intrauterine insemination (IUI) with and without letrozole in couples with unexplained or mild male factor infertility feasible?

Summary answer: We showed that an RCT comparing IUI with letrozole versus unstimulated IUI in couples with unexplained or mild male factor infertility is feasible.

What is known already: IUI is the treatment of first choice for couples with unexplained or mild male factor infertility, but it is unclear whether ovarian stimulation improves fertility outcomes. In our recent retrospective cohort study, we found that in couples undergoing IUI ovarian stimulation with letrozole increased live birth rate as compared to unstimulated IUI without increasing multiple pregnancy rates. There are no RCTs comparing IUI with letrozole versus unstimulated IUI and therefore we plan a large RCT to test the hypothesis. Here, we present the results of our pilot study to evaluate the feasibility of a subsequent RCT.

Study design, size, duration: We performed a randomized pilot study in the Reproductive Medicine Centre of Peking University Third Hospital in China. This pilot study included 100 couples with unexplained or mild male infertility. There was no masking. The study was registered under trial number NCT03455426.

Participants/materials, setting, methods: Couples with unexplained or mild male factor infertility scheduled for IUI were randomized to IUI with or without ovarian stimulation (letrozole) for up to 3 IUI cycles within a time horizon of 4 months. Women in the letrozole group received letrozole 5 mg oral tablets daily starting from cycle day 3-5 for 5 days. Women in the unstimulated IUI group did not receive ovarian stimulation before IUI. The primary outcome was ongoing pregnancy.

Main results and the role of chance: Between March 2018 and January 2019, 158 couples were eligible to participate after initial screening, and 100 (63.3%) couples agreed to participate in this study. Of the 100 recruited couples, 50 were randomly allocated to IUI with letrozole and 50 to unstimulated IUI. The women's mean age was 31.8 years (SD3.3) and 30.3 years (SD3.3) in the letrozole group and unstimulated group, respectively. At the moment, we have follow up data of 81% of the recruited couples (41 in letrozole group and 40 in unstimulated group). Ongoing pregnancy occurred in 29.2% in the letrozole group and 20.0% in the unstimulated group (RR 1.46 (95% CI 0.67 to 3.20)). Clinical pregnancy rates were 34.1% and 22.5% (RR 1.41 (95% CI 0.68 to2.92)). There were no multiple pregnancies. There was one ectopic pregnancy and one miscarriage in the group with letrozole versus one miscarriage in the unstimulated group.

Limitations, reasons for caution: This is a pilot study with a limited sample size. Full follow up data will be available before July 2019. Based on these data, we plan a large randomised clinical trial in our centre.

Wider implications of the findings: This pilot study confirmed the feasibility of a well powered clinical trial. In couples with unexplained or mild male infertility scheduled for IUI, we hypothesise letrozole results in higher ongoing pregnancy rate without substantially increasing multiple pregnancy rate.

Trial registration number: NCT03455426

P-605 Patients < 38 years old and diminished ovarian reserve: Qualitative reduction in IVF success?

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Study question: Do infertile women aged <38 years old with quantitative evidence of diminished ovarian reserve have also diminished live birth rates or no?

Summary answer: There is no significantly difference in live birth rates between young women with AMH< 1ng/ml compared with young women controls with AMH> 1ng/ml.

What is known already: Previous studies are conflicted as to whether young women with evidence of diminished ovarian reserve are also at increased risk of poor oocyte quality.

Study design, size, duration: This was a retrospective cohort study at a single center between 2012 and 2017. We included 4150 women ongoing IVF cycle in the center in this period. For each group of women < 38 years old and ≥ 38 years old, we compared live birth rates regarding AMH level as AMH<1ng/ml versus AMH≥1ng/ml.

Participants/materials, setting, methods: We included 4211 women. The primary outcome was live birth rate. To analyze ovarian reserve, we included women with AMH level available. We defined diminished ovarian reserve as AMH< 1ng/ml. For the group < 38 years old, there were 2496 women with AMH level ≥ 1ng/ml and 250 women with AMH < 1ng/ml. For the group ≥ 38 years old, there were1263 women with AMH level ≥ 1ng/ml and 202 women with AMH < 1ng/ml.

Main results and the role of chance: Live birth rate was not significatively different in young women from a cycle with ovarian reserve parameters AMH < 1 ng/ml compared with women with good AMH values(18% versus 23.7%, p=0.1) whereas in older women ≥ 38, AMH≥ 1ng/ml is positively correlated with live birth rates (14.17% versus 5.4%, p=0.001).

Limitations, reasons for caution: Some biases are likely to be present because of the retrospective nature of the study. We also defined diminished ovarian reserve as AMH level < 1ng/ml as defined in many other studies but this definition is not consensual.

Wider implications of the findings: Our study suggests that low ovarian reserve do not impact live birth rates in young women: an oocyte retrieved from young women with diminished ovarian reserve performs similarly to that with age matched controls with normal ovarian reserve. Our observation represents valuable information for treatment counseling and cycle planning.

Trial registration number: not applicable

P-606 Is oocyte maturation rate associated with the dose of triptorelin used for triggering final oocyte maturation in high-risk for severe OHSS patients, undergoing ovarian stimulation.

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Study question: Is oocyte maturation rate associated with the dose of triptorelin used for triggering final oocyte maturation in high-risk for severe OHSS patients, undergoing ovarian stimulation?

Summary answer: Triptorelin doses of 0.1, 0.2 and 0.4mg, used for triggering final oocyte maturation, result in similar maturation rates, in high-risk for OHSS patients undergoing ICSI.

What is known already: Gonadotrophin releasing hormone (GnRH) agonist triptorelin is widely used instead of human chorionic gonadotrophin (hCG), for triggering final oocyte maturation, to eliminate the risk of severe OHSS in patients undergoing ovarian stimulation for IVF/ICSI. Several different types and doses of GnRH agonists have been used to trigger final oocyte maturation, including subcutaneous administration of triptorelin (0.2mg) buserelin (0.2-0.5-1mg), leuprolide acetate (0.5-1-1.5mg) , nafarelin 400μg and intranasal administration of buserelin 0.2mg. Although the most commonly used GnRH agonist for triggering is triptorelin, limited data are currently available regarding the optimal dose used for this purpose in high risk for OHSS patients.

Study design, size, duration: Retrospective study, performed between 2015 and 2017, in 131 infertile patients at high risk for severe OHSS (defined as the presence ≥19 follicles ≥11mm) undergoing ovarian stimulation for ICSI. The primary outcome measure was oocyte maturation rate, defined as the number of mature (metaphase II) oocytes divided by the number of cumulous oocyte complexes (COCs) retrieved. Patients received 0.1 (n=42) or 0.2 (n=46) or 0.4 mg (n=43) of triptorelin for triggering final oocyte maturation.

Participants/materials, setting, methods: Ovarian stimulation was performed with recombinant follicle stimulating hormone (FSH) and GnRH antagonists. Hormonal evaluation of FSH, luteinizing hormone (LH), estradiol (E2) and progesterone (PRG) was carried out on the day of triggering, 8 and 36 hours later as well as on days 3, 5, 7, and 10 post-triggering. During this period, all patients were assessed for symptoms and signs indicative of severe OHSS development. Results are expressed as median (interquartile range).

Main results and the role of chance: No significant differences in patient baseline characteristics were observed among the 0.1mg, the 0.2mg and the 0.4mg groups. Regarding the primary outcome measure, no differences were observed in oocyte maturation rate among the three groups compared [82.6 (17.8)% vs. 83.3 (18.8)% vs. 85.1 (17.2)%, respectively, p=0.686].

In addition, no significant differences were present among the 0.1 mg, 0.2 mg and 0.4mg groups, regarding the number of mature (metaphase-II) oocytes [21 (13) vs. 20 (6) vs. 20 (11), p=0.582], the number of oocytes retrieved [25.5 (13) vs. 24.5 (11) vs. 23 (12), p=0.452], oocyte retrieval rate [81.0 (17.7)% vs. 76.5 (23.5)% vs. 75.0 (22.5)%, p=0.088), the number of fertilized (2PN) oocytes [12.5 (9) vs. 14.5 (7) vs. 14.0 (8), p=0.985], fertilization rate [71.7 (22)% vs. 77.1 (19.1)% vs. 76.6 (23.3)%, p=0.525] and duration of luteal phase [7 (1) vs. 8 (2) vs. 7 (1) days, p=0.632] respectively.

No significant differences were present among the different triptorelin doses compared regarding serum levels of LH, FSH, E2, PRG, at any of the time points assessed following triggering of final oocyte maturation.

No patient was diagnosed with severe early OHSS in any of the three groups of triptorelin compared.

Limitations, reasons for caution: This is a retrospective study and although there were no differences in the baseline characteristics of the three groups compared, the presence of bias cannot be excluded.

Wider implications of the findings: It appears that triggering final oocyte maturation with a lower (0.1 mg) or a higher dose (0.4mg) of triptorelin, as compared to the most commonly used dose of 0.2mg, does not confer any benefit considering oocyte maturation rate in high risk for severe OHSS patients.

Trial registration number: Not required

P-607 The Effect of Controlled Ovarian Stimulation on Polycystic Ovary Syndrome-Related Chronic low-grade Inflammation

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Study question: Are polycystic ovary syndrome (PCOS) patients at increased risk for Controlled Ovarian Stimulation (COS)-related inflammation during ART cycle?

Summary answer: PCOS patients show a greater leukemoid reaction to COS, unravelling one potential co-factor in the pathogenesis of their increased risk for ovarian hyperstimulation syndrome (OHSS)

What is known already: Increasing evidence suggests that PCOS may be characterized by a chronic low-grade inflammation, independently from other comorbidities such as insulin resistance and obesity. COS is also known to induce an inflammatory response which, in severe cases, may contribute lead to the development of OHSS, a dreaded complication of IVF treatments. As PCOS is a common indication for IVF and a risk factor for the development of OHSS, we sought to investigate whether the inflammatory drive of COS is increased in PCOS patients.

Study design, size, duration: Retrospective cohort study analyzing n=2800 first-attempt IVF cycles performed on n=250 PCOS patients and n=2550 non-PCOS controls at the San Raffaele Hospital between January 2010 and February 2018.

Participants/materials, setting, methods: Venous blood samples from 2800 patients undergoing first-cycle IVF were taken twice, within 3 months before COS (baseline) and on the day of oocyte pick up, and analyzed for total blood cell count including total and differential white blood cell counts. Blood parameters were compared using Student's T test. Multivariate linear regression analyses controlling for BMI, age, and number of oocytes retrieved was also performed.

Main results and the role of chance: Baseline absolute lymphocytes (LYM) and basophil counts – but not total white blood cell (WBC) and absolute neutrophil (NEU) counts - were significantly higher in PCOS patients compared to controls ($p<0,01$). In both groups, WBC, NEU counts and neutrophil-to-lymphocyte ratio increased significantly on the day of pick up with respect to baseline values - but the increase was greater in PCOS patients ($p<0,01$).On the day of pick up, absolute counts of WBC, NEU and LYM counts - but not BAS counts - were all significantly higher in the PCOS group compared to non-PCOS patients ($p<0,01$).

Thus, even though COS lead to a leukemoid reaction both in PCOS patients and controls, both the increase and the absolute values of white blood cells on the day of pick up were greater in the study group. These resulta suggest that PCOS-patients are more susceptible to the inflammatory drive of COS than non-PCOS patients.

Limitations, reasons for caution: The retrospective nature of the study and heterogeneity of the population limits our study. Moreover, information on ovulation trigger used was available only for a small proportion of the population - preventing us from performing a stratified analysis.

Wider implications of the findings: This finding is especially interesting in face of the increased risk for OHSS seen in PCOS, which may be partly due to the enhanced inflammatory response caused by COS in these patients.

Trial registration number: n/a

P-608 Serum metabolites as molecular predictive markers of ovarian response to controlled stimulation

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Study question: Whether the serum metabolite composition may predict the ovarian response to controlled ovarian stimulation (COS)?

Summary answer: The serum levels of Glycerophospholipid and Phenylsulfate species are associated with high and poor responses to COS, respectively.

What is known already: COS is an essential step of IVF. However, the balance between the retrieval of too few or too many oocytes is difficult to strike, and is decisive to reduce the success rate or increase the risk of negative outcomes. It is nearly impossible to accurately predict ovarian response based on parameters such as maternal age, antral follicle count, antimüllerian

hormone or FSH levels, or a previous poor or excessive response to COS. The development of noninvasive techniques, capable of predicting the ovarian response would allow individualized treatment and significantly increase treatment success, and decrease patients' physical, emotional and economic burden.
Study design, size, duration: For this case-control study, serum samples from 30 patients, undergoing COS, from January to August 2017, in a university-affiliated assisted reproduction centre were analysed. Samples were split into groups depending on the response to COS: Poor-Response-Group: < 4 retrieved oocytes (PR group, n=10), Normo-Response-Group: ≥ 8 and ≤ 12 retrieved oocytes (NR group, n=10), and Hyper-Response-Group: > 25 retrieved oocytes (HR, n=10). The metabolic profiles of the serum samples were compared between the groups.
Participants/materials, setting, methods: Samples were collected before the beginning of COS. After extraction, metabolites were injected into a TOF-QII mass spectrometer, equipped with an Apollo-I-electrospray ion source and coupled to a UFLC-liquid chromatograph. Spectra were acquired in the positive mode and data for NR group were compared with the other groups. A ROC curve was built considering the ions with higher predictive power, and a cross-validation test was conducted. Metabolites were tentatively identified in online Human Metabolite databases
Main results and the role of chance: Considering the components 1 and 2, the principal component analysis (PCA) was able to clearly distinguish the PR, NR and HR groups. The ROC curve considering PR and NR groups presented an area under the curve (AUC) of 99.6% (CI 95%: 88.9 – 100%, p<0,001). As for the identified metabolites, phenylsulfate was hiperrepresented in the PR group (AUC: 100%, CI 95%: 89.4 – 100%, p=0.01) and glycerophospholipid was increased in the HR group (AUC: 86.7%, CI 95%: 60.8 – 100%, p=0.05). Glycerophospholipid was also increased in the NR group. Altogether, our evidence suggests a higher membrane stability, cell proliferation potential and cellular growth capacity among patients in the NR and HR groups when compared with those in the PR group.
Limitations, reasons for caution: Although these results may favour the implementation of individualized COS protocols and despite its high sensitivity, the model did not allow the exact identification of all metabolites due to the limited available human metabolite databases.
Wider implications of the findings: The identification of metabolites correlated with ovarian reserve and ovarian response to COS may allow migration towards the era of personalized treatment in the field of reproductive medicine. This will offer a valuable opportunity for achieving the treatment success with reduced discomfort, emotional and financial costs for the patients.
Trial registration number: None

P-609 the prevalence of cervical insufficiency in Chinese women with polycystic ovarian syndrome after ART treatment: a retrospective analysis

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Study question: the relationship between cervical insufficiency(CI)and polycystic ovarian syndrome(PCOS) in Chinese women with the second trimester miscarriage after ART.
Summary answer: A high prevalence of CI in PCOS women may originate from insufficient uterine development and may be related to higher serum T levels.
What is known already: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting 6–10% of women of reproductive age. PCOS is associated with menstrual irregularities, hyperandrogenism, insulin resistance and subfertility. Adverse pregnancy outcomes, including early pregnancy loss and preterm birth, are also related to PCOS.Cervical insufficiency (CI) is a major cause of miscarriage and premature birth that affects 0.1%–1.0% of all pregnancies and accounts for approximately 8%-25% of recurrent spontaneous abortions during the second trimester.Few studies have investigated the risk of CI among PCOS women, just one study reported that CI occurred with a higher frequency in PCOS women.
Study design, size, duration: The present retrospective study included all the women who underwent ART treatment who experienced second trimester miscarriage at the Reproductive Medicine Center at The Six Affiliated Hospital of Sun Yat-sen University between May 1st, 2010, and December 31st, 2017.Patient characteristics including age, body mass index, basal hormone level, infertility duration, parity, uterine diameter and any history of spontaneous abortion were obtained by detailed review of the pre-pregnancy, antenatal and delivery records.
Participants/materials, setting, methods: PCOS was defined by Rotterdam criteria. Women were diagnosed as CI based on cervical dilation and/or cervical shortening in the absence of other clear causes without contractions or labor. The prevalence of CI and the clinical characteristics of these women were analyzed. Chi-square, Fisher's exact test, Student's t-test, point biserial correlation analysis and multivariable logistic regression were used for data analysis. A P<0.05 indicated significant result.
Main results and the role of chance: A total of 490 women with a second trimester miscarriage were included, among which 67 women were diagnosed with CI (13.67%). 26 PCOS women were diagnosed with CI (30.23%) compared with 41 of non-PCOS women (10.15%), P<0.001. PCOS women had a significantly smaller uterus and higher serum T level compared with non-PCOS women. Point biserial correlation analysis showed that negative correlation existed between CI pregnancy and the average uterine length(r=-0.242, P <0.001).The ROC curve analyses of the average uterine length showed the area under curve was 0.710, P <0.001,in which the optimal cut-off point was 4cm. The CI frequency of women with a mean uterine length shorter than 4.0 cm was 25% (17/68),while the rate of women with a mean uterine length longer than 4.0 cm was 11%(48/422),P=0.02.After adjusting for maternal age, body mass index (BMI), infertility duration, number of fetuses and basal hormone levels, PCOS status (OR: 3.93, 95%CI 1.54-10.02), uterine size(OR: 4.323, 95%CI 1.699-10.002) and serum testosterone levels (OR: 1.02, 95%CI 1.01-1.03) were associated with an increased risk of CI.
Limitations, reasons for caution: The cohort was limited to Chinese women who underwent ART treatment due to infertility, and miscarriage is defined as fetal delivery earlier than 28 weeks of gestational age in China which is different from other western countries. The relatively number of PCOS women included in the miscarriage group is limited.
Wider implications of the findings: Therapeutic measures should be taken to reduce the influence of hyperandrogenism in PCOS patients. Pre-pregnancy physical examinations and regular prenatal examinations are recommended for PCOS women. Regularly monitoring cervical length during pregnancy may benefit PCOS patients and lead to the early diagnosis and treatment of CI to prevent fetal loss.
Trial registration number: 2018ZSLYEC-043

P-610 Short term effects of metformin, myo-inositol or combination on metabolic and endocrine profile of infertile women with polycystic ovarian syndrome (PCOS)

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Study question: What is the effect of of different insulin sanitizers (metformin, myo-inositol or combination) on metabolic and endocrine profile in infertile women with PCOS?
Summary answer: Metformin, **m**yo-inositol and combination of both improve the endocrine and metabolic profile in infertile PCOS. Myo-inositol is significantly better in lowering high AMH levels.
What is known already: Use of insulin sensitisers including metformin and myo-inositol lead to an improvement in insulin sensitivity, restoration of ovulation, improvement in oocyte quality and a reduction in hyperandrogenism through the reduction of insulin plasma levels, which may help to ameliorate clinical symptoms and increase their fertility. Both improve outcome when used alone. There is little knowledge on their metabolic and endocrine benefits when used in combinations.

Study design, size, duration: Data from two randomised trials were analysed to compare the effects of metformin in combination with myo-inositol or each drug alone on some of the metabolic [BMI, insulin levels, HOMA-IR (Homeostatic Mode Assessment of Insulin Resistance) and lipid profile] and endocrine (LH, FSH, testosterone, SHBG and AMH) profile in infertile PCOS women undergoing IUI (combinations versus metformin) or IVF (myo-inositol versus metformin) cycle. Both trial were conducted over 2 years (December 2016-December 2018)

Participants/materials, setting, methods: One hundred and seventy infertile PCOS women (Rotterdam's criteria) were randomised to Group 1 (n=60) who received combination of metformin (1500mg) + myo-inositol (1800mg); Group 2 (n=60) received metformin (1500mg) and group 3 (n=50) received myo-inositol 4g/day. Insulin sensitisers were given for 3 months and groups were compared in terms of improvement in metabolic and endocrine profiles with respect to baseline within and between different groups.

Main results and the role of chance: The baseline parameters including BMI, FG score, global acne score, antral follicle count, ovarian volume were comparable among the three groups. The number of women with abnormal BMI (> 25 kg/m2) decreased to 73% from 93% (p=0.001), 78% from 87% (p=0.05) and 58% from 88% (p=0.002) in group 1, 2 and 3 respectively. The percentage of women having HOMA-IR >2.4 at 3 months was significantly higher in group 3 (28%) compared to group 2 (11.7%) and group 1 (8.3%) with p=0.017.The change in total, LDL and HDL cholesterol at 3 months from baseline was not statistically significant among the three groups. The percentage change in LH was significantly higher in group 1 (31.1%) than group 2 (27.2%) and group 3 (11.2%) with p< 0.001. Serum AMH levels were significantly reduced after 3 months of therapy in all the groups but the reduction was significantly higher in group 3. The percentage change was significantly higher in group 3 (44%) versus group 1 (33.4%) with p=0.033 whereas group 1 (33.4%) versus group 2 (32.8%), showed no significant difference (p=0.0779).

Limitations, reasons for caution: The participants were taken from two RCT with similar characteristics and primary outcomes but different interventions. This study analysed the metabolic and endocrine improvements with insulin sensitisers in PCOS. Further the doses of myo-inositol varied between two groups (Group I and III).

Wider implications of the findings: Both insulin sensitisers (metformin and myo-inositol) are effective in improving the metabolic profile in PCOS women. Myo-inositol has better potential in reducing AMH level, perhaps due to its local action at ovary and may be more beneficial in infertile PCOS with high AMH levels.

Trial registration number: CTRI/2018/05/014196
CTRI/2017/07/009021

P-611 Assessment of ovarian reserve using the SonoAVC antral tool

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Study question: To assess the reliability and potential advantages of automatic antral follicle count (AFC) using the SonoAVC*antral* tool compared to manual count.

Summary answer: Automatic AFC using the new SonoAVC*antral* tool is reliable and reproducible compared to manual count.

What is known already: AFC is a rutine parameter for ovarian reserve assessment. Three-dimensional(3D) US has added precision to follicular monitoring with the introduction of the SonoAVC follicle software (GE Healthcare, Austria). This software allows quick and precise measurement of follicular volumes and these correlate with oocyte maturity. More recently a new software has been designed that is specific for evaluation of small antral follicles. Studies are nessesary to assess the reliability of this method in clinical practice. FORT (follicular output rate) is the ratio of preovulatory follicle count on the day of trigger×100/baseline AFC and is an indicator of follicular response to gonadotropin.

Study design, size, duration: Prospective study of 43 women undergoing assessment of ovarian reserve prior to an IVF cycle (36 egg donors and 7 infertility patients with good ovarian reserve). 35 women underwent ovarian stimulation during the study period and an antagonist protocol was used in all cases. Data were collected from May to October 2018 in a single private infertility clinic. All exams were performed by one of 4 operators and then analyzed by a single operator (AR).

Participants/materials, setting, methods: A Voluson S10 ultrasound device (GE Healthcare, Austria) with a RIC 5-9 vaginal probe and the SonoAVC*antral* tool was used for all assessments. Manual(2D) and automatic(3D) ovarian volume and AFC and the time needed to perform each measurement were recorded. On the day of trigger the number of follicles 16-22 mm was recorded to assess FORT. FORT was also calculated using number of follicles >0,7cc x100/automatic AFC. The interobserver variability of automated measurements was calculated.

Main results and the role of chance: Manual and automatic AFC were 38,11 and 34,74 respectively (P>0,05). Ovarian volume measured using two-dimensional(2D) and 3D US was: Right Ovary 5,28 vs 4,77 (P>0.05); Left Ovary 5,53 vs 4,65 (P=0,036). The time needed for manual(2D) AFC and ovarian volume assessment was 4,02 minutes. The total time needed for measurement of ovarian volume and AFC using 3D US and the sonoAVC*antral* tool was 9,91 minutes (P=0,00). This includes the time spent by the operator to perform the exam (volume adquisition) (1,91 minutes) and the time necessary for automatic volume calculation and post-processing (8 minutes). Improvements in the software and increasing experience of the operators will reduce the time needed for post-processing. The interobserver variability for automatic assessment was not statistically significant. The number of follicles between 16 and 22 mm on the day of trigger was 8,77 manual and 10,43 automatic (P=0,025). The calculated FORT was not significantly different using manual diameters and AFC (27,34) or automatic diameters by SonoAVC*follicle* and AFC by SonoAVC*antral* tool (29,4). When FORT was calculated using number of follicles >0,7cc x100/baseline automatic AFC (59,02) the result was statistically significantly greater that "conventional" FORT.

Limitations, reasons for caution: These results are limited by the small number of subjects included and we are collecting more data to confirm these findings. Patients with poor quality of image limit the application of automatic measurements. Improvements of the automatic softwares are needed to increase accuracy of automatic measurements.

Wider implications of the findings: AFC is widely used in fertility clinics to assess ovarian reserve and the availability of an automatic tool is promising. The SonoAVC*antral* tool may be useful to achieve the final goal of determining what is the ideal follicular size to be included in the AFC.

Trial registration number: Not applicable.

P-612 No evidence of different miRNomic profiles in the follicular fluids retrieved after stimulation conducted in follicular and luteal phase of the same ovarian cycle.

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Study question: Is there any difference in the miRNomic profile of follicular fluids retrieved after follicular-phase-stimulation (FPS-FFs) and luteal-phase-stimulation (LPS-FFs) conducted in the same ovarian cycle?

Summary answer: Pooled FPS-FFs and paired-LPS-FFs from 15 poor-prognosis women resulted in similar miRNomic profiles.

What is known already: Multiple waves of follicular growth can arise during an ovarian cycle, an evidence that questioned the classic "single recruitment" theory of folliculogenesis. The "wave theory" paved the way for the implementation in IVF of stimulation protocols conducted in the luteal-phase of the ovarian cycle, theoretically anovulatory. Nevertheless, LPS-derived cohorts of oocytes were reported larger and competent as paired FPS-derived ones. DuoStim (FPS+LPS in the same ovarian cycle) was therefore suggested to treat poor-prognosis women. Still, more data are needed to investigate putative

differences in the follicular environment. MiRNomic signatures of FFs after FPS+paired-LPS represent a promising tool to this end.

Study design, size, duration: Exploratory study. Fifteen poor-prognosis women (AMH≤1.5ng/mL and/or AFC≤6 and/or ≤5 oocytes retrieved from a previous cycle and/or ≥35yr) completed FPS and paired-LPS with recombinant-gonadotrophins in an antagonist protocol between September-2016 and April-2017. Three pools of FPS-FFs (N=15) and three pools from paired-LPS-FFs (N=15) were collected. Mean maternal age and number of follicles were balanced between the pools. The miRNomic profiles of FPS-FFs and paired-LPS-FFs were compared to define putative differences.

Participants/materials, setting, methods: RNA-isolation was performed with the Plasma/Serum RNA Purification Kit (Norgen-Biotek). MicroRNAs were analysed with the Bioanalyzer2100 using the Agilent Small RNA Chip. 50ng of total-RNA were pooled and processed with the TrueSeq Small-RNA Library Prep Kit (Illumina) for library indexing. The Illumina HiSeq2500 was used for cluster generation and sequencing in a 1x36 single-end format. After quality-control, differential expression was reported as Fold-Change with adjusted *p*-values. Gene-ontology analysis was performed with IPA software.

Main results and the role of chance: Intra- and inter-variability among and between FPS-FFs pools and paired-LPS-FFs were limited in terms of maternal age and number of cumulus-oocyte-complexes collected (pool-A: 39.2±3.0yr, FPS 5.4±3.6 COCs and LPS 5.8±3.2; pool-B: 39.6±2.5yr, FPS 5.2±2.1 COCs, LPS 5.8±4.3 COCs; pool-C: 39.6±2.3yr, FPS 5.0±2.3, LPS 6.0±5.1). The transcripts identified clustered in the following classes: Small-Nucleolar RNAs, RNA5S ribosomal pseudogenes, mitochondrial genes and miRNAs. A comparative analysis revealed no statistically-significant difference between FPS-FFs and paired-LPS-FFs (paired t-tests conducted). When focusing on miRNAs, 57 were identified in both FPS-FFs and LPS-FFs (filtering parameters: detected in ≥2/3 FPS-FFs and LPS-FFs pools with ≥10 reads). No miRNA was found either only in FPS-FFs or only in LPS-FFs. The Pearson's correlation among and between FPS-FFs and paired-LPS-FFs was always ≥0.9, thereby testifying the consistency in the design of the pools and in the miRNomic profiles. Some of the 57 miRNAs identified contribute to molecular pathways involved in cell proliferation, regulation of progesterone concentration, follicular and post-fertilization development. Also key-miRNAs previously outlined as biomarkers of conditions such as polycystic-ovarian-syndrome (e.g. miR-182,-30a,-193b,-222,-483) or IVF outcomes (e.g. miR-10b,-16-1,-320a) did not show differences.

Limitations, reasons for caution: More studies are required to confirm the safety of LPS. This study suggests a similar miRNomic profile in FFs after FPS and paired-LPS. Although molecular investigations, possibly proteomic, should be performed on a wider population to draw clear conclusions.

Wider implications of the findings: LPS in a DuoStim-approach is promising for poor-prognosis or oncological patients, as it allows to collect large cohorts of competent oocytes in a short timeframe. Comprehensive assessments of DuoStim safety, like the one adopted in this study, are crucial among the requirements for a widespread implementation of this strategy.

Trial registration number: None.

P-613 BMI MIGHT BE A RISK FACTOR FOR LOW PROGNOSIS IN PATIENTS WITH PCOS UNDERGOING ART

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Study question: To find the risk factor for low prognosis in patients with PCOS undergoing ART.

Summary answer: BMI might be the possible risk factor for POR in patients with PCOS.

What is known already: Polycystic ovary syndrome (PCOS) affects 5–10% of women in reproductive age and is characterized by chronic anovulation and hyperandrogenism. IVF-ET is the main treatment for ovulation induction therapy failure of PCOS patient, but there is still a few part of patients present as a poor ovarian response (POR), which always leads to a "low prognosis". What is the risk factor of these patients for the POR remains unclear. This retrospective study is going to find the possible risk factors for POR of women with PCOS.

Study design, size, duration: 334 patients with PCOS who underwent assisted reproductive technology (ART) in Peking University People's Hospital Reproductive Center from Jan 1st , 2016 to Dec 31st , 2017 were enrolled into this study.

Participants/materials, setting, methods: The number of oocytes were calculated, and the pregnancy rate and live birth rate related to the oocytes number were also estimated. Patients with POR were grouped according to the POSEIDON stratification. Multivariate logistic regression analysis were used to value the risk factors for the POR of patients with PCOS.

Main results and the role of chance: The mean number of oocytes in the enrolled 334 patients was 16.28. Forty-two patients were diagnose as POR with the mean oocytes number of 2.8. The pregnancy rate in patients with POR was similar with other patients (56.5% vs. 41.3%, P=0.194), but the live birth rate was significantly decreased (13.0% vs. 21.3%, P=0.047). The duration of infertility (P=0.022), BMI (P=0.010) and duration of GnRH use (P=0.027) was significantly higher in patients with POR than that in other patients. After the multivariate logistic regression analysis, BMI remained to be the risk factor for the POR of patients with PCOS (RR=1.101, 95%CI: 1.002-1.209, P=0.045).

Limitations, reasons for caution: The scale of the study is still small, and more larger, multicenter researches are needed to confirms this conclusion.

Wider implications of the findings: The results of this study show that obesity (BMI>24) is indeed the main risk factor for POR in patients with PCOS, which is consistent with the results reported in foreign literature.This study firstly revealed a direct link between MIB and POR or low prognostic factors in patients with PCOS.

Trial registration number: Not applicable.

P-614 Can GnRH agonist-trigger reduce multiple pregnancy risk without affecting live birth in clomiphene and letrozole resistant PCOS women undergoing letrozole-gonadotropin stimulation?

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Study question: Can GnRH agonist (GnRHa) trigger reduce multiple pregnancy risk and associated complications in clomiphene citrate (CC) and letrozole resistant PCOS women undergoing letrozole-gonadotropin stimulation?

Summary answer: GnRHa trigger lowers multiple pregnancy rate and associated complications without affecting live birth in CC/Letrozole resistant PCOS women when compared with hCG trigger

What is known already: When CC resistant women are treated with letrozole, many turn out to be resistant to letrozole also. The next option for these cases remains gonadotropins which are expensive, associated with increased risk of ovarian hyperstimulation (OHSS) and multiple pregnancies. Letrozole-gonadotropin co-treatment was reported to be more cost-effective in CC resistant PCOS women than CC-gonadotropin or gonadotropin alone. GnRHa trigger has been successfully used for follicular maturation in both IUI and IVF cycles

Study design, size, duration: This non-randamised prospective study was conducted at the Institute of Reproductive Medicine, Kolkata, India between March 2016 and September 2018. The study was approved by the Institute Ethics Committee. 591 PCOS women (1374 cycles) resistant to CC (150mg) and letrozole (7.5mg) were recruited for the study. On the day of trigger, 273 women (673 cycles) were given GnRHa trigger, whereas 318 women (701 cycles) were given hCG trigger following ovulation induction .

Participants/materials, setting, methods: Ovulation was induced with 5mg letrozole (day 3-7) and uFSH (75-150 IU) stimulation (day 7 onward) until follicular size reached ≥14mm. Ovulation was triggered when leading follicle reached ≥18mm and endometrial thickness >7mm. Following confirmed ovulation, patients were advised for timed intercourse. All the patients received luteal support. Number of follicles, endometrial thickness, terminal E_2, clinical pregnancy rate, multiple pregnancy, miscarriage rate, obstetric complications and live birth were compared between GnRHa and hCG trigger groups.

Main results and the role of chance: 152 cycles were cancelled due to poor ovarian respone (4.12%), hyper response (when >4 follicles reached ≥14

mm, 2.68%), premature LH surge (0.52%) and poor endometrial thickness on day of trigger (2.03%). Mean age, BMI, duration of infertility, baseline E2, AMH and AFC were comparable in both the GnRHa and hCG groups. Dose of gonadotropin, days of ovulation trigger, endometrial thickness on the day of trigger, number of follicles ≥ 14mm and less than <14mm were also observed to be comparable between the two groups. A non-significant, but higher clinical pregnancy rate was observed in hCG trigger group (19.83%) when compared with GnRHa trigger group (17.26%), whereas significantly higher miscarriage rate ($p<0.05$) was observed in hCG group (19.83%) compared to that of GnRHa (10.63%). Multiple pregnancy were significantly low ($p<0.05$) in GnRHa group (one triplet and four twins, 5.88 %) than in hCG group (One quadruplet, three triplet and 11 twins ,15.46 %). Live birth was found to be comparable between the GnRHa (12.33%) and hCG group (12.69%). One patient in GnRHa group and three in hCG group had mild OHSS. Fewer obstetric complications were also observed in women of GnRHa trigger group when compared to hCG trigger group.

Limitations, reasons for caution: A limitation of this study is non-randomized design. Due to dearth of any similar reported study, the present observations were important to precede any multicentric, randomized studies with larger sample to check feasibility of using letrozole-gonadotropin co-treatment with GnRHa trigger to reduce multiple pregnancy risk without compromising live birth rate.

Wider implications of the findings: To reduce multiple pregnancies and its complications associated with gonadotropin stimulated cycle, expensive option like IVF with elective single embryo transfer is offered. GnRH agonist trigger instead of hCG in letrozole-gonadotropin cycle can reduce multiple pregnancy and provides cost effective and safe treatment to these CC/letrozole resistant PCOS patients

Trial registration number: Not applicable

P-615 Pilot observational study evaluating the effect of L-Leucine pretreatment on ovarian response and embryo quality in Poor Ovarian Response (POR) patients undergoing controlled ovarian stimulation

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Study question: Is there any change in the ovarian response and embryo quality after L-Leucine (800 mg MTorr capsule) therapy in POR patients undergoing Controlled Ovarian Stimulation for IVF/ICSI ?

Summary answer: 90 days L-Leucine therapy showed an improvement in mean AMH, AFC, more retrieved oocytes, higher peak estradiol levels and more high-quality embryos in poor responders.

What is known already: Leucine is an essential amino acid and a nutrient signal that activates complex 1 of the mammalian target of rapamycin (mTORC1). mTOR signalling integrates extracellular and intracellular signals to regulate protein translation, cell metabolism, growth, proliferation, and survival. Thus, mTOR is activated during cellular processes that use energy and it also regulates primordial follicle activation.

The association of the mTOR pathway in folliculogenesis and the involvement of leucine in the regulation of mTOR pathway has been studied in animal models. However, no human study has investigated whether pre-treatment with L-leucine could improve the ovarian response and embryo quality in poor responders.

Study design, size, duration: Pilot Observational longitudinal cohort study of 30 POR patients pretreated with L-leucine for 90 days before undergoing Controlled Ovarian Stimulation(COS) for IVF/ICSI in a tertiary care Reproductive Medicine Institute. The total study duration was 120 days. 30 POR patients with one previous failed IVF cycle were supplemented with Mtorr 800 capsules containing 800mg of L-leucine once a day after meals for 90 days before undergoing COS. Treatment non-compliant patients were excluded from the study.

Participants/materials, setting, methods: Pilot Observational cohort study of 30 POR patients after 1 failed ART(Assisted Reproductive Technology) cycle undergoing COS for IVF/ICSI in a tertiary care Fertility Centre and Reproductive Medicine Institute. These patients were supplemented with Mtorr 800 capsules containing 800mg of L-leucine once a day after meals for 90 days before undergoing the second cycle of COS. The baseline characteristics, changes in AFC,AMH,Peak E2 levels,Oocyte and Embryo details were collected and analysed using MS EXCEL and SPSS software.

Main results and the role of chance: The mean AFC(antral follicle count) before treatment was 6.9 and the mean AFC after 90 days of treatment with Mtorr 800 capsules was 8.2 (p value 0.01).

The mean AMH(anti Mullerian hormone) before treatment was 1.3ng/ml and mean AMH after treatment was 1.8ng/ml which showed a rising trend.

The Peak Estradiol(E2) levels on the day of trigger before treatment were 1308pg/ml and Peak Estradiol levels after treatment were 1473.4pg/ml. An increasing trend in mean peak E2 levels was observed after 90 days of treatment with L-leucine.

The average oocyte number before treatment was 4.53 and after treatment was 5.53(p value 0.06).Total Metaphase 2(M2) oocytes retrieved were observed to increase by an average of 1 oocyte after 90 days of treatment with L-leucine, which could reduce cycle cancellation.

The average number of day3 cleavage Grade A Embryos before treatment were 1.4 and Grade A Embryos after treatment were 2.23 (p value 0.01).Increase in total grade A embryos improves likelihood of an embryo transfer in a poor responder.

An increasing trend in the mean AMH,AFC,Peak Estradiol levels, oocytes retrieved and number of Grade A embryos was observed.For a poor responder, one extra oocyte or embryo could increase the chance of pregnancy and possibly avoid donor egg program.

Limitations, reasons for caution: The limitation of our study was the small sample size as it was an observational pilot study. A randomised controlled trial would better assess the effect of L-leucine on ovarian response and live birth rate.

Wider implications of the findings: Mtorr 800 capsules containing 800mg of L-leucine could offer hope to POR patients where improvement in ovarian response and embryo quality could offer better chances of pregnancy.After 90 days of therapy, an improvement in mean AMH,AFC, more retrieved oocytes and high-quality embryos in poor responders could avoid donor egg program.

Trial registration number: Institutional Ethics Committee Project Number 042/P/19/01

P-616 A randomised controlled trial assessing the effect of micronised progesterone versus medroxyprogesterone acetate on the coagulation cascade of women with premature ovarian insufficiency

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Study question: Do progestogen preparations impact the thrombotic profile of women with premature ovarian insufficiency (POI) through markers of haemostatic activation when controlled for route of administration?

Summary answer: Fluctuations in traditional haemostatic biomarkers is not reproduced by thrombin generation changes, a marker of global coagulation, demonstrating no increased thrombotic risk with either progestogen.

What is known already: Natural menopause is associated with a pro-thrombotic state secondary to coagulation pathway changes. Limited evidence exists on venous thromboembolic (VTE) disease risk in POI. Hormone replacement therapy (HRT) has been implicated in the development of VTE disease, with oestrogen route of administration and progestogen preparation being independent causative factors.

The thrombin generation curve measures four main parameters, lag time (LT), time to peak (ttP), peak height and endogenous thrombin potential (ETP), differing from classical clotting assays in its ability to globally reflect all three phases of coagulation in contrast to the initiation phase only, better characterising the underlying prothrombotic states.

Study design, size, duration: Prospective open-label randomised trial conducted over 2 years. Subjects were randomised to one of two treatment arms.

Venous blood was collected for thrombin generation and coagulation profile. Samples were taken at baseline and 3-months.

Seventy-one subjects were consented. 44 (21 randomised to Utrogestan; 23 to MPA) underwent sampling at baseline and 3-months for thrombin generation. 61 underwent a baseline coagulation screen and 43 (20 allocated to Utrogestan; 23 to MPA) had sampling repeated at 3-months.

Participants/materials, setting, methods: POI subjects were recruited from two tertiary Reproductive Clinics. Both groups received 50mcg/day transdermal oestradiol (Evorel® Patches) in conjunction with either cyclical oral Utrogestan® (200mg for 12 days) or medroxyprogesterone acetate (MPA) (10mg for 11 days).

Independent sample t-test was used to assess the percentage difference between the groups. Paired t-test was used for continuous variables. ANOVA and MANOVA were performed to determine the impact of dependent and independent variables (age/BMI): $p<0.05$ considered statistically significant.

Main results and the role of chance: The baseline characteristics of the two study populations were not statistically different. The data has been presented as mean±standard deviation (SD).

The coagulation profile included an assessment of the following parameters: Protein C, Protein S, Antithrombin III, Lupus anticoagulants (DRVVT), Clauss fibrinogen, D-dimer, APC Resistance, Factor VIII, international normalised ratio (INR) and Activated Partial Thromboplasti Time Ratio (APTR).

Utrogestan was noted to result in a reduction in Protein S (-9.24±15.53%; 95% confidence interval [CI] -17.22 to -1.25; p=0.03) and Antithrombin III (-8.78±11.25%; 95% CI -14.37 to -3.18; $p<0.001$) levels at 3-months from baseline. Changes in Protein C levels were not found to be significant.

MPA use was associated with a reduction in Protein C levels at 3-months from baseline: -11.64±20.47%; 95% CI -20.71 to -2.56; p=0.01. Non-significant changes occurred in Protein S and Antithrombin III levels.

Thrombin generation analysis demonstrated no statistical change in the measured parameters, 3-months from baseline for both groups.

Limitations, reasons for caution: The study did not achieve statistical power with wide confidence intervals for the demonstrated differences, therefore, clinical relevance of these small differences requires further evaluation in larger studies.

Wider implications of the findings: The absence of statistically significant changes in the global coagulation assay confers no additional adverse effect with either progestogen preparation when combined with transdermal oestrogen in the management of women with POI. This finding helps create an evidence-based choice for women without increasing their overall thrombotic risk.

Trial registration number: Ethical approval and patient consent were obtained.

REC Number: 12/LO/1957.

EudraCT Number: 2012-004511-30.

P-617 Comparative results of IUI performed 24 hours and 40 hours after HCG-Trigger

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Study question: Is an IUI at 24 hours post HCG Trigger as effective as an IUI after 40 hours ?

Summary answer: Results of 24 hours IUI post HCG Trigger are comparable to 40 hours IUI without compromising efficacy and convenience.

What is known already: When natural intercourse occurs, before ovulation, it yields a good probability of pregnancy in fertile couples.

Therefore an IUI performed in Pre-Ovulatory period is as effective as post ovulatory IUI.

Study design, size, duration: 326 infertile women, 25-40 years of age (2016-2018) , were randomized equally to a single IUI, either 24 hours or 40 hours after receiving hCG trigger .

All patients had evidence of tubal patency .

Participants/materials, setting, methods: 326 infertile women, 25-40 years of age, were randomized equally to a single IUI, either 24 hours or 40 hours after receiving hCG trigger. Study duration: 2016-2018.

All patients had evidence of tubal patency, with a Day- 2 FSH < 12 mIU /ml.

1 to 4 IUI cycles were performed per couple.

Primary outcome measured was the clinical pregnancy rate.

Main results and the role of chance: The 24 hour group pregnancy rates were similar to the 40 hours group.

The clinical pregnancy rate was 11% in the 24 hour IUI group (n-163) and 13% in 40 hours IUI group (n-163)

Limitations, reasons for caution: Timing of IUI is most frequently performed with human chorionic gonadotropin (hCG) injection. Limitations of timing by ultrasound and hCG administration are frequent hospital visits and the occurrence of premature LH surges or the possibility of triggering ovulation in the presence of an immature follicle.

Wider implications of the findings: IUI performed 24 hours after administration of hCG yields similar pregnancy rates compared to an IUI performed 40 hours after hCG.

The comparative clinical pregnancy rates seen in both timings of IUI suggests that efficacy and similar results can be obtained along with convenience to patient and treating physician .

Trial registration number: not applicable

P-618 Machine Learning approaches to Predicting Personalized Response to Controlled Ovarian Hyperstimulation (COH) During In Vitro Fertilization (IVF).

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Study question: Statistical methods in machine learning (ML) provide exciting tools for developing patient specific prediction systems. Can a supervised learning framework predict ovarian response to Controlled Ovarian Hyperstimulation (COH)?

Summary answer: Our study demonstrates that an ensemble framework for ML models provides an efficient framework for predicting follicular development in women undergoing COH during IVF.

What is known already: IVF is a widely utilized method in the treatment of sub-fertile couples. Central to the IVF cycle is COH of the female, thereby stimulating the development of numerous follicles, yielding multiple oocytes at the time of retrieval. The stimulation process involves a concerted program of interleaving hormone and drug administration. While these protocols have been developed over many decades, unexplained divergent responses are frequently observed in women undergoing identical stimulation regimes.

Study design, size, duration: The ovarian response to controlled hyperstimulation in patients seeking IVF treatment was analyzed retrospectively for 36 months (02/2015-02/2018). A total of 6072 patient data were initially examined. The cohort was reduced to 2746 records after eliminating any incomplete sets. 80% of data records were randomly used to train the network and 20% were used for the validation set.

Participants/materials, setting, methods: An extensive medical record library from one large IVF clinic was used to create a training (80%) and prediction (20%) database. The training database contained baseline data from all IVF patients including age, FSH, estradiol, progesterone and other anthropometric data.

Main results and the role of chance: The ML approaches have been successfully applied for several biological signaling models in the recent years. However, a successful IVF cycle presents a complex challenge which involves a multitude of latent modifiers including genetic, anthropometric and age-related components. Accordingly, we set up several ML schemas to examine the predictive accuracy for the ovarian stimulation. In order to limit predictability to

a female factor, we chose endometrial lining thickness, mature follicle number and oocyte number as the outcomes. The mean age of the cohort was 36.3 years (range 22-46), had an average BMI of 26.8 and 2.2% were self-reported smokers. The patient characteristics (E2, P4, LH) had a minimum root-mean-square error (RMSE) of 0.4, 0.3 and 0.4, respectively) and were found to be log normal except for the endometrial lining, which was normally distributed. Of the several machine learning algorithms (random forest, SVM, Neural Networks), the ensemble approaches were found to be most accurate in predicting ovarian response outcomes. The ensemble model used to generate correlations plots displayed excellent concordance with predicted trajectories of E2, LH, P4, and Follicular size >14mm (log normal RMSE of 0.4, 0.7, 0.4 and 0.4 respectively).

Limitations, reasons for caution: Machine learning approaches, while extremely powerful in modeling latent modifiers, suffer from an intrinsic bias towards the quality of training set and its correspondence with the test set. ML based applications must be validated for sensitivity analysis of input parameters for the treatment seeking population at large.

Wider implications of the findings: The model presented here uses the laboratory tests routinely performed in IVF clinics. For a wider adoption of this ML framework, it is imperative that clinicians use harmonized laboratory values as input parameters prior to expanding it to other centers.

Trial registration number: The de-identified data from patients' charts was analyzed under the IRB protocol number 2018P000062.

P-619 the novel potential molecule involved in polycystic ovary: overexpression of phosphodiesterase 4D (Pde4d) disturbs antral follicle development

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Study question: what's the possible molecular mechanism underlying the arrested antral follicle development in Polycystic ovary syndrome (PCOS) women?

Summary answer: Novel candidate pathogenic genes of polycystic ovary were identified, among which the overexpression of Pde4d was investigated to impair the antral follicle development.

What is known already: Most PCOS women have polycystic ovary, with excessive arrested small antral follicles. However, when stimulated with gonadotropins, these stunted follicles could continue to mature. Many key molecules, participating in primordial to preantral follicles growth as well as ovulation, have been well reviewed. Although cAMP/PKA/pCREB, activated by gonadotropins on G-protein-coupled receptors (GPCR), is widely admitted that it's vital in gonadotropin-dependent follicle growth, it is few studied on the potential molecular mechanism of the arrested antral follicle development.

Study design, size, duration: RNA-Seq was performed on granulosa cells (each group n=4) from three kinds of rat polycystic ovary (PCO) models (each group n=20). The overlapped differentially expressed genes (DEGs) were matched in three groups and further screened for candidate PCO pathogenic gene (Pde4d). The RNA-Seq result was then verified. Moreover, the pathophysiology of upregulated Pde4d and its role in arrested antral follicle development were investigated.

Participants/materials, setting, methods: 21-day-old SD rats were induced respectively by estradiol valerate (EV), testosterone propionate (TP), constant light (CL) for 8 weeks. The vaginal opening time, estrous cycle, and ovary morphology were observed. RNA-Seq was performed with Illumina Hiseq 2500 and the DEGs were screened by cuffdif2 test. The Pde4d expression was verified by PCR and Western Blot. Pde4d hydrolysis function was detected by cAMP level, folliculogenesis-related genes by PCR, and signal pathway by Western Blot.

Main results and the role of chance: The EV/TP/CL induced PCO rats had advanced vaginal opening time, disturbed estrous cycle, and polycystic ovary (arrested antral follicle development), compared to their own control rats. In EV group, there were 367 DEGs (189 up-regulated, 178 down-regulated). In TP group, there were 447 DEGs (226 up-regulated, 221 downregulated). In CL group, there were 886 DEGs (399 up-regulated, 487 down-regulated). 86 DEGs were overlapped within all three PCO groups, of which only 16 DEGs had the same tendency of upregulation or downregulation in all three groups. As Areg, Ereg, Lyve1, Ptger2, and Tnfaip6 have been investigated to be involved in follicular physiology, candidate genes were reduced to 10. As to Pde4d, its protein level was verified increased in PCO rats. When stimulated with FSH, the cAMP level was comparable between PCO and control rats after 30m, while markedly decreased in PCO rats after 60m. Consequently, the intensity of pCREB, usually phosphorylated by cAMP-activated PKA, was obviously weakened in PCO rats. Moreover, genes involved in early immature follicle growth were upregulated, while genes associated with follicular maturation was downregulated.

Limitations, reasons for caution: Rats are multi-ovulatory, inconsistent with mono-ovulatory female, implying difference in follicle selection process. So, our results need to be further verified in human samples before applied to women.

Wider implications of the findings: The newly found molecules are strong candidates for investigations of their roles in dysfunctional folliculogenesis in PCOS, which could be diagnostic markers or therapeutic targets in clinic. Pde4d, one of them, was initially investigated to shed light on the possible pathophysiological mechanism participating in arrested antral follicle growth in PCOS.

Trial registration number: not applicable

P-620 Correlation between serum and follicular fluid levels of oxidative stress markers in women who undergo in vitro fertilization/intracycoplasmatic sperm injection

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Study question: Is the serum level of oxidative stress markers representative of ovarian levels in assisted reproduction techniques patients at different times?

Summary answer: Lipoperoxidation level is similar both in serum and follicular fluid (FF) and there was a strong correlation in antioxidant defense measured in serum and FF.

What is known already: The role of oxidative status in women´s reproductive physiology has not yet been clearly elucidated. Although we know a correct oxidative status is necessary for normal endocrine function, there are few studies in human reproduction about this topic.

The pathological effects of reactive oxygen species result basically from damage to lipids.

Finding out a correlation between serum and FF would involve the power to predict the ovarian situation with a simple blood extraction.

Study design, size, duration: This study is a prospective pilot study, a total of 20 patients were recruited twice in the period between april 2017 and february 2018.

Patients undergoing IVF/ICSI and embryo transfer were stimulated with recombinant FSH and monitored by regular ultrasound and serum estradiol assays.

The follicular fluid (FF) was collected the recovery day and the serum sample was collected 48 hours before. Both samples were centrifugated and frozen at -80º until analysis.

Participants/materials, setting, methods: Patients underwent treatment twice and the anaysis was carried out in both cycles to increase consistency of the results.

Lipid peroxidation (LP), total antioxidant activity (TAA) and ratio LP/TAA were measured by spectrophotometry, using N-metil-2-phenilindol as chromogen which reacts with derivative lipids, and the acid ABTS to form a radical with a characteristic spectrum at 414 and 730 nm. The t-Student test and Pearson or Spearman correlation coefficients were calculated depending on the normality condition.

Main results and the role of chance: Means (+SD) were: for age of women 35.56 (+2.83) years, for BMI 23.92 (+5.04) Kg/m^2 and for time between

treatments 7.28 (+1.5) months. The sterility causes were: 33.33% male factor, 27.78% mixed factor, 22.22% unknown factor and 16.67% female factor.

Serum vs FF results showed as (mean + DS) followed by p:

1st cycle: LP (62.12±25.21) vs (50.57±24.06), p=0.109, TAA (3.89±2.70) vs (6.21±4.25), p<0.001, ratio LP/TAA: 17.84(12.31-28.96) vs 9.46(6.33-15.15), p<0.001.

2nd cycle: LP (75.54±36.01) vs (49.58±20.58), p=0.033, TAA (4.75±2.05) vs (6.45±2.92), p=0.002, ratio LP/TAA: 17.56(8.59-21.66) vs 8.21(4.37-12.13), p=0.001.

TAA (mg Tx/g prot) is the only one parameter greater in FF in both cycles. The antioxidant activity is expressed as Trolox equivalents which produce the same antioxidant effect tan the studied sample. LP is expressed as µmol MDA + 4HNE/g of protein or µM of MDA + 4 HNE.

To predict ovarian values based on serum, lineal regression models were put up when lineal relationship were statistically significant. The achieved coefficients were: 1st cycle: TAA (1.38, p<0.001). 2nd cycle: TAA (1.034, p<0.001).

Limitations, reasons for caution: These results, should be confirmed in a larger study population.

Funding by hospital/clinic(s). The study was partially supported by a grant from Jose Luis Castaño Foundation and Spanish Society of Clinical Biochemistry, FJLC-SEQC. The authors declare no conflicto of interest.

Wider implications of the findings: We find strong correlation between serum and FF in TAA, on contrary ratio LP/TAA does not show a statistically significant lineal correlation between serum and FF although it shows significant lower levels in FF. Finding correlations as this one is important to predict ovarian situation using blood samples.

Trial registration number: None

P-621 High basal estrogen levels (≥40 pg/ml) affect the clinical pregnancy rate of a cryo-thawed single blastocyst transfer during Hormone Replacement following Oocyte Retrieval

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Study question: Could cryo-thawed blastocyst transfer (thawed BT) be performed following oocyte retrieval (OR)?

Summary answer: Cryo-thawed blastocyst transfer in a hormone replacement cycle (HRC) can follow the OR cycle when the basal estradiol (E2) level is lower than 40 pg/ml.

What is known already: Currently, the practice of freezing all cases has increased in order to avoid OHSS. Moreover, in Japan the use of fresh BT has resulted in lower pregnancy rates compared with using thawed BT. Therefore, both clinicians and patients tend to prefer a freeze-all policy. But, there obviously is no standard as to whether thawed BT can be used following the oocyte retrieval cycle. Additionally, few studies have focused on the influence of remaining follicular cysts and basal E2 levels on day 3 following the OR cycle.

Study design, size, duration: This is a prospective case-control study. From January 2018 until December 2018, patients who accounted for 173 thawed BT cycles were recruited at Yanaihara Women's Clinic. All patients followed our "freeze-all policy" and none received fresh blastocyst transfers. Thawed BTs were used for the first cycle of treatment and were performed immediately after either the OR or the spontaneous cycle. This study was approved by the Local Ethics Committee of our clinic.

Participants/materials, setting, methods: Endometrial preparations for thawed BT were accomplished via estrogen supplementation, which was begun on day 3. The morphologically good blastocysts of either Gardner A or B grade were transferred. On day 3, all patients had their serum E2 levels checked for the presence of remaining follicular cysts via transvaginal ultrasound. Despite the results, all patients started HRC, and the clinical pregnancy rates were evaluated accordingly.

Main results and the role of chance: Out of 173 cycles, 96 achieved pregnancy for a clinical pregnancy rate of 55.5%. Among them, 119 cycles were performed using thawed BT just after OR cycles (after group), and the others (n=54) were performed after one cycle interval following OR (interval group). The clinical pregnancy rate in the after group was 57.9% (69/119) and that in the interval group was 50.0% (27/54), and there were no significant differences.

Follicular cysts remained in 37 cycles (cyst group) and did not exist in the other groups (non-cyst group; n=136), the clinical pregnancy rates in the cyst and non-cyst groups were 48.6 (18/37) and 57.4% (78/136), respectively, with no significant differences (p=0.3449).

On day 3, there were 74 cycles (high E2 group) that showed an elevated E2 level (≥40 pg/ml); the others (n=99) showed normal E2 levels (<40 pg/ml) on day 3 (normal E2 group). The clinical pregnancy rate for the high E2 group was 41.9% (31/74), which was significantly lower than that for the normal E2 group (65.7% [65/99], p=0.0019).

Limitations, reasons for caution: Although the follicle growth was checked on the day of progesterone in all cycles, the progesterone level was not measured on that day. This study was an observational study, and all transferred blastocysts were evaluated by morphological analysis rather than by a chromosome of analysis such as PGT-A.

Wider implications of the findings: The high E2 group showed a lower pregnancy rate because this condition might shorten the period before progesterone elevation compared with the normal E2 group in HRC. The thawed BT could be performed following OR via HRC, when patients showed a normal E2 on Day 3 with or without cyst.

Trial registration number: This study has no RCT status, and, therefore, it did not receive a trial registration number.

P-622 The endocrine milieu in naturally matured follicles is different in women with high serum AMH concentrations

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Study question: Is the endocrine milieu in follicular fluid (FF) different in women with high serum Anti-Mullerian hormone (AMH) concentrations, and what regulates follicular endocrine parameters?

Summary answer: FF AMH and A2 concentrations are higher in women with high serum AMH levels. FF AMH does not seem to be regulated by FF hormones.

What is known already: Polycystic ovarian syndrome is associated with high serum androgen and AMH concentrations, which is assumed to be due only to a high number of antral follicles. LH stimulates FF androgens which are converted into E2. FF androgens and E2 have been suggested to regulate FF AMH secretion. The intrafollicular concentration of AMH has been shown in Natural Cycle IVF (NC-IVF) to be positively correlated with oocyte fertilisation and implantation rates. It is unknown what regulates AMH synthesis.

Study design, size, duration: Cross-sectional study involving 4 groups with each 21 NC-IVF treatment cycles with the aspiration of one oocyte, performed in 2013 – 2016. Groups were defined by serum AMH concentration. In group A AMH was not detectable, in group B AMH concentrations were detectable but <7.14 pml/L (<1ng/ml), in group C AMH was 7.14-35.7pmol/L (1-5ng/ml) and in group D AMH concentrations were >35.7 pmol/L (>5ng/ml).

Participants/materials, setting, methods: Mean age was 37.6 ± 4.2 (SD, range 27–42) years in all participants and age did not differ between the four groups. FF was collected from the leading follicles. AMH, Androstendione (A2), total Testosterone (T), Estradiol (E2), Luteinizing hormone (LH) and Follicle stimulation hormone (FSH) were determined by immunoassays. Hormone concentrations were compared between groups and associations were analysed by correlation and regression analysis.

Main results and the role of chance: FF AMH and A2 concentrations were highest in group D (p<0.0001 and 0.0025 by one-way ANOVA, respectively). Median FF AMH concentrations were 9.54, 13.2, 37.7, and 36.2 pM in groups A to D, respectively and median A2 concentrations were 9.8, 9.4, 18.8, and 63.2nM, respectively. Regression analysis confirmed the association of serum AMH with FF AMH concentrations (p<0.0001, r^2=0.2933).

FF LH concentrations were positively correlated with T (p=0.05 over all groups), and with A2 (p=0.032 in group D only) concentrations. T and A2 were correlated with increasing E2 concentrations (p<0.001 and 0.010, respectively, over all groups). Neither LH, T, nor E2 were correlated with FF AMH concentrations. None of the hormone parameters were significantly correlated with follicle diameter, oocyte maturity, embryo quality and pregnancy rate.

Limitations, reasons for caution: As NC-IVF follicles were stimulated by HCG the endocrine milieu in these patients does not exactly correspond to the physiological situation. The study was not powered to analyse the pregnancy rate in relation to AMH concentration.
Wider implications of the findings: The endocrine follicular milieu is significantly different in women with high (>5ng/ml) serum AMH concentrations. High serum AMH concentrations do not only seem to reflect a high number of antral follicles but are apparently also due to overproduction of follicular AMH. Women with high serum AMH have a follicular dyfunction.
Trial registration number: Not applicable

P-623 Lack of predictive value of ovarian reserve tests for pregnancy likelihood. The huge difference between quantity and quality

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Study question: Are ovarian reserve tests predictive of pregnancy and, thus, should they be used as criteria for assisted reproduction exclusion?
Summary answer: Ovarian reserve tests are important to predict ovarian response to COS, but reproductive potential is more related to factors associated to oocyte quality.
What is known already: As a consequence of delayed childbearing, poorer results can be expected in terms of pregnancy rates due to two different factors related to age: declining of follicular pool (quantity) and oocyte quality. Whereas number of oocytes may be predicted by using ovarian reserve tests, chance of pregnancy resulting in a healthy newborn depends on factors not directly related to number of oocytes.

A myriad of markers of ovarian reserve have become part of routine diagnostic testing performed prior to IVF. Moreover, such tests have been used "predictors of successful full term pregnancies" or even as "fertility tests" in many centers.
Study design, size, duration: Cohort study carried out by analyzing prospectively data of 718 consecutive patients who underwent their first IVF treatment during 2017-18 period at a university associated private AR center.

First, we tried to identify factors capable of predicting the quantitative ovarian response (number of MII oocytes). Second, we assessed whether the same parameters were useful for identifying chances of successful ongoing pregnancy.

The study was conducted according to the principles expressed in the Declaration of Helsinki.
Participants/materials, setting, methods: First: Multiple stepwise linear regression analysis. Number of MII oocytes as dependent variable. Age, BMI, AFC, AMH, basal FSH, LH and Estradiol on day 3 to 5 and administered rFSH during stimulation as dependent variables. (P=0.05 significant (Hosmer and Lemeshow).

Second: multivariable logistic (binomial) analysis. Ongoing pregnancy as dependent variable. Number of MII oocytes included as independent variable. The AUC (ROC curves) was used to assess the discriminative power of the logistic regression models.
Main results and the role of chance: The only factor with no influence on the number of retrieved of mature oocytes by applying a simple linear regression analysis was BMI (R2=0,001;p=0,989).

The other variables were evaluated by a multiple stepwise linear regression. Over 78% of the variance of the dependent variable (MII oocytes) is explained by AFC (R2=0,786;t=32,514;p=0,000). By adding the value of AMH to the predictive model, the prediction in the number of oocytes to be obtained improves less than 0,1% (Increase of R2 from 0,786 to 0,787;t= 1,511;p=0,132). Furthermore, the inclusion of all the assessed variables in the multiple lineal regression analysis after AFC does not increase significantly the ability to predict the number of oocytes (From R2=0,786 to 0,794 (t=0,099;p= 0,921).

In 299 cycles(41,64%) an ongoing pregnancy was achieved. Age represented the most important factor influencing ongoing pregnancies (38,8% of the variance;R2: 0,388). AFC and AMH values (the most important variables predicting number of oocytes) increase the predictability by only a 0,6% and 0,7% respectively (R2 increase of 0,006 and 0,007;p=0,170 and p=0,160.

By using ROC curves, the only variable that showed a good accuracy in predicting ongoing pregnancy was age (AUC=0,817). Other variables showed poorer accuracy even though a statistical significance is present.
Limitations, reasons for caution: The descriptive nature of the study may be a limitation for the results of the present study.

A downfall of AFC as predictor of ovarian reserve may be lack of standardization in ultrasound equipment, technique, and inter-observer variability, which results in challenges with cross-comparing results from studies and different centers.
Wider implications of the findings: AMH measurement adds little value to AFC determination in predicting the number of retrieved oocytes. It is worthless to determine basal FSH,E2 or LH levels.

However, ovarian reserve tests should not be offered as "fertility tests" since they are not independently related to pregnancy chance,neither after sexual intercourse nor ART.
Trial registration number: Not applicable

P-624 Evaluation of the Second Follicular Wave Phenomenon in Natural Cycle Assisted Reproduction: A Key Option for Poor Responders Through Luteal Phase Oocyte Retrieval

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Study question: What is the role of Luteal Phase Oocyte Retrieval (LuPOR) in the management of poor responders undergoing natural cycles?
Summary answer: Emergence of LuPOR may revolutionize the practice regarding the time-sensitive nature of poor responders ascertaining a higher number of good quality oocytes faster.
What is known already: Observation of a second follicular wave (SFW) in the luteal phase of a menstrual cycle has altered the traditional theory on folliculogenesis. This led the In Vitro Fertilization (IVF) specialists to the implementation of LuPOR, with favorable results regarding the management of poor responders in stimulated cycles.
Study design, size, duration: The present study represents a retrospective observational clinical study including 136 patients during the time period of 2015 to 2018.
Participants/materials, setting, methods: Collection and statistical analysis was performed based on data from 136 poor responders who underwent follicular oocyte retrieval (FoPOR) and subsequent LuPOR in natural cycles, during IVF treatment. All 136 participants were diagnosed with poor ovarian reserve (POR) according to Bologna criteria. The 272 cycles were categorized as follows: 136 natural cycles with only FoPORs (Control Group) and 136 natural cycles including both FoPORs and LuPORs.
Main results and the role of chance: Our primary results indicate no statistically significant differences with regards to the mean number of oocytes, the maturation status, and fertilization rate between FoPOR and LuPOR in natural cycles. Secondarily, we demonstrate a statistically significant higher yield of oocytes (2.50±0.78 vs 1.25±0.53), better oocyte maturity status (1.93±0.69 vs 0.95±0.59) and higher fertilization rate (1.31±0.87 vs 0.61±0.60) in natural cycles including both FoPOR and LuPOR, when compared to cycles including only FoPOR.
Limitations, reasons for caution: Our study constitutes a retrospective single center analysis.
Wider implications of the findings:

Our study may contribute towards the establishment of an efficient poor responders' management through the natural cycle approach. This novel clinical practice may ascertain the opportunity to employ oocytes and embryos

originating from a luteal phase follicular wave for this time sensitive cohort of patients.

Trial registration number: Not applicable.

P-625 Female obesity does not impact live birth rate after frozen thawed blastocyst transfer

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Study question: Does female obesity affect live birth rate after frozen-thawed blastocyst transfer?

Summary answer: Live birth rate was not statistically different between obese and normal weight patients after frozen thawed blastocyst transfer (FBT)

What is known already: Obesity is a major and increasing health problem, especially in women of reproductive age. It impacts spontaneous fertility and increases delay to conception. Although some studies yielded discordant results, most authors found that female obesity impacted negatively all assisted reproductive technology outcomes. Among them, pregnancy rate after fresh embryo transfer in IVF were generally found to be lower than in normal weight women, probably because of reduced uterine receptivity. Surprisingly, only one study evaluated the effects of female body mass index (BMI) on implantation rate after FBT. The authors did not find any association between female BMI and FBT outcome.

Study design, size, duration: This retrospective case control study was conducted in all consecutive frozen thawed autologous blastocyt transfer (FBT) cycles conducted between 2012 and 2017 in our center. FBT cycles in obese women (BMI>30 kg/m^2) were considered as cases (n=252), while FBT cycles conducted in normal weight women (BMI=18.5-24.9 kg/m^2) were considered as controls (n=1415).

Participants/materials, setting, methods: This study was conducted in a single University-based hospital. A total of 252 FBT cycles performed in obese women and 1415 FBT cycles performed in normal weight women were included in the analysis. Endometrial preparation was based on hormonal replacement therapy. Vaginal progesterone was started when endometrial thickness was >7mm. One or 2 blastocyst were transferred after 7 days of progesterone administration. Descriptive analysis of both groups, followed by univariate and multivariate analysis was performed.

Main results and the role of chance: Both female and male age and smoking status, and type of infertility were comparable in both groups (obese and normal weight). Anti mullerian hormone level was statistically higher in obese women than in normal weight women (5,9±5,2µg/L vs 4,8±4,1µg/L, p<0,0002). Concerning FBT cycles, the duration of hormonal treatment, the stage and number of embryos used for transfer were comparable between both groups. Mean endometrium thickness was significantly higher in obese than in normal weight group (8,7±1,8mm vs 8,1±1,6mm, p <0,0001). Concerning FBT cycle outcome, live birth rate was comparable in obese and in normal weight groups (19,05% vs 20,35% respectively, p=NS). The implantation rate (14% vs 19% respectively) and pregnancy rate (21,83 vs 22,05%) were also comparable between two groups

Limitations, reasons for caution: Patient's weight data may have been declarative in some cases. The anthropometric parameters such as hips waist ratio were not used. Polycystic ovarian syndrome status was not systematically available and was not included in the analysis. Neither FBT cycles after ovarian stimulation nor natural cycle were included.

Wider implications of the findings: Our study showed that live birth rate after frozen thawed blastocyst transfer was not statistically different in obese and in normal weight women. Although this needs confirmation, this suggests that the impairment of uterine receptivity observed in obese women might be associated with ovarian stimulation and its hormonal perturbations.

Trial registration number: not applicable

P-626 Progesterone dose adjustment and transfer postponement in patients with low progesterone levels following hormonal replacement therapy for frozen thawed embryo transfer.

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Study question: Is progesterone (P) dose adjustment and postponement of frozen thawed embryo transfer (FET) effective to improve outcome in patients with low P levels following hormonal replacement therapy?

Summary answer: Dose adjustment to obtain serum P levels >10 ng/ml and 1 day transfer postponement gave similar outcome to those of normal P "in phase" transfers.

What is known already: Several studies dealing with HRT FET cycles have demonstrated that low serum P levels on the day of FET or on the day of pregnancy test were associated with decreased pregnancy and live birth rates. However, in our hands, P dose adjustment after ET to obtain P levels >10 ng/ml was ineffective to correct impaired outcome. Therefore, we decided to measure serum P levels and adjust P dose to obtain a serum P level >10 ng/ml prior to ET and to postpone ET by one or two days accordingly.

Study design, size, duration: This is a retrospective analysis of 481 prospectively collected HRT FET cycles from March 2017 to October 2018. Endometrial preparation was achieved by sequential administration of vaginal estradiol until endometrial thickness >7 mm, followed by transdermal estradiol combined with 600 mg/day vaginal micronized P (200 mg three times a day).

Participants/materials, setting, methods: This study was conducted in a university hospital. Serum P was measured on D2 following exogenous P introduction in the evening (referred as D0). When P levels were >10 ng/ml, ET was performed on D2, D3 or D5 depending on embryo stage at cryopreservation. When P levels were <10 ng/ml, P dose was increased to 1200 mg/day. Serum P level was checked one day later and the next day if necessary. ET was postponed accordingly.

Main results and the role of chance: Mean serum P level on D2 was 12.4 + 4.29 ng/ml and serum P <10 ng/ml were observed in 28% of cycles. Cycles were cancelled in 39 patients because of inability to obtain P >10 ng/ml despite increased P dose to 1200 mg/day (n=36) or failure in performing P measurement timely (n=3). Therefore, 442 FET were performed with P >10 ng/ml prior to ET: 346 FET were in phase according to embryo stage, 80 FET were postponed by 1 day and 16 FET by 2 days (P >10 ng/ml on the first or second day following increased P dose to 1200 mg/day respectively). Characteristics of patients in the 3 groups were similar except for mean E2 levels prior to P onset and mean P levels of the last measurement before transfer that were significantly lower in postponed transfer groups. This strategy led to similar positive pregnancy test (38.7 % vs 47.5 %, NS), heartbeat activity at 8 weeks (29.7 % vs 31.2 %, NS) and ongoing pregnancy rates at 12 weeks (29.5 % vs 28.7 %, NS) between "in phase" and "postponed by 1 day" ET while results in "postponed by 2 days" were poor (12.5 %, 6.2 %, 6.2%).

Limitations, reasons for caution: The number of cycles with FET postponed by 1 day following P dose adjustment has to be extended to confirm these preliminary data. In contrast, when serum P levels are not corrected 1 day after P dose adjustment, waiting 1 more day does not seem a suitable option.

Wider implications of the findings: These results suggest that serum P measurement prior to ET and further adjustment of exogenous P dose and postponement of transfer might optimise the outcome of FET cycles performed using HRT and avoid cancellation of a large number of cycles with low serum P levels.

Trial registration number: Not applicable.

P-627 Female obesity significantly impairs live birth rate following IVF: a systematic review and meta-analysis

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Study question: Does female obesity, defined as Body Mass Index (BMI) ≥ 30 kg/m^2, impact live birth rate following in vitro fertilization (IVF)?
Summary answer: Female obesity negatively and significantly impacts live birth rates following IVF.
What is known already: Female obesity has been clearly associated with impaired spontaneous fertility and evidence from the literature shows that it seems also to impact clinical outcomes following In Vitro Fertilisation (IVF) procedures. However, as published studies are heterogenous in terms of populations, groups definition and outcomes no formal conclusion can be established. A previous meta-analysis published in 2011 (Rittenberg et al., 2011) concluded to a marginal but significant negative effect of female Body Mass Index (BMI) on IVF results. Since several studies, including large cohort from national registries, have been published later, an updated review and meta-analysis was performed.
Study design, size, duration: A systematic review was performed out using the following key words: ('obesity', 'body mass index', 'live birth', 'IVF', 'ICSI'). Searches were conducted on MEDLINE, EMBASE, Cochrane Library, Eudract and clinicaltrial.gov from January 01, 2007 to November 30, 2017.
Participants/materials, setting, methods: Study selection was based on title and abstract, and then by utilizing the quality assessed using the Newcastle-Ottawa Quality Assessment Scales. Two independent reviewers carried out study selection and data extraction according to Cochrane methods. Random-effect meta-analysis was performed using Review Manager software on all data (overall analysis). Sub-groups analysis according to ovulatory status, oocytes origin, fresh or frozen embryo transfer and cycle rank were also performed.
Main results and the role of chance: After review of 407 abstracts, 54 full-text articles were assessed for eligibility. A total of 21 studies were included in the meta-analysis, corresponding to 682,532 IVF cycles. Compared to normal weight women (BMI between 18.5 and 24.9kg/m^2), the risk ratio (RR) for live birth rate after IVF was 0.85 (95%CI=0.82;0.87) in obese women. Sub-groups analyses demonstrated that prognosis was poorer when obesity was associated to Polycystic Ovary Syndrome (RR=0.78; 95%CI=0.74-0.82). The oocytes' origin did not modify the overall interpretation: when only studies including only donated oocytes were analysed, the RR was 0.80 (95%CI=0.68-0.94). Only one study included frozen-thawed only transfers, then conclusions cannot be drawn.
Limitations, reasons for caution: The numerous confounding factors that potentially influence the chance of live birth after IVF procedures were not evaluated in this meta-analysis. Heterogeneity among studies included in the meta-analysis was moderate.
Wider implications of the findings: Our meta-analysis demonstrates that female obesity impairs live birth rate irrespective of PCOS status and oocytes origin raising the hypothesis of endometrial role. The impact of weight loss through lifestyle modifications or bariatric surgery for reversing this effect should be further evaluated.
Trial registration number: PROSPERO: CRD42018090645

P-628 Mifepristone influences natural killer cells function mediated by macrophages

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Study question: The mechanism of mifepristone influencing the development of maternal-fetal interface is still unknown.
Summary answer: The migration ability and cytotoxicity of NK cells can be increased by macrophages treated with mifepristone.
What is known already: Mifepristone, a modulator of progesterone receptor has been the primary choice for first trimester abortion in some parts of the world. Additional studies have also observed its role in achieving contraception.
Study design, size, duration: we collected decidua tissues from 40 women who had undergone surgical termination of unwanted pregnancy. 20 patients were treated with 150mg mifepristone 24 hours before the surgery. And another 20 patients who not using mifepristone before the surgery were control group.
Participants/materials, setting, methods: The decidual tissues from all study participants were collected to analyze the effect of mifepristone on NK cells and macrophages. In addition, in a separate in vitro assay, NK cells were co-cultured with macrophages pre-treated with different concentrations of mifepristone, to test their migration ability using transwell system, and cytotoxicity by MTT assay. SiRNA was used to determine whether CD48 and TGF-β were involved in the mifepristone influencing the functions of NK cells.
Main results and the role of chance: Our study revealed that the distribution and proximity of NK cells and macrophages changed after mifepristone treatment. Moreover, the migration ability and cytotoxicity of NK cells were significantly increased by macrophages treated with mifepristone in a dose-dependent manner. Furthermore, the numbers of CD56+NK cells and CD206+ macrophages significantly decreased in women who received mifepristone compared to those in the control group. After interfering with CD48 expression, mifepristone has a reduced effect on macrophage migration and cytotoxicity. After interfering with TGF-β expression, mifepristone has a increased effect on macrophage migration and cytotoxicity.
Limitations, reasons for caution: further in vivo studies would be required to decipher the exact mechanism of mifepristone action in inducing abortion.
Wider implications of the findings: These results indicated that mifepristone may mediate the function of NK cells via macrophages.
Trial registration number: not applicable

P-629 Identification of microRNAs associated with an impaired metabolic profile in women with polycystic ovary syndrome

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Study question: Can we identify microRNAs associated with an altered metabolic profile in women with Polycystic Ovary Syndrome (PCOS), which can serve as biomarkers for early diabetes?
Summary answer: Circulating miR-122-5p is a possible marker of prediabetes in women with PCOS and is associated with the severity of metabolic disease.
What is known already: It is well established that women with PCOS are at greater risk of developing metabolic syndrome and type 2 diabetes. Recent discoveries indicate that miRNAs play an important role in development of diabetes in general and reflect the clinical status of patients. However, to date there are no existing studies of circulating miRNAs as biomarkers for a challenged metabolism and a prediabetic phenotype in women with PCOS.
Study design, size, duration: A prospective follow-up-study with a cohort of 46 women with PCOS diagnosed according to Rotterdam 2003 criteria and nine healthy controls, aged 18 to 39 years. The women were examined twice 6 years apart. Circulating miRNA levels were analyzed at both visits and compared with clinical data. A cross sectional cohort of 162 PCOS patients (133 PCOS and 29 controls) were investigated for biological validation of findings.
Participants/materials, setting, methods: Relevant clinical data of PCOS status and measurements of metabolic disease were obtained at both visits. Peripheral fasting serum samples collected at both time points were used for microRNA analysis. Total RNA was extracted using TriReagent. MiRNA levels were measured using custom Taqman® Array cards of 96 selected miRNAs. MiRNA relative levels were quantified by the $2^{-\Delta\Delta Ct}$ method. MiR-122-5p were analyzed with individual qRT-PCR in the validation cohort.
Main results and the role of chance: During the 6-year follow-up, more participants became prediabetic (defined by Impaired Glucose Tolerance (IGT) or Impaired Fasting Glucose (IFG)) (Six (10.9%) participants at baseline vs 10 (18.2%) participants at follow-up). Analysis of miRNA did not reveal significant differences in circulating levels of any of the tested miRNAs comparing PCOS

with controls neither at baseline nor at follow-up. However, comparing the non-prediabetic with prediabetic, we found that miR-122-5p was significantly upregulated in the prediabetic group both at follow-up (p=0.03) and at baseline, as predictor of prediabetic status at follow-up (p=0.03, OR=7.7 (95%CI: 1.2-51.3) adjusted for BMI, smoking and age). Next, the participants were evaluated for degree of metabolic disease by numbers of metabolic factors present (0-5: hypertension, dyslipidemia, triglyceridemia, increased waist circumference and IGT or IFG). Applying linear regression on the levels of miR-122-5p vs the degree of metabolic disease, the models were statistically significant both at baseline (R^2=0.10, B=0.51, p=0.05) and follow-up (R^2=0.17 B=0.9, p<0.01). In the validation of findings in the cohort of 162 participants, we found significantly elevated levels of miR-122-5p in participants with IFG both in PCOS patients alone (p<0.05) and in the cohort as a whole (p<0.01) after adjustment for BMI and age.

Limitations, reasons for caution: Few participants with prediabetes in the follow-up study causes weak modelling.

Wider implications of the findings: Our results are in line with earlier studies of miR-122-5p, which have demonstrated strong correlation with both metabolic and hepatic disease. Further, validation in the larger cohort strengthen the findings, but suggests that miR-122-5p is probably more a general marker of metabolic disease, than a PCOS-specific marker of metabolic disease.

Trial registration number: ClinicalTrials.gov. Identifier: NCT03142633

P-630 A comparison of IVF outcomes transferring a fresh single ideal blastocyst in women with polycystic ovary syndrome and normal controls with normal ovarian reserve

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Study question: To assess the effects PCOS on live birth rates when transferring a single fresh ideal blastocyst.

Summary answer: After controlling for confounders, when transferring a fresh single ideal blastocyst, live birth rates were lower among the women with PCOS than normal ovulatory controls.

What is known already: PCOS is the most common cause of anovulatory infertility. Although PCOS patient have higher number of obtained oocytes, the quality and maturity of these oocytes might be compromised, leading to reduce fertilization, cleavage, implantation, pregnancy and live birth rates, compared to controls. Stimulated PCOS may have endometrial sub-competence, since ovulatory controls demonstrated similar pregnancy and live birth rates in frozen cycles while PCOS, FET resulted in a higher rate of live birth than do fresh embryo transfer, in prospective randomized studies.

Study design, size, duration: This is a retrospective cohort study performed between August 2010 and March 2014 at the McGill University Health Centre Reproductive Centre. Women with PCOS who underwent their first fresh embryo transfer were included in the study. A control group of women who underwent fresh IVF cycles in the same study period was included in 1:3 ratio.

Participants/materials, setting, methods: 71 PCOS and 272 normal ovulatory controls were included in the study. Statistical analysis was performed with independent-T or chi-squared tests where appropriate. Stepwise multivariate logistic regression was used to calculate pregnancy outcomes while controlling confounders. Data is presented as mean±SD, percentages and confidence ratios (CI). Two-sided p values $\leq$ 0.05 were accepted as significant.

Main results and the role of chance: PCOS patient were younger (31.0± 3.7 vs. 33.1± 3.2, p=0.0001), with higher antral follicle counts (40.0± 9.3 vs. 13.3± 4.6, p=0.0001), required lower dose of gonadotropins to stimulate (1198±786 vs. 1891± 1224, p=0.0001), and had higher serum total testosterone levels (2.3± 0.7 vs. 1.1± 0.3, p=0.0001).

No significant different was fount between the two groups regarding the number of previous pregnancies, the number of previous full-term pregnancies, the level of basal serum FSH and the BMI. Garder's grade of transferred embryo did not differ. When compared by chi-squared testing pregnancy rates, clinical pregnancy rates and live birth rates did not differ. However, when controlling (with multivariate stepwise logistic regression) for female age, total gonadotropin dose used, previous number of pregnancies and previous number of live births, live birth rates was lower among the women with PCOS (p=0.035, CI:0.18-0.92)

Limitations, reasons for caution: It was a retrospective cohort and as such undetected biases may be present.

Wider implications of the findings: This finding suggests that in PCOS patients, the pre-existing hyperandrogenic milieu may have a negative impact on the oocytes or the endometrium which may translate into lower embryo potential at fresh cycles.

Trial registration number: not applicable

P-631 A novel and simple method to schedule GnRH antagonist cycles with a short course of oral estradiol in the early follicular phase

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Study question: Does scheduling GnRH antagonist cycles with a short course of estradiol in the early follicular phase affect oocyte yield?

Summary answer: Early follicular phase estradiol scheduling(FES) enables collection of similar numbers of oocytes without a clinically significant increase in gonadotropin consumption as compared with unscheduled cycles.

What is known already: Widely used GnRH antagonist protocol has limited flexibility regarding to timing of oocyte retrieval, sometimes unable to accommodate the needs of the patient and the medical staff. While various methods have been put to use, they either gain only one or two days, increase gonadotropin/GnRH antagonist injections, require advance planning/monitoring, or risk impairing implantation rates.

Study design, size, duration: A retrospective analysis of 118 cycles from 59 oocyte donors between 2015 and 2018. Each donor underwent two GnRH antagonist stimulation cycles within 6 months, one with and one without FES, and served as her own control to ensure similar baseline and biological characteristics, as in a randomized controlled trial.

Participants/materials, setting, methods: Women aged between 20-30 years who had no medical contraindication to oocyte donation that underwent karyotype analysis/routine screening for infectious diseases were recruited as oocyte donors. FES was achieved by giving 6 mg/day estradiol valerate orally from the 2nd or 3rd day of menstrual cycle until the desired day of gonadotropin start. Gonadotropin was started on the 2nd or 3rd day of menstrual bleeding in unscheduled cycles. Daily gonadotropin dosage was 225-300 IU recombinant FSH.

Main results and the role of chance: A total of 118 cycles, 59 FES and 59 unscheduled GnRH antagonist, were included in the study. Median duration of estradiol administration was 3 days (25th – 75th percentile, 2-4) in FES cycles. The longest duration of estradiol use was 7 days. Median gonadotropin starting dosage was similar at 225 IU/day in FES and unscheduled cycles. In the FES group, stimulation lasted significantly longer by one day (11 vs. 10 days, p=0.03) and total gonadotropin consumption (2497 vs. 2404 IU, p=0.03) was statistically significantly higher, albeit minimal absolute difference, which is probably short of clinical significance. Number of GnRH antagonist injections were similar between FES and unscheduled cycles (5 vs 5). Numbers of COC (21 vs 20) and metaphase two oocytes (17 vs 17) were similar between the two groups.

Limitations, reasons for caution: Although this is a non-randomized retrospective study, each woman serving as her own control assures similar characteristics. Participants or clinicians were not blinded, however, performance bias is unlikely. Pregnancy outcomes are not available, yet prior studies of estradiol scheduling does not suggest a harmful effect on implantation or pregnancy rates.

Wider implications of the findings: Our results suggest similar oocyte yield following FES. FES does not require advance planning, involves shorter use of estradiol/oral contraceptive tablets and can be advantageous to scheduling with luteal estradiol/oral contraceptive administration. If further studies provide reassurance for pregnancy rates, FES can be the method of choice for cycle scheduling.

Trial registration number: None.

P-632 Comparison between prepubertal and postpubertal patients with Obstructed hemivagina and ipsilateral renal anomaly (OHVIRA) syndrome

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Study question: How does prepubertal and postpubertal patients with OHVIRA syndrome differ in terms of clinical presentation, management, and long-term outcome?

Summary answer: Contrast to postpubertal, prepubertal patients showed ectopic ureter with multicystic dysplastic kidney and were conservatively managed through serial evaluation of hemivaginal obstruction and renal function.

What is known already: OHVIRA syndrome is one of the most common causes of obstructive Mullerian anomaly in menstruating female, with a hallmark presentation of progressively worsening, severe dysmenorrhea at 2 months to 2 years after menarche. Most commonly suspected mechanism is an arrest in development of Mullerian structure and in reciprocal formation of renal structure, leading to a triad of renal agenesis, uterine didelphys, and hemivaginal obstruction. However, with an increased use of prenatal and neonatal sonography, prepubertal cases of OHVIRA syndrome have been reported.

Study design, size, duration: We retrospectively reviewed all patients with the diagnosis of OHVIRA syndrome at Severance Hospital from January 2004 to September 2018. A total of 68 patients were included for final analysis: postpubertal (n=24) and prepubertal (n=44).

Participants/materials, setting, methods: First, subgroup analysis was performed in postpubertal and prepubertal cohorts separately. In postpubertal group, prognosis was compared according to the degree of suspicion for complete (n=12) versus incomplete (n=12) hemivaginal obstruction based on chief complaint. In prepubertal group, patients with ectopic ureter and multicystic dysplastic kidney (n=30) were compared to those without (n=14). Then, as a whole postpubertal and prepubertal OHVIRA patients were compared in terms of clinical presentation, radiological finding, management, and long-term outcome.

Main results and the role of chance: Median age at diagnosis was 20 years old (range: 11-26) for postpubertal (n=24) and 8 days old (range: 0-817) for prepubertal (n=44). All postpubertal patients were symptomatic, and those with abdominal pain or palpable mass (n=12) were more likely show a large amount of obstruction (p=0.046) and up to a higher anatomical level (p=0.029). A majority of prepubertal patients were incidentally diagnosed by ultrasound (81.8%). Among prepubertal patients, those with ectopic ureter were more likely to develop urinary incontinence (33.3% vs. 0.0%, p=0.022) and recurrent urinary tract infection (50.0% vs. 7.1%, p=0.006). Compared to postpubertal who presented with ipsilateral renal agenesis, prepubertal patients presented with ectopic ureter (68.2% vs. 20.8%, p<0.001) and multicystic dysplastic kidney (61.4% vs. 16.7%, p<0.001) and developed contralateral kidney hypertrophy (54.5% vs. 20.8%, p=0.002). Most postpubertal patients underwent primary operation (91.7%), whereas only 8 prepubertal patients (18.2%) underwent operation: nephrectomy in 6 patients and aspiration of hemivaginal obstruction in 4 patients. Serial sonographic evaluation of the amount of hemivaginal obstruction in prepubertal patients showed no change at minimal amount in 44.4% and at least a sign of improvement in 27.8%, reaching a complete resolution in another 25.0%.

Limitations, reasons for caution: Limitations include retrospective design and a relatively small sample size. Sample size could have been larger had we decide to include the anatomical variants of OHVIRA syndrome in a multicenter setting. A future prospective study on prepubertal patients would help provide an effective guideline for follow up and consultation.

Wider implications of the findings: With the recent inclusion of prepubertal patients, clinical spectrum of OHVIRA syndrome has drastically widened in terms of presentation, anatomical features, management, and prognosis. Contrast to postpubertal patients who require timely surgery, prepubertal patients benefit from an individualized approach in the context of maturing gynecologic and urologic organs in children.

Trial registration number: Not applicable

P-633 A slower age-related decline in treatment outcomes associated with in vitro fertilization in women with polycystic ovary syndrome

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Study question: what is the exact impact of age on pregnancy outcomes of women with PCOS?

Summary answer: The decline in treatment outcomes with age is slower in PCOS women .Older PCOS patients (≥35 years) show better pregnancy outcomes than age-matched controls.

What is known already: The age-related improvement of endocrine disturbance and relatively good ovarian reserve does not indicate that PCOS women will definitely acquire reproductive superiority over normal women, as other factors that affect female fertility cannot be ignored. For example, obesity, insulin resistance, and metabolic disorders may persist or even worsen in PCOS women with advancing age. Some extra- and/or intra-ovarian factors may also negatively affect oocyte quality and embryonic development potential and thus result in unsuccessful pregnancy outcomes in patients with PCOS.

Study design, size, duration: To study the effect of age on pregnancy outcomes of in vitro fertilization and embryo transfer (IVF-ET) in patients with polycystic ovary syndrome (PCOS). A retrospective cohort study involving 3502 patients with PCOS and 18596 age-matched patients with tubal factor infertility who had undergone their first attempted IVF-ET between January 2010 and July 2015.

Participants/materials, setting, methods: Among the 22098 patients, 3502 were diagnosed with PCOS, and the remaining 18596 were diagnosed with infertility due to tubal factors. The diagnosis of PCOS was based on the Rotterdam criteria. Women in the non-PCOS group exhibited normal menstrual cycles and ovulation and had no evidence of polycystic morphology. SPSS 17.0 (IBM) was used for statistical analyses.

Main results and the role of chance: In both the PCOS and non-PCOS groups, the clinical pregnancy rate and live-birth rate were consistently more favorable in younger than in older women. In the control group, both the clinical pregnancy rate and live-birth rate showed a sharp decline with age (20 to 50 years), while in the PCOS group, the decline in the clinical pregnancy rate and live-birth rate with age (20 to 50 years) was relatively slow. For women over 35 years old, the PCOS group showed a higher implantation rate, clinical pregnancy rate, and live-birth rate than the age-matched control group.

Limitations, reasons for caution: Only a relatively small number of patients at an advanced age were included, especially in the group of older PCOS women. We cannot exclude that the different treatment outcomes between the younger and older patients are the result of this limited sample size.

Wider implications of the findings: PCOS patients manifest a significant but slow decline in fecundity associated with IVF. An advantage in fecundity can be expected in PCOS women of an older age, while early pregnancy loss still cannot be ignored in this period.

Trial registration number: not applicable

P-634 Randomised controlled trial of connected ovulation test system demonstrates double the chances of pregnancy in first cycle and reveal other factors affecting pregnancy likelihood.

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Study question: Does using an ovulation predictor test connected to an App increase chances of pregnancy, and what other factors are related to likelihood of natural conception?

Summary answer: The test arm (connected ovulation test system) had double the odds of conception compared to the control arm (no ovulation test) in first cycle.

What is known already: Natural conception requires intercourse during the fertile period of the cycle, and this period can be conveniently identified using ovulation tests. Most ovulation tests measure only luteinising hormone, so detect the day before and day of ovulation. However, intercourse the day before the LH surge is associated with highest likelihood of pregnancy. Monitoring of estrogen enables identification of the wider fertile phase; indeed the only randomised controlled study on ovulation tests considered a fertility monitor, which found an 89% increased chance of pregnancy. Apps are now very popular, but calendar based Apps provide inaccurate information on fertile phase prediction.

Study design, size, duration: Randomised, controlled study of 844 volunteers seeking to conceive. Volunteers were randomized 1:1 into the test or control arm. Randomization was stratified by the age of the volunteers, with two cohorts (<35 and ≥35 years of age). Volunteers participated for two full cycles if pregnancy was not achieved. Volunteers conducted digital pregnancy tests (Clearblue), collected urine samples (hCG measurement, AutoDELFIA, Perking Elmer), and a diary of menses to determine pregnancy status.

Participants/materials, setting, methods: Home based study of women between the age of 18–40 years. Test group used the Clearblue Connected Ovulation Test System, which measures lutenising hormone and estrogen to detect the wider fertile window, and results are synced to an App. The control group were instructed not to use ovulation tests. Both groups were able to use other methods to time intercourse. Admission questionnaires collected demographics, and post study questionnaires examined behaviour during the study.

Main results and the role of chance: More women became pregnant after one cycle using the test system (25.4%) compared to the control group (14.7%; $P<0.001$), with an odds ratio of 2.0. After two cycles pregnancy rate was still higher in the test group (36.2% vs 28.6%; $P=0.026$), with an odds ratio of 1.4. The test group reported less frequent intercourse per cycle compared to controls (9 vs 10; $P=0.027$), however, the test group did report greater targeting of intercourse (88.5% vs 57.8%; $P<0.001$). The control group indicated they had used methods, most commonly Apps (40.0%) and cervical mucus (35.2%). Neither, use of a non-study App or cervical mucus monitoring was associated with a higher pregnancy rate.

Other factors related to likelihood of pregnancy were folic acid use (p=0.004), average cycle length (p=0.021) and intercourse/month (p=<0.0001). The odds ratio for pregnancy was 2.0 for folic acid users vs non-users. This is likely to be a non-causative observation, linked to overall healthy lifestyle and higher fertility knowledge. Shorter (≤25 days) cycles had lower conception probability. Interestingly, the higher number of intercourse acts, the lower the likelihood of conception; odds ratio for pregnancy 5.9 for ≤5 acts, 4.0 for 6-10, 2.6 for 11-15 and 1.0 for 16-20 vs >20 acts.

Limitations, reasons for caution: An exclusion criteria was trying to conceive for >6months, because a considerably higher sample size would have been required due to low conception rates in this group. Therefore, findings may not extrapolate to those who have been trying for a long period of time.

Wider implications of the findings: With women delaying pregnancy and desiring control over their future, tools demonstrated to help conception, such as the Clearblue Connected Ovulation Test System, are of great relevance today. High levels of intercourse without conception probably indicates infertility, so could be used to guide couples for infertility investigation.

Trial registration number: NCT03424590

P-635 No increased risk of adverse perinatal outcomes in singleton pregnancy of polycystic ovary syndrome(PCOS): a propensity score matching study

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Study question: To evaluate the effect of the PCOS on the pregnancy complications compared with control cases of women undergoing IVF or ICSI.

Summary answer: Women with PCOS have a higher incidence of pregnancy complications in twin pregnancies and a lower incidence of pregnancy complications in singleton pregnancies than non-PCOS.

What is known already: PCOS is an endocrine disorder and accounts for 18-25% of infertility cases. Many women with PCOS pregnancies through IVF or ICSI, which may increase the risk for multiple pregnancies.To evaluate the effect of the PCOS on the pregnancy and perinatal complications of singleton and twin births compared with control cases of women undergoing IVF or ICSI.

Study design, size, duration: We did a retrospective cohort study of the data using propensity score-matching, which included of data of 8066 deliveries in women conceived after their first fresh IVF/ICSI from January 1, 2009 to December 31, 2016.

Participants/materials, setting, methods: The 8066 women were divided into four groups in accordance with the number of fetuses delivered and the diagnosis: group A (277 PCOS women with twin pregnancy), group B (2038 non-PCOS women with twin pregnancy), group C (687 PCOS women with singleton pregnancy), and group D (5064 non-PCOS women with singleton pregnancy).

Main results and the role of chance: After propensity score matching, the risks of gestational diabetes mellitus, preterm birth, and small for gestational age were comparable between the PCOS and control groups for both singleton and twin births. Nevertheless, in the twin pregnancy group, women with PCOS had higher risks for hypertensive disorders of pregnancy (odds ratio [OR] 3.17, 95% confidence interval [CI]: 1.33-7.60, P=0.010) and preterm premature rupture of membranes (OR 4.70, 95% CI: 1.90-11.62, P=0.001) than the control subjects. In the singleton pregnancy group, women with PCOS had lower risks for cesarean section (OR 0.58, 95% CI: 0.43-0.77, P<0.001) and iatrogenic preterm birth (OR 0.39, 95% CI: 0.19-0.80, P=0.010) than the control subjects.

Limitations, reasons for caution: Because of the design of our study, we cannot explain the mechanism behind these phenomena. Due to individual differences between PCOS cases, we did not analyze them according to PCOS phenotypes, although we did enroll cases with various phenotypes.

Wider implications of the findings: Additional monitoring and attention to singleton births from women with PCOS after ART is unnecessary; however, twin births in women with PCOS need to be more closely watched and monitored than those in women without PCOS. Therefore, singleton pregnancies should be recommended to women with PCOS undergoing IVF/ICSI.

Trial registration number: Not applicable

P-636 Follitropin delta for controlled ovarian stimulation: a one-year analysis

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Study question: How is the ovarian response and the clinical pregnancy rate (CPR) of an individualized controlled ovarian stimulation (COS) with follitropin delta in the daily clinical routine?

Summary answer: The mean number of oocytes retrieved was 11. In fresh embryo transfer (ET) cycles the CPR was 35.7 % with 1.18 embryos being transferred.

What is known already: Follitropin delta, a new human recombinant follicle-stimulating hormone (rFSH), was introduced for controlled ovarian stimulation (COS) in 2015 and shown to be non-inferior to a COS with conventional rFSH by the randomized, multicentre ESTHER-1 trial. The unique glycosylation pattern of follitropin delta leads to a higher level of sialylation with more acidic isoforms, displaying a higher exposure and pharmacodynamic response. According to the patient's body weight and antimüllerian hormone (AMH) level, an individualized dosing regimen is calculated to aim for an optimal ovarian response (8-14 oocytes) with a minimized risk for an ovarian hyperstimulation syndrome (OHSS).

Study design, size, duration: Single centre cohort study with 98 patients who underwent COS with follitropin delta for in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles at the reproductive unit UniKiD of the women's university hospital in Duesseldorf. The study period comprises 1st of August 2017 to 31st of August 2018.

Participants/materials, setting, methods: Patients undergoing COS with follitropin delta at the women's university hospital in Duesseldorf. The primary endpoint was clinical pregnancy rate (CPR). Cycle characteristics, mean number of oocytes retrieved and cases of OHSS were also analysed.

Main results and the role of chance: 89 patients were included in the analysis (mean age: 34.3 years, mean weight: 65.3 kg). The mean duration of stimulation was 10.4 days (range: 6-22 days) with a mean dose of follitropin delta of 81.8 µg (range: 45-181.2 µg) injected. The mean number of retrieved oocytes, metaphase II oocytes and two pronuclear zygotes was 11, 8 and 6, respectively. The time interval between the measurement of AMH and the start of COS did not show any impact on ovarian response. 64% of patients received a fresh ET with a single embryo transfer (SET) in 82.5%. Elective frozen embryo transfer (eFET) was performed in 27% of the cases to prevent an OHSS. Of the 24 cases receiving OHSS preventive measures, 2 presented an OHSS grade II and 2 an OHSS grade III. CPR was 35.7% in fresh ET cycles with a mean number of 1.18 embryos per transfer. In comparison the age-adjusted CPR in Germany for 2017 is 31.5% with 1.80 embryos per transfer.
Limitations, reasons for caution: Single centre descriptive study with an unselected patient group. Small sample size.
Wider implications of the findings: The present study confirms that individualized follitropin delta dosing is efficient and safe in the daily clinical routine. Furthermore, a comparable CPR is possible with a lower number of embryos transferred compared to the age-adjusted data of the German national registry.
Trial registration number: N/A

P-637 Modified natural cycle IVF versus conventional stimulation in advanced-age Bologna poor responders

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Study question: Do reproductive outcomes differ between modified natural cycle (MNC) and conventional high-dose ovarian stimulation (HDOS) in advanced-age poor responders?
Summary answer: In advanced-age poor ovarian responders as defined by the Bologna criteria, MNC and HDOS appear to be equally effective.
What is known already: Poor ovarian response (POR) remains one of the main therapeutic challenges in women undergoing ovarian stimulation for in vitro-fertilization, associated with low live birth rates and high cancellation rates. Traditionally, the stimulation protocol in POR includes high doses of gonadotropins. MNCs with minimal stimulation have been proposed as an alternative for conventional ovarian stimulation and have become increasingly popular in this challenging population with POR. However, although MNCs have been shown to result in acceptable cumulative pregnancy rates in normal responders, evidence is sparse in POR and, more specifically, in women of advanced age.
Study design, size, duration: This is a retrospective cohort study including patients with POR attending a tertiary referral University Hospital from 1st January 2011 until 1st March 2017.
Participants/materials, setting, methods: All women who fulfilled the Bologna criteria for POR and aged ≥ 40 years who underwent their first intracytoplasmic sperm injection (ICSI) cycle in our center were included.
Main results and the role of chance: In total, 476 advanced-age Bologna poor responder patients were included in the study: 189 in the MNC group and 287 in the HDOS group. MNC resulted in significantly fewer oocytes (0.8 vs. 4.1; P <0.001), lower embryo transfer rates (45% vs. 75%; P <0.001) and lower top embryo quality rates (83% vs. 94%; P = 0.002) compared with HDOS. Biochemical (5.0% vs. 19.0%; P <0.001), clinical (4.8% vs. 17.4%; P <0.001) and ongoing pregnancy rates (OPR) (2.6% vs. 10.0%; P = 0.002) were significantly lower in the MNC group as compared with the HDOS group. Live birth was achieved in 3/189 (1.6%) MNC and in 23/287 (8.0%) HDOS cycles. However, multivariate logistic regression analysis for relevant confounders showed that the type of treatment strategy (HDOS or MNC) was not significantly associated with OPR (OR 0.6, 95% CI 0.2-2.07).
Limitations, reasons for caution: In spite of its large sample size, the main limitation is the retrospective design of our study, with an inherent risk of bias. OPR was selected as primary endpoint due to the low number of live births encountered in this population, which precluded the application of a valid statistical approach.
Wider implications of the findings: In advanced-age poor ovarian responders as defined by the Bologna criteria, MNC and HDOS appear to be equally effective. Since MNC is a more patient-friendly approach, this strategy emerges as a reasonable alternative in these poor prognosis patients, when they reject the option of oocyte donation.
Trial registration number: not applicable

P-638 A shorter duration of estrogen administration yields equivalent live birth rates in non-downregulated hormone replacement frozen thawed embryo transfer cycles

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Study question: Does a shorter duration of estradiol (E) exposure prior to progesterone (P) administration decrease live pregnancy rate (LBR) in frozen thawed embryo transfer (FTET) cycles?
Summary answer: Shorter E exposure does not significantly affect LBR in FTET cycles.
What is known already: There is a little consensus regarding the necessary minimal duration of E supplementation in FTET cycles. The usual practice is to administer E for 12-14 days prior to initiation of P.
Study design, size, duration: A retrospective cohort study was conducted in the American Hospital (Koc University affiliated private hospital) between October 2015 and October 2017. All FTET cycles with a single blastocyst transfer using artificial endometrial preparation without GnRH agonist pretreatment were included. Only G1 and G2 blastocysts had been cryopreserved. Women >43 years of age, treatment cycles with incomplete data and women who were lost to follow-up were excluded.
Participants/materials, setting, methods: All women received oral E treatment starting on day 2 of menstrual bleeding in a fixed dose of 6mg/day. The duration of E administration varied from 8 to 14 days due to concerns regarding embryo transfer scheduling. A total of 241 frozen thawed single blastocyst transfer cycles were analyzed according to the duration of E administration (Group 1: <12 days, n=105; Group 2: >12 days, n=136). The main measure outcome was LBR per transfer.
Main results and the role of chance: Among a total of 241 frozen thawed blastocyst transfers, E exposure prior to P supplementation was as follows: 8 days (3%), 9 days (8%), 10 days (16%), 11 days (16.5%), 12 days (22.5%), 13 days (20.5%), 14 days (13.5%). LBR was not significantly affected when the E treatment was administered for less than 12 days (OR=1.25; 95%CI [0.75-2.1], p=0.38). Mean endometrial thickness was similar among the groups (9.5mm). When Group 1 was sub-divided according to the duration of E treatment, early loss and clinical loss rates were likewise similar.
Limitations, reasons for caution: Retrospective design of the study may have inevitably resulted in bias that may affect the conclusions.
Wider implications of the findings: Shorter than the normally accepted duration of E administration yields similar live birth rates in FTET cycles, thus questioning the need to adhere strictly to an untested protocol.
Trial registration number: Not applicable

P-639 Live baby rate per oocyte with no stimulation

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Study question: What is the age adjusted intrinsic fertility rate of human oocytes with no stimulation or with only clomiphene citrate stimulation?
Summary answer: Live baby rate per oocyte with no stimulation is similar to that with minimal stimulation, is age dependent, and 4-5 times greater than with hyperstimulation.
What is known already: Ovarian hyperstimulation has been found to yield a live baby rate per oocyte of only about 4-6%. Thus on average more than 20 to 25 oocytes would be required to produce a single live baby. But this is in stimulated cycles. The live baby rate per oocyte in a natural cycle or a minimal stimulation cycle with single embryo transfer IVF would estimate better the intrinsic fertility of the human oocyte.
Study design, size, duration: 13,949 oocyte retrievals were performed in a natural cycle program with single embryo transfer in women from age 29 to 47 years, and 839 women (mean age: 38.4±0.1 y; 2,488 cycles) underwent clomiphene citrate only minimal stimulation cycle IVF (mini-IVF). Live birth rate per oocyte retrieved were retrospectively analyzed. The total live birth rate per oocyte was calculated and compared to what has been previously reported for controlled ovarian hyperstimulation (COH).
Participants/materials, setting, methods: In a private Japanese IVF center, infertile women underwent natural cycle with a single embryo transfer or clomiphene citrate only mini-IVF. The primary data of live baby rate per oocyte was approximated with a logistic curve r +1 (a+exp[b(t-c)]) where r is live baby rate per oocyte and t is age in years. The coefficients were evaluated using gradient method as implemented in statistical package R (version 3.2.5).
Main results and the role of chance: The fertility per oocyte was similar for both natural cycle and clomiphene citrate stimulation, and 4-5 times greater than reported for hyperstimulation. For women ≤42 years, the overall live baby rate per oocyte was 18%, which translated into only 5.5 oocytes needed to produce one baby. For women 42 years of age, every oocyte would have a 4% chance of becoming a baby, which means for a 42 year old woman it would require 22.7 rather than 5.5 eggs to produce a baby. The drop in intrinsic fertility per oocyte is summarized remarkably robustly in a logistic curve. There is at first a steady (almost horizontal) maintenance of fertility per oocyte (26%), followed after age 34 with a sharp linear decline until age 42 (4%), with a 10% loss of fertility every year. This decline slows down after age 43, with only 3% of original fertility remaining at age 45.
Limitations, reasons for caution: This is a retrospective study, and the hyperstimulation cohort is just from the reported literature. Also there is the inevitable likelihood of variation in treatment techniques by different physicians.
Wider implications of the findings: Overstimulating IVF patients may create extra expense and complications without improvement in success rate. Furthermore IVF of itself without any other factors, even in the absence of ovarian stimulation, can help infertile couples become successfully pregnant, possibly correcting problems of ovum pickup, sperm transport, tubal dysfunction, and even timing.
Trial registration number: None

P-640 Lipid Profiling of Endometrium at the Window of Implantation in Patients with Premature Progesterone Rise on the day of human chorionic gonadotrophin administration

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Study question: Do patients with premature progesterone rise at the end of the follicular phase affect endometrial lipids metabolism at the window of implantation?
Summary answer: We found the lipid profiling alteration at the window of implantation in patients with high progesterone level (P ≥1.5) on the day of HCG administration.
What is known already: Premature progesterone rise in stimulated in vitro fertilization (IVF) / intracytoplasmic sperm injection (ICSI) cycles negatively affects the outcome of assisted reproductive techniques due to worsen endometrial-embryo asynchrony. But, there is still no study to investigate the effect of abnormally elevated progesterone level on endometrial lipid metabolism during the window of implantation.
Study design, size, duration: This was a retrospective cohort study with 43 patients undergoing IVF/ICSI by the reason of tubal factor or male factor in Center of Reproductive Medicine, the Sixth Affiliated Hospital, Sun Yat-sen University from 2014 to 2016.
Participants/materials, setting, methods: The endometrium tissues were obtained by pipelle biopsy 7 days after human chorionic gonadotropin (hCG) administration from the patients underwent GnRH-agonist long protocol or GnRH antagonist protocol and cancelled embryo transfer due to high estradiol level or lack of embryo. We evaluated lipidomics variation of endometrium by ultra high performance liquid chromatography coupled with electrospray ionization high-resolution mass spectrometry (UHPLC-ESI-HRMS).
Main results and the role of chance: The patients were divided into high progesterone group (15 patients) and control group (28 patients) on the day of hCG administration. There were no significant differences in age, BMI, basal FSH, LH and E2 level between the two groups. A total of 1026 ions were identified and 25 lipids were showed significantly up-regulated. The endometrium lipid profile of endometrium at the window of implantation was characterized by significant increase in concentration of phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), diacylglycerol (DG), ceramide (Cer), phosphatidylinositol (PI), phosphatidylserine (PS) in patients with premature progesterone rise at the end of the follicular phase. The correlation analysis between P level with the lipids showed stronger negative correlation between PE(18:0p/18:1)+H with P level(r= -0.5235, P= 0.0003), PE(18:1p/18:1)-H with P level (r= -0.6060, P= 0.0006), PE(18:0p/18:1)-H with P level (r=-0.5003, P= 0.0006) and PS(16:0/19:0)-H with P level (r= -0.6340, P= 0.0063). In order to rule out the effects of confounding factors, we distinguished estrogen levels. We found the stronger negative correlation only between PS (16:0/19:0)-H with P level (r=-0.6527, P= 0.0083) and PE (18:1p/18:1)-H with P level (r= -0.5697, P= 0.0045).
Limitations, reasons for caution: Limitations include the retrospective design and small sample size in low estrogen patients on HCG day to exclude the effect of high level estrogen on lipids metabolism.
Wider implications of the findings: We investigated the lipid profiling alteration at the window of implantation in patients with premature progesterone rise for the first time. The alterations of lipids at the window of implantation suggest an adverse effect of elevated progesterone on endometrial receptivity. Our findings also provide potential targets for endometrial receptivity.
Trial registration number: N/A

P-641 improvement of ovarian function and oocyte quality by water-extracted perilla frutescens in aged female mice

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Study question: To investigate whether administration of water-extracted Perilla frutescens (PF) rescues ovarian function and oocyte quality in aged female mice
Summary answer: The beneficial effects of PF on ovarian function and oocyte quality may be attributed to the activation of ovarian angiogenesis and follicular development.
What is known already: Women of advanced reproductive age have a high risk of infertility. A recent study showed that water-extracted Perilla frutescens (PF) increases endometrial receptivity though leukemia inhibitory factor-dependent expression of integrins. In addition, PF has been well known to contain various chemical compounds, such as phenolic compound, flavonoid, and anthocyanin, which exhibited antioxidant properties. However, there are no reports on the effects of PF on ovarian function, oocyte quality, and oocyte development competency, especially for reproductive aged female.
Study design, size, duration: Study design is controlled experimental study.
Participants/materials, setting, methods: Female mice were treated orally with PF of 26.5mg/kg (n=7) and 53mg/kg (n=7) for 4 weeks. The control group (n=7) was treated with normal saline. Ovaries and serum were collected for the H&E stain and the evaluation of ROS levels. After another 21 female mice were

administered with PF, followed by mated with male. Zygotes were retrieved and cultured for 4 days. Ovaries were provided for examination of expressions of target genes.

Main results and the role of chance: PF significantly increased numbers of surviving follicles (primary, secondary, and antral), numbers of zygotes retrieved, embryo development rate, and ovarian expression of VEGF, visfatin, cKit, BMP-15, and GDF-9 at both doses. However, ovarian expression of Sirt1 and Sirt2 was increased at PF of 53mg/kg. Serum ROS levels were not affected by PF.

Limitations, reasons for caution: This study experimented on 8-monthold mice. This age may be around 40 years of age in humans considering the sexual maturity. Therefore, it is necessary to investigate whether PF improves ovarian function, oocyte quality and further fertility in older mice aged corresponding to around 50s of women.

Wider implications of the findings: The beneficial effects of PF may be attributed to the activation of ovarian angiogenesis and early follicular development, but may not associated with the reduction of agerelated increase in oxidative stress and anti-aging events. This study can be implicated to aging related infertility.

Trial registration number: not applicable

P-642 Accumulation of advanced glycation end products in follicles is associated with poor oocyte quality: inducing inflammation in granulosa cells via activation of unfolded protein response

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Study question: Does advanced glycation end products (AGE) accumulated in follicles induce inflammation via unfolded protein response activation in granulosa cells (GCs) and thus affect oocyte quality?

Summary answer: Follicles containing poor-quality oocyte showed higher free AGE levels, and AGE stimulated interleukin-6 and 8 production in GCs via activation of activating transcription factor 4.

What is known already: The levels of soluble receptor for AGE (sRAGE), that antagonizes the deleterious effects of AGE, in serum and follicular fluid serve as positive predictive markers of the number of oocytes retrieved in IVF. The accumulation of AGE is reported in GCs of women in their late reproductive age and of PCOS patients. The close relationship between AGE, proinflammatory cytokine production, and induction of endoplasmic reticulum (ER) stress is observed in various pathologies, including atherosclerosis and osteoarthritis. ER stress activates a group of signal transduction cascades termed unfolded protein response (UPR), which includes activating transcription factor 4 (ATF4).

Study design, size, duration: AGE and sRAGE concentrations in follicular fluid were examined in 42 follicles of 21 patients (37.1 ± 5.1 years old, average ± S.D.) who underwent ICSI treatment for male factor infertility. AGE and sRAGE concentrations in each follicle were analyzed according to the quality of enclosed oocyte, namely, whether the oocyte yielded poor or good-quality embryo. For *in vitro* experiments, human granulosa-lutein cells (GLCs) were obtained from pooled follicular fluids from patients undergoing oocyte retrieval.

Participants/materials, setting, methods: The concentrations of AGE and sRAGE were examined by ELISA and quality of embryos was retrospectively reviewed in medical charts. Human GLCs were incubated with AGE for 24 or 48 hours, while GLCs were transfected with siRNA prior to the treatment with AGE to examine the intermediary role of ATF4. Interleukin (IL)-6, IL-8, and ATF4 mRNA expression was examined by quantitative RT-PCR. Protein secretion of IL-6 and IL-8 in the supernatant was examined by ELISA.

Main results and the role of chance: The levels of AGE in follicular fluid were increased with a marginal significance in follicles with poor-quality oocytes, compared with those with good-quality oocytes (9.32 ± 1.47 μg/mL vs 5.97 ± 1.81 μg/mL, average ± SEM; p=0.0852), while there was no significant difference in the levels of sRAGE, a decoy receptor for AGE, between the two groups (3.65 ± 0.34 μg/mL vs 4.21 ± 0.38 μg/mL; p=0.1573). The resultant AGE/sRAGE ratios, which indicate the intrafollicular level of free AGE that can bind to membranous receptor for AGE (RAGE) and activate intracellular signaling cascades, were significantly higher in follicles with poor-quality oocytes, compared with those with good-quality oocytes (2.65 ± 0.39 vs 1.36 ± 0.36; p=0.0166). Treatment of cultured human GLCs with AGE increased ATF4, IL-6 and IL-8 mRNA expression in a dose-dependent manner. Knockdown of ATF4 abrogated the stimulatory effects of AGE on mRNA expression and protein secretion of IL-6 and IL-8. AGE stimulated the production of inflammatory cytokines including IL-6 and IL-8 in cultured human GLCs via activation of ER stress, especially the UPR branch of ATF4.

Limitations, reasons for caution: Small sample size for the evaluation of AGE and sRAGE concentrations in follicular fluids is a limitation of this study. The direct effect of increased production of IL-6 and IL-8 by granulosa cells, stimulated by AGE, on oocyte quality was not examined in the present study.

Wider implications of the findings: AGE in follicles affects oocyte quality by inducing inflammation in follicular microenvironment via activation of ER stress in granulosa cells. Administration of RAGE antagonists and/or ER stress inhibitors could improve embryo quality in patients with high ovarian AGE, including those in their late reproductive age and PCOS patients.

Trial registration number: not applicable

P-643 rescue of age-related decline in fertilty of female mice aged 18 months by visfatin

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Study question: This study investigated whether visfatin stimulates the mTOR/PI3K and Hippo signaling pathways associated with the activation of primordial follicles and OGSCs and rescues age-related decline in fertility in mice.

Summary answer: The present study shows that administration of visfatin improves ovarian function and fertility potential at different doses in female mice aged 12 and 18 months.

What is known already: Visfatin activates migration, invasion, and tube formation in human umbilical vein endothelial cells (HUVECs). Moreover, visfatin evokes activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) in endothelial cells, which is closely linked to angiogenesis. Inhibition of ERK activation markedly decreases visfatin-induced tube formation of HUVECs and visfatin-stimulated endothelial cell sprouting from rat aortic rings.

Study design, size, duration: age-course (C57BL/6 female mice aged 12 and 18 months),

control versus treatment (intraperitoneally injected with 0.1 ml of 500 ng/ml or 1,000 ng/ml of rmVisfatin for three times at intervals of two days),

Participants/materials, setting, methods: C57BL/6 female mice aged 12 and 18 months were intraperitoneally injected with 0.1 ml of 500 ng/ml or 1,000 ng/ml of rmVisfatin for three times at intervals of two days. The mice were superovulated with 5 IU PMSG and 5 IU hCG injection 48 hours later. Zygotes were cultured for 4 days after mated with male. Then, we analyzed the expression of 4EBP1, S6K1, RPS6, MST!, OCT4, VEGF, visfatin and SDF1a in ovaries by RT-PCR.

Main results and the role of chance: In both ages, visfatin treatment significantly increased the number of zygotes retrieved and embryo development rate to blastocyst and finally improved fertility potential compared to the control at different doses depending on age ($P < 0.05$). Visfatin increased ovarian expression of S6K1, RPS6, MST1, VEGF, visfatin, and SDF-1α just in mice aged 18 months, but not in mice aged 12 months. There was no difference in ovarian expression of Oct4 in both age groups.

Limitations, reasons for caution: The limitation of this study is the 12 and 18 months aged C57BL6 mice in vitro.

Wider implications of the findings: The present study shows that visfatin increases ovarian expression of molecules associated with angiogenesis as well as the mTOR/PI3K and the Hippo signaling pathways at the same dose that enhanced ovarian function and fertility in mice aged 18 months, but not in mice aged 12 months.

Trial registration number: Not applicable

P-644 Self-detection of the endogenous LH surge using urine testing as a simple and efficient confirmation of successful GnRH agonist trigger in IVF.

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Study question: How can we ascertain that an endogenous LH surge was efficiently induced by a GnRH agonist (GnRHa) trigger in order to avoid oocyte retrieval failure?
Summary answer: Urine LH testing performed at home by the patient is a simple, cheap, and efficient way to confirm the efficacy of a GnRHa trigger.
What is known already: Since GnRHa trigger was introduced in daily practice, questions have arisen about how to detect involuntary errors in the administration of the medication or patient compliance, particularly in oocyte donors. When hCG is used as a trigger, a urine pregnancy test can easily detect if hCG was administered or not. As for GnRHa trigger no recommendations exist, however, some clinics advocate LH testing in blood the morning after the trigger. From a practical point of view this is inconvenient for the patient/donor as she needs to visit the center again.
Study design, size, duration: Prospective cohort study including a total of 371 oocyte donors from May 2017 till November 2018, and approved by our IRB (1701-MAD-006-JG). Urine LH testing was performed at home, using the same test (Ruedafarma, Spain, sensibility 25 mIU/mL) and urines from the first micturition in the morning after the GnRHa trigger. Donors had to send a picture of the LH test outcome to the nurse by WhatsApp as soon as the test was performed.
Participants/materials, setting, methods: A total of 371 oocyte donors were included in the study. A single dose of 0.2 mg GnRHa (Decapeptyl 0.1 daily, Ipsen Pharma, Spain) was administered to trigger final oocyte maturation when at least three follicles reached a mean diameter of 17 mm. The nurse instructed the donor to self-administer the GnRHa at night according to the time of retrieval, and to perform the urine LH test next morning with first micturition.
Main results and the role of chance: Of a total of 371 donors included in the study, 12 forgot to perform it, leaving a total of 359 oocyte donors and oocyte retrievals for analysis. A total of 355 donors had (98.88%) positive LH testing and an uneventful oocyte retrieval with good retrieval rates. One test was positive, however, no oocytes were retrieved (false positive rate 0.28%). A total of three tests were negative (false negative rate 0.85%). In one of these cases, LH was tested in serum and values confirmed an LH rise consistent with a GnRHa trigger. In two cases, the pickup was rescheduled after hCG trigger and a good number of mature oocytes were retrieved.
Limitations, reasons for caution: Failure to respond to a GnRHa trigger in terms of an adequate LH rise is a relatively rare phenomenon, and a future larger sample size is needed to confirm the findings of this study.
Wider implications of the findings: This new approach of LH surge self-testing in urine and communication via a picture provides a simple and cheap method to confirm that LH rise was induced by a GnRHa trigger. It may help to detect errors in the administration, improving patient compliance and minimizing failure to retrieve oocytes.
Trial registration number: not applicable

P-645 Endometrial fluid aspiration with diosmin intake in ART cycles: A randomized controlled trial

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Study question: To evaluate the effect of aspiration & diosmin intake where endometrial fluid is present at ovum pick up in ART cycles.
Summary answer: Endometrial fluid was decreased in cases where aspiration & diosmin was given with increased chance of fresh embryo transfer and without affecting the pregnancy rate.
What is known already: Endometrial fluid accumulation in controlled ovarian stimulation occurred in 2.9% - 8.2% of ART cycles. When present without hydrosalpinx, the mechanism is not well understood. Theories include: over response of the endometrium to gonadotropin therapy, subclinical endometrial infection & obstruction of the cervical canal with the hCG administration.
Diosmin was widely used for the treatment of varicose veins & pelvic congestion syndrome. It causes a reduction in plasma levels of endothelial adhesion molecules, neutrophil activation, inflammatory mediators, such as prostaglandin E2 (PGE2) and thromboxane A2 (TxA2). Thus, providing protection against microcirculatory damage.
Study design, size, duration: This was a prospective randomized single blinded controlled trial in 100 cases presented with endometrial fluid at the day of ovum pickup in their ART cycles from 2014-2018.
Participants/materials, setting, methods: The study was conducted at a private fertility center. Cases were divided into 2 groups. Group 1 (50 cases): conservative management with follow up by ultrasound at day 5. Group 2 (50 cases): aspiration of the fluid was done by an intrauterine insemination catheter then the cases were given diosmin 500 mg thrice daily till day 5. In both groups, fresh embryo transfer was conducted if fluid resolution occurred or freeze-all policy if still present.
Main results and the role of chance: Our main outcome was the presence of endometrial fluid at Day 5 embryo transfer. It persisted in 31 case in group1 (conservative management) versus 2 cases in group 2 (aspiration+diosmin) with a highly significant difference (P value < 0.0001). All the cases where endometrial fluid persisted at Day 5, underwent freeze-all policy. If no endometrial fluid was detected at Day 5, fresh embryo transfer was done. That included 19 cases in group1 versus 48 cases in group 2. Clinical pregnancy was detected in 10 cases in group 1 versus 23 cases in group 2 (52.63% vs 47.92%). The difference was not statistically significant between both group s (p value= 0.73).
Limitations, reasons for caution: 1- Single blind, not double blind study.
2- The number of cases is small.
Wider implications of the findings: Aspiration of endometrial fluid & diosmin have a role in decreasing the presence of endometrial fluid. However, this was a pilot study on a small number of cases due to the low incidence of the condition. A wider application on a bigger sample size is warranted.
Trial registration number: NCT02158000

P-646 The pretreatment with collagenase before vitrification improves the ovarian reserve due to the maintenance of the cell-cell attachments with oocyte and granulosa cells

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Study question: Why is the ovarian reserve decreased in the frozen-thawed ovary? How are ovarian functions kept after vitrification?
Summary answer: The cell-cell adhesion in ovary was broken by the high-osmotic solution. The pretreatment with collagenase kept the communications in the vitrificated ovary.
What is known already: The auto-transplantation of cryopreserved ovary is used for fertility preservation of women before anti-cancer chemotherapy. The slow-freezing method is commonly used for ovarian cryopreservation and the vitrification is also adapted. More than 100 live births were achieved after transplantation of cryopreserved human ovarian tissue. However, most of the developed follicles induced atresia when the frozen-thawed ovary was cultured or transplanted. The ovarian reserve judged by the circular AMH level was much lower when frozen-thawed ovary was transplanted as compared with the transplanted fresh ovary. Thus, the method of ovarian cryopreservation has been insufficient to become the general medical.
Study design, size, duration: To examine the influence of vitrification treatment on the ovarian morphology, the mouse ovaries were exposed to hyper-osmotic vitrification solution and were made for paraffin sections. To loosen the tight connections between ovarian stroma and follicles, ovaries were treated with the different dose of collagenase before vitrification procedure. After ovarian replacement-transplantation were done to adult female mouse,

the estrus cycle, the reproductive performance and the circular level of AMH were analyzed up to 6 months.

Participants/materials, setting, methods: The vitrification of mouse ovaries was performed using Ova Cryo Kit. The morphology was observed by HE staining and the localization of cadherin was detected by immunofluorescence staining. After transplantation of frozen-thawed ovaries, the estrus cycle was observed by vaginal smear test. To evaluate the reproductive performance, the female mice in which ovarian transplantation was done were mated with male mice. The circular level of AMH was analyzed by mouse AMH ELISA kit.

Main results and the role of chance: The space between oocyte and granulosa cells was observed in secondary follicle and antral follicles of ovaries exposed to hyperosmotic vitrification solution. The space was disappeared in frozen-thawed ovaries under the isotonic condition. However, the positive signals of anti-pan cadherin antibody were not detected between them, suggesting that the cell-cell adhesions were broken during the vitrification process. To relax the follicles from ovarian stroma, especially collagen-rich membrane, ovaries were treated with different doses of collagenase. When ovaries were treated with 10 μg/ml of collagenase for 5 min, the collagen-rich membranes were disappeared in stroma. The treated ovaries were exposed to hyperosmotic vitrification solution and then moved to liquid nitrogen. The frozen-thawed ovaries were transplanted to the receipient adult female mouse. The estrus cycles were recovered at 8 days after transplantation of frozen-thawed ovaries that were pretreated with collagenase, and the period was 4 days earlier than the frozen-thawed ovaries without the collagenase pretreatment. Furthermore, the circular levels of AMH at 6 months after transplantation of frozen-thawed collagenase pretreated ovaries were similar to those in same age mice without the ovarian transplantation. The average numbers of pups for 6 months were significantly increased by the collagenase treatment.

Limitations, reasons for caution: Because the characteristics of ovarian stroma were different among species, it is necessary to find the optimal condition of collagenase pretreatment in each animal species.

Wider implications of the findings: The cell-cell adhesions between oocyte and granulosa cells were disrupted by the vitrification procedure in ovary. The different shrink speeds between oocyte and follicular somatic cells would be induced by the connection with ovarian stroma, which might be a possible reason for the disruption.

Trial registration number: not applicable

P-647 Interleukin-34(IL-34) and macrophage colony-stimulating factor(M-CSF) regulates macrophage colony-stimulating factor receptor(M-CSFR) and natriuretic peptide receptor2(NPR2) in human Granulosa Cells : potentially serving a role in ovulation

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Study question: Do IL-34 and M-CSF form a novel heteromeric cytokine and regulate the M-CSFR and NPR2 signaling in human granulose cells?

Summary answer: IL-34 and M-CSF regulate signal transduction associated with M-CSFR and NFR2 in human GC, which can affect the ovulation process.

What is known already: M-CSF, a hemopoietic growth factor with classic function of controlling the proliferation and differentiation of macrophages, has recently been shown to involve oocyte maturation and ovulation. It has been clear that action of M-CSF is exerted via a high-affinity transmembrane tyrosine kinase receptor(M-CSFR). M-CSF can modulate generation of natriuretic peptide precursor type C(NPPC). NPPC and its receptor(NPR2) have been assumed to be involved in female reproduction and have recently been shown to play an essential role in maintaining meiotic arrest of oocytes. IL-34, recently recognized as another ligand for M-CSFR, is a cytokine identified as protein present in human tissues.

Study design, size, duration: In a previous study, we found that the M-CSFR gene expressed in the GC of IVF patients was associated with a clinical outcome. It was also confirmed that M-CSF is involved in the expression of MCSFR and NPR2 genes. In this study, we tried to confirm whether IL-34 could play the same role as M-CSF. Compared to M-CSF, IL-34 facilitates phosphorylation of M-CSFR.

Participants/materials, setting, methods: We investigated expression levels of M-CSFR genes in luteinized GCs(210 IVF patients). M-CSFR expression patterns were divided into two groups and their results were compared with clinical outcome. To determine the effect of M-CSF and IL-34 on GC during folliculogenesis, GC was cultured in the culture medium containing rhM-CSF and rhIL-34(GC from 30 IVF patients). Cell proliferation was assessed by MTT assay and the expression of M-CSFR and NPR2 mRNA were analyzed by RT-PCR.

Main results and the role of chance: Expression of M-CSFR gene in GC obtained from 210 IVF patients was confirmed by RT-PCR. We compared the clinical outcome with two groups according to the presence or absence of M-CSFR gene expression. The pregnancy rate of the group in which M-CSFR was expressed was significantly higher than the group in which the M-CSFR was not expressed(72.7% ***vs.*** 17.12%). These groups were comparable with respect to patients' characteristics such as age of patients, infertility duration. GC culture was performed to confirm the effect of M-CSF and IL-34 on GC. In GC culture, 10 g/ml of rhM-CSF, rhIL34 and both were added to the culture medium. We determined the changes in expression of M-CSFR and NPR2 and proliferation of cell. The growth rate of GC cells grown in the medium supplemented with rhM-CSF, rhIL-34 and both was increased and M-CSFR gene expression was increased, while NPR2 gene expression was decreased($p<0.01$, respectively). We also examined the changes in GC according to Estrogen level; less than low-response, appropriate response and high-response group. Comparing three groups, we confirmed that the same change was not shown by the hormone response.

Limitations, reasons for caution: This experiment was carried out in human GC cultured in vitro. Unlike animal experiments, the use of human oocytes is limited. So we have not identified the outcome of oocyte maturation and did not know whether the same results would be obtained in vivo.

Wider implications of the findings: We confirmed that the expression of M-CSFR has positively correlation with IVF-ET pregnancy outcome. In rhM-CSF and rhIL-34, GC proliferation rate and M-CSFR expression were increased and NPR2 expression was decreased. Our study suggests that M-CSF and IL-34 influence folliculogenesis through direct action on GCs, also may regulate follicular development.

Trial registration number: Not applicable.

P-648 Do reproductive ageing and parity modulate the expression of VEGF/VEGFR2 and cell cycle control proteins?

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Study question: Can age- and parity-dependent comparative analysis of VEGF/VEGFR2 and of cell cycle control proteins help to identify alterations predisposing to ovarian cancer onset?

Summary answer: Nulliparity and parity can differentially modulate expression levels of VEGF/VEGFR2 and of cyclin D1, cyclin E, Cdk4, Cdk2, cdc25A in an age-dependent manner.

What is known already: Ovarian cancer (OC) is the third most common gynecological cancer, characterized by tardive diagnosis and high mortality. Women's advanced age plays a role in OC onset, and older patients show higher risk of peritoneal metastasis in comparison to younger patients. Important risk factors are considered also infertility *per se* and nulliparity. In fact, this is supported by the observation that OC is more frequent in infertile or nulliparous women than in those with at least one child, a correlation confirmed by the extreme example of catholic nuns.

Study design, size, duration: To point out how parity can exert a protective effect against OC onset, expression levels of VEGF/VEGFR2 and of proteins controlling cell cycle progression (cyclin D1, cyclin E, Cdk4, Cdk2, cdc25A) were determined in both nulliparous (Virgins, V; n=40) and parous (Mothers,

M; n=40) mice aged up to reach late (L)- or post (P)-reproductive age. Each group, i.e. LM, LV, PM, PV, consisted of 20 mice.
Participants/materials, setting, methods: CD1 female mice 8 weeks-old were either kept unmated (V) or mated (M). After the 1 full-term pregnancy, M were kept alone. Animals were housed up to 40-42 weeks or 66-70 weeks, corresponding to late-reproductive or post-reproductive age, respectively. Ovaries were collected from 4 different groups: LM, LV, PM, PV mice. Expression levels of VEGF-A, VEGFR-2, Cyclin E1, cyclin D1, Cdk4, Cdk2, cdc25A were detected by Western blot. Experiments were repeated at least 4 times.
Main results and the role of chance: VEGF expression was similar in L ovaries (M vs V, P>0.05), but in older M mice it decreased to low/undetectable levels (PM vs PV, P<0.05). Although total VEGFR2 content was unchanged, phospho-receptor content decreased significantly only in PM mice (P<0.05). Cyclin D1 content decreased significantly in post-reproductive ovaries, especially in M ovaries (PV, PM vs LM, LV, P<0.05). Cdk4 level was always higher in V than M mice (LM, PM vs LV, PV, P<0.05). A 3-fold increase in cyclin E1 content was recorded in PV but not in PM ovaries, where it was undetectable (PV vs LM, LV, PM, P<0.05). Also Cdk2 expression decreased drastically in PM (LM, LV, PV vs PM, P<0.05). Cdc25a expression was not altered in all L ovaries (P>0.05), but in older ovaries a 1.5-fold increase was recorded in PV (L vs PV, P<0.05). The above results evidence that ovarian levels of VEGF/VEGFR2 and cell cycle control proteins are modulated in a parity-dependent manner, and that older parous M mice show the lowest contents of VEGF/VEGFR2, cyclins and Cdks. Therefore, parity should exert a protective role by down-regulating expression levels of proteins that are commonly altered in OC.
Limitations, reasons for caution: The study was performed in a mouse model, and the correlation with human should be considered. Results on protein expression levels should be confirmed with human ovaries, before being utilized as useful markers of alterations predisposing to OC onset.
Wider implications of the findings: This report suggest that parity can exert a protective effect not only on mammary gland but also on ovary. The differences here recorded could be part of a more complex mechanism that, at least in mice ovaries, is activated after the first pregnancy and maintained throughout whole life.
Trial registration number: None

P-649 A multicenter, randomized study comparing the efficacy of follitropin alpha biosimilar and the original follitropin alpha

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Study question: To investigate the therapeutic equivalence between a novel follitropin alpha biosimilar and the reference medication in women undergoing IVF.
Summary answer: This study demonstrated similar therapeutic equivalence and safety profiles between two follitropin solutions in women who underwent controlled ovarian hyperstimulation (COH) in GnRH-antagonist cycles.
What is known already: Biosimilars are not exact copies of the reference molecule due to the differences in genetic modifications of host cell lines and the manufacturing process. The manufacturer of biosimilars is required to conduct randomized pharmacokinetic/pharmacodynamic studies aiming to demonstrate that two medicines are equal at clinical level. The results of randomized, crossover, comparative study on the bioequivalence of follitropin alpha biosimilar indicated that a 300 IU single dose of biosimilar exhibits pharmacokinetic and safety profiles comparable to those of original follitropin in healthy young women.
Study design, size, duration: A multicenter, randomized (1:1), embryologist-blinded, parallel-group, therapeutic equivalence study of two solutions of follitropin alpha. All of the subjects underwent COH using a GnRH-antagonist protocol. Over the 5-day fixed-dose regimen, the women received 150 IU/day of follitropin, followed by dose adaptation. A study power of 80% at a significance level of α=0.05, clinical equivalence margin of ±3.4 oocytes, the required sample size was 55 subjects per group and 110 subjects in total (intention-to-treat [ITT] population).
Participants/materials, setting, methods: The inclusion criteria were: women aged 20-35 years old, established causes of infertility: tubal and/or male factors, first or second attempt at IVF/ICSI; 18 ≤ BMI ≤ 30 kg/m^2; FSH 10< IU/l and oestradiol level <50 pg/ml; AMH≥1.0 ng/ml. The exclusion criteria were: women with established contraindications to the use of ART methods; PCOS; endometriosis. Of 118 women screened, 110 were randomized into the trial. Demographic and clinical characteristics were comparable between the treatment groups.
Main results and the role of chance: The number of oocytes retrieved is the recommended primary endpoint as stated by European Medicinal Agency for the development of biosimilars containing follitropin. Similar numbers of oocytes were retrieved in both treated groups: 12.16±7.28 in the follitropin alpha biosimilar group and 11.62±6.29 in the reference group, with mean difference of 0.546±1.297 oocytes (95% confidence interval [CI]: -2.026, 3.116), p-value for equivalence of p=0.002 (ITT population). Additionally, no statistically significant differences were found for secondary endpoints: (1) in the number of follicles (≥16 mm) on the day of trigger injection 12.09±6.159 and 11.38±4.965 [95% CI: -1.405, 2.824], p=0.709; (2) in the number of MII oocytes 9.64±6.27 and 9.86±5.55 [95% CI: -2.455, 2.019], p=0.617; (3) and 2PN zygotes 8.13±6.61 and 8.76±5.85 [95% CI: -2.995, 1.723], p=0.445 (ITT population). The mean total follitropin doses (IU) per treatment cycle were 1532.7±267.2 and 1517.9± 255.2 [95% CI: -83.9, 113.6] (p=0.488), duration of treatment (days) 9.75±1.08 and 9.73±1.03 [95% CI: -0.379, 0.416] (p=0.629) were similar in both groups. Ovarian hyperstimulation syndrome was observed in subjects with a positive pregnancy test in 0% and 3.64% of cases and after triggering ovulation in 7.27% and 3.64% for the biosimilar and reference medication groups, respectively.
Limitations, reasons for caution: Normogonadotrophic patients enrolled in this study were representative, showing the ability of exogenous FSH to the stimulate development of multiple follicles in women without endocrine and ovarian disturbances during COH. Additional comparative studies are needed to confirm efficacy of follitropin alpha biosimilar in patients with other causes of infertility.
Wider implications of the findings: In this study, we demonstrated the therapeutic equivalence in terms of oocytes retrieved in women undergoing COH with a GnRH-antagonist cycle. Further post-authorization studies will be conducted to evaluate the efficacy of biosimilar in patients undergoing ART in GnRH-agonist cycles and with other causes of infertility: endometriosis, PCOS, poor response.
Trial registration number: ClinicalTrials.gov: NCT03088137. Date of registration: 2 March, 2017, retrospectively registered; conducted between 08.02.2017 and 17.08.2018.

P-650 Comparison between adjuvant 1500I.U. hCG + GnRH agonist on trigger day or 1500 I.U. hCG 35-36h later, in OHSS high-risk patient with peak E2 level<4000pg/mL

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Study question: Study question is to compare the ongoing pregnancy and live birth rates between 1500IU.of hCG at the time of GnRH agonist triger day or 35-36h later on the OPU day.
Summary answer: Results suggest that adjuvant1500IU of hCG at the time of GnRH agonist trigger day significantly improve clinical pregnancy and live birth rates in high-risk patient.
What is known already: Ovarian hyperstimulation syndrome is serious complication of controlled ovarian hyperstimulation.The use of GnRH- a for final oocyte maturation in GnRH antagonist cycle significantly decrease the incidence of OHSS, but there have been studies showing lower pregnancy rates in patients triggered with GnRHa compared with hCG in autologous cycles.

The lower pregnancy rates after GnRH-a trigger have been attributed to a defective luteal phase. By providing intensive luteal-phase support satisfactory pregnancy rates can be sustained,but the probability of conception after GnRH-a trigger was significantly lower in patients with estradiol peak<4,000pg/mL despite intensive luteal phase support.

Study design, size, duration: This single center prospective study encompassed the period from March2016 to March 2018 year. The initial inclusion criteria were:women age ≥18 years and ≤35 years, with FSH <10mIU/mL, AMH≥4 ng/ml and have PCOSy/PCOM.All patients have ≥14 follicles and peak estradiol level <4000pg/ml, on trigger day. Patients with peak estradiol ≥4000pg/ml on trigger day, patients with significant risk for OHSS, where freeze all of oocytes or embryos were performed or OPU with no ET, were excluded.

Participants/materials, setting, methods: Total of 217patients were entered for final analysis, underwent antagonist protocol, ICSI and ET on 3th or 5th day.Patients were randomized in one of two groups:Group A- Dual trigger group -1500IU. of hCG at the time of GnRH agonist trigger day and Group B-1500IU. of HCG 35-36 h later, on the OPU day. For comparison of the groups 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 of man and women, previous IVF attempts, AMH, PCOSy / PCOM, normospermia, oligospermia and duration of COS. Peak estradiol level were significantly higher in group B(2851.3 vs 2262.9 pg/ml, p < 0,05), peak LH level were non significantly higher in group B(2,55 vs 2,17) on the trigger day. There is approximately to or no significant difference between the two(A vs B) groups according to average number of retrieved oocytes(13.08 vs 14.41 p < 0,08), M II oocytes(10.5 vs 10.95 p<0.47),GV(1.24 vs 1.52 p<0.09),fertility rate(68.46 % vs 64.04 % p<0,07).The dual trigger group(A) had a higher live birth rate(62,29 % vs 40,68%), and ongoing pregnancy rate(64,29% vs 45,76%) compared with GnRH-a trigger group(B). When the LH level on the day of trigger is≤1,0 IU/L the dual trigger group(A) had a significantly more MII oocytes(11,61 vs 9,95), higher fertility rate(66,75% vs 59,72%) ,significantly higher percentage of blastocyst transfer (74,49 % vs 54,24%) , higher live birth rate (60,00% vs 29,41%), and higher ongoing pregnancy rate(60,00% vs 35,29%) compared with GnRH-a trigger group(B). There were no cases of moderate or severe OHSS in both groups.

Limitations, reasons for caution: Our study should be further investigated.Prior to the dual trigger's routine implementation, further large prospective studies are needed.Dual trigger in GnRH antagonist protocols should be advocated as a safe approach but in undetected high risk patients reasons for caution is developing clinically significant OHSS.

Wider implications of the findings: Adjuvant low dose of hCG(1,500 IU) at the time of GnRH agonist trigger day improve clinical pregnancy and live birth rates in high responders with peak E2<4,000pg/mL without increasing the risk of clinically significant OHSS.Protocol of dual trigger and freezing all oocytes or embryos is promising technique in everyday practice.

Trial registration number: N/A

P-651 Quality Approaches in Adipose Tissue; Differences in Fatty Acid Profiles of Subcutaneous Adipose Tissue among Pregnant Women with PCOS and Non-PCOS

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Study question: Are there differences between fatty acid (FA) profiles and desaturase indices in subcutaneous adipose tissue (AT) of PCOS and non-PCOS pregnant women?

Summary answer: Eicosapentaenoic acid (*EPA*; C20:5 n-3) were 10-times lower in AT of PCOS than non-PCOS. In contrast, monounsaturated FAs and desaturase indices were elevated in PCOS.

What is known already: Whereas the crucial roles of AT quantity were understood, little information exists on the quality of AT in PCOS. FA profiles of AT are established long-term biomarker for FA status, but AT samples are not easily available. Limited studies illustrated the association between FA profiles and gene expression in AT of non-PCOS women which warrants further studies for FA profiles in AT of PCOS. Moreover, the mother must meet her own needs as well as meeting the requirements of the developing infant by her AT supports and FA profiles imbalance in AT of PCOS mother may deal with infant's health.

Study design, size, duration: Following permission from the Royan Institute ethics committee and after written consent was obtained from all subjects, demographic data and abdominal subcutaneous AT samples were collected. The subjects were twenty-four age-matched and BMI-matched Iranian pregnant women underwent cesarean section (12 PCOS and 12 non–PCOS; age 31.8 ±1.1 and BMI at delivery day 30.5±1.5 kg/m^2). Intake of any medication affecting lipid metabolism and diabetic, alcoholic, smoking defined as exclusion criteria.

Participants/materials, setting, methods: At the time of cesarean section, three grams of AT were collected, washed, segmented, floated in liquid nitrogen and storage. Gas chromatography with a 100 meters capillary column was utilized to determine the concentration of FA. Data were initially tested for normal distribution using Kolmogorov-Smirnov test. Data with normal distribution were analyzed using *t* test. Data that did not have normal distribution were analyzed using Mann–Whitney U test. All analyses were conducted in SAS.

Main results and the role of chance: No significant differences were found with respect to age and BMI at delivery day. Total monounsaturated FA (MUFA), EPA, C18:1/C18:0 and C16:1/C16:0 index in subcutaneous AT were significantly different among experimental groups. Total MUFA and desaturase indices were significantly lower in non-PCOS than PCOS (P<0.05). In contrast, total n-3 FA in non-PCOS group was significantly higher than PCOS (1.11 *vs.* 0.97%; P<0.05). Interestingly, EPA (C20:5n3) percentage of non-PCOS was also much higher (10-fold) than observed in PCOS (0.10% *vs.* 0.01%, P<0.001). Saturated FA, unsaturated FA, odd chain FA, total n-6 FA, total poly unsaturated FA (PUFA), n-6/n-3 ratio and saturated: unsaturated ratio were similar in PCOS and non-PCOS groups. Palmitic acid (C16:0) was the most prevalent with 23 % and 21.7% for non-PCOS and PCOS, respectively that were similar among PCOS and non-PCOS. Unsaturated FA accounted for more half (68.6% of total fatty acids), the major being oleic acid (18:1 n-9 *cis*). Saturated: unsaturated ratio were 0.43 for non-PCOS and 0.40 for PCOS which they were unaltered in PCOS and non-PCOS groups. The mean composition of total PUFA also were present no significant differences between two groups (22.5 % and 20 % for non-PCOS and PCOS, respectively).

Limitations, reasons for caution: Although we collected 24 samples during one year, the small sample size is a limitation. Nevertheless, the strength of the observed differences in aged-matched and BMI-matched subjects suggests that power was not an important issue and that a larger study would be able to detect same findings.

Wider implications of the findings: Our study opens the road for exploration regarding FA and AT metabolism for PCOS. This is the first report for Iranian mother's FA profiles in AT and this attitude may improve the aspects of maternal nutrition in Iran.

Trial registration number: -

P-652 A novel nomogram for individualized gonadotropin starting dose in GnRH antagonist protocol: a prospective cohort study

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Study question: To develop a predictive nomogram for individualized gonadotropin starting dose in gonadotropin releasing hormone (GnRH) antagonist protocol.
Summary answer: We developed a clinically practical nomogram for prediction of appropriate gonadotropin starting dose.
What is known already: AMH, AFC and BMI are the most importent fact affect the outcome of the control ovarian stimulation. Starting dose is one of the most imprtant aspect in GnRH antaonist protocol.
Study design, size, duration: This is a single-center prospective cohort study between April 2018 and July 2018.A total of 198 women aged 20-45 years underwent in vitro fertilization/ intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) cycles.
Participants/materials, setting, methods: The women undergoing COS with a GnRH antagonist protocol at the Reproductivecenter of the First Affiliated Hospital of Sun Yat-sen University.

All patients received ovarian stimulation using GnRH antagonist protocol. Univariate and multivariate analysis were performed to identify predictive factors of ovarian sensitivity (OS). A nomogram for gonadotropin starting dose was developed based on the multivariate regression model. Validation was performed using concordance statistics and bootstrap resampling
Main results and the role of chance: A multivariate regression model based on serum anti-Müllerian hormone (AMH) level, antral follicle count (AFC), and body mass index (BMI) was developed and accounted for 59% of the variability of OS. An easy-to-use predictive nomogram for gonadotropin starting dose was established with excellent accuracy. The concordance index (C-index) of the nomogram was 0.833 (95%CI, 0.829-0.837). Internal validation using bootstrap resampling further showed the good performance of the nomogram.
Limitations, reasons for caution: If the patient's BMI is too higher such as more than 30, and the patient has FSH recepor mutation gene, the predictive nomogram may be not **accuracy. Because the** predictive nomogram was developed from normal BMI women(BMI less than 30).
Wider implications of the findings: In the antagonist protocol, it is estimated that using the predictive nomogram to determining the Gn starting dose could help to obtain a satisfactory number of eggs.
Trial registration number: ChiCTR1800015081

P-653 Natural estradiol in combined oral contraceptive has a favorable inflammation and lipid profile compared with preparation containing ethinyl estradiol– a randomized controlled trial

Abstract withdrawn by the authors

P-654 Impact of supraphysiologic estradiol serum levels on birth weight in singletons born after fresh embryo transfer

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Study question: Does estradiol level at trigger-day impact low-birth weight incidence in singleton born after fresh transfer in a center practicing 'freeze-all' strategy to prevent ovarian-hyperstimulation syndrome?
Summary answer: In controlled ovarian stimulation followed by a fresh embryo transfer, estradiol (E2) level at trigger-day is not associated with low-birth weight (LBW) in singleton babies.
What is known already: Assisted reproductive technology is known to increase the risk of LBW. Factors involved are not yet clearly identified but high level of E2 on the day of hCG trigger could be implicated. Since the expansion of "freeze all" policy to prevent the ovarian hyperstimulation syndrome, embryos are mostly cryopreserved in case of high E2 level.
Study design, size, duration: An observational retrospective cohort study was conducted in a tertiary care university hospital between November 2012 and January 2017. All women having a live singleton birth after fresh embryo transfer were included. In case of high risk of ovarian hyperstimulation syndrome women did not have a fresh transfer but benefited from a "freeze all" strategy.
Participants/materials, setting, methods: The main inclusion criteria was having a live singleton birth (≥24 Weeks of gestation (WG)) after a fresh embryo transfer. Four groups were defined according to E2 level on the day of hCG trigger, into quartiles of the whole population. The main measured outcome was the LBW rate. Statistical analysis was conducted using univariate and multivariate logistic regression models.$p < 0.05$ was considered significant.
Main results and the role of chance: During the study period, a total of 1538 embryo transfers had led to a live birth (LB). Among them, 497 LB were obtained after a fresh embryo transfer. The mean E2 level on the day of hCG trigger was 1608,4 ± 945,5 pg/ml. Repartition between groups according to the E2 level, dividing into quartile was as followed: 124 LB in the Group E2<25 percentile (p) (1106.5 pg/ml), 124 LB in the Group E2 [25p-50p](1106.5 -1440 pg/ml),124 LB in the Group E2 [50p-75p] (1440 -1915 pg/ml) and125 LB in the Group E2 > 75p (> 1915 pg/ml). There was no significant difference for LBW rate according to the E2 level (GroupE2 <25p, n= 8/124, (6.5%), Group E2 [25p-50p], n= 15/124, (12.1%), Group E2 [50p-75p], n=13/124, (10.4%) and Group E2>75p, n= 10/125, (8.1%); (p= 0.43)). Others neonatal outcomes were not significantly different among groups. After multivariate analysis, E2 level on the day of hCG trigger did not have a significant effect on LBW in this study. Mother's age over 35 years old and the presence of a gestational pathology were found as significantly associated with LBW.
Limitations, reasons for caution: The main limitation of our study is its retrospective and monocentric nature. Further, practice of freeze all cycles is carried out in our center since November 2012, we do not dispose of data corresponding to the previous period when only fresh transfers were performed for comparison.
Wider implications of the findings: The fresh transfer strategy, in case of moderate E2 levels on the day of trigger, remains one possible approach considering the absence of increased risk of low birth weight.
Trial registration number: NA

P-655 Dual Triggering versus hCG final maduration in patients over 38 years old, our experience in IVI Santiago

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Study question: The addition of gonadotropin-releasing hormone agonist (GnRH-a) to the hCG final triggering in low responder patients enhance the percentage of mature oocytes (MII) ?
Summary answer: In patients with advanced maternal age and low ovarian reserve, the use of dual triggering do not increase the percentage of mature oocytes
What is known already: In the recent years, the use of the dual triggering in ovarian stimulation has been increased in patients with high rates of immature oocytes in previous cycles in order to obtain higher number of MII and fecundation rate. Also, it has been described higher number of good quality embryos with this technic for oocyte´s maturation induction. These Hypothesis are still controversial
Study design, size, duration: This is a retrospective study in patients older than 38 years old with low ovarian reserve measured as AMH < 1 ng/ml, ICSI cycles and normal semen or moderate oligoastenoteratozoosperm (OAT). It was divided in 2 groups. The first group has 40 cycles in which the induction was made with hCG and the other group has 58 cycles inducted with dual triggering. Data where obtained between January 2014 and March 2018.
Participants/materials, setting, methods: This clinical study was performed in IVI Santiago, a private reproduction Clinic with authorization of Ethics Committee of Santiago de Chile. Our patients had no previous ovary pathology like surgery or endometriosis or ovarian cysts. There was no hormonal stimulation in previous cycles. Couples had normal sperm or OAT moderate. We divide them in two groups:

Group A: Triggering with hCG 6500 UI
Group B: Triggering with dual triggering: hCG 6500 UI and a-GnRH 20 UI

Main results and the role of chance: There was no statistical difference between both groups in the selection criteria like Age, days of stimulation, or AMH value. We couldn´t determinate a statistical difference in number

of aspirated oocytes triggered with DT vs hCG 4.45+2.26 (3.85; 5.04) vs 5.1+3.36 (4.03; 6.17), in the number of mature oocytes retrieved with dual trigger: 3.2+1.66 (2.74-3.67) vs hCG alone 3.9+2.01 (3.11-4.69). There was also no statistical difference on fecundation rate in DT 2,36+1.76 (1.93- 2.80) vs hCG 2.83+2.47 (2.18- 3.47), or good blastocysts formation DT 0.91+0.96 (0.66-1.17) vs hCG alone 1.02+1.35 (0.59-1.46). With these results we can conclude that there is no benefit in adding a-GnRH to hCG to final induction for oocyte maturation in patients with advance maternal age and low ovarian reserve.

Limitations, reasons for caution: A limitation of this study was the low statistical power, because we had only 98 cycles studied in this period. Whether dual triggering favors embryo implantation or not is still controversial. In this study the implantation rate was not an objective because we included cycles with and without PGT-A

Wider implications of the findings: Our results agree with the study of Ding et al, who did not find significant difference in number of oocytes, MII or fecundation rate. High-quality RCTs are warranted to define more-eligible evidence of the efficacy of dual triggering in the GnRH antagonist cycle for IVF in low responders.

Trial registration number: 1806-SCL-051-ES

P-656 EFFICACY OF DOUBLE STIMULATION IN FOLLICULAR AND LUTEAL PHASE (DUOSTIM PROTOCOL) IN PATIENTS WITH LOW OVARIAN RESPONSE: SYSTEMATIC REVIEW AND META-ANALYSIS

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Study question: Can follicular and luteal phase ovarian stimulation (double stimulation) be effective in patients with low ovarian reserve?

Summary answer: Double stimulation may be effective in patients with low response

What is known already: Since the work by Baerwald who found the existence of two to three waves of follicular development during the ovarian menstrual cycle, it has been studied the possibility to take advantage of these waves for dual stimulation (in follicular and luteal phase) in the same cycle, the DuoStim protocol

Study design, size, duration: Systematic review and meta-analysis

Participants/materials, setting, methods: A systematic literature search was conducted in: Pubmed/MEDLINE, EMBASE, Web of Science and clinicalTrials.gov from 2014 until today. We identified a total of 14 papers related to , 4 met the inclusion criteria and were included in the meta-analysis. The statistical analysis was done with RevMan 5.3.

Main results and the role of chance: The duration of the ovarian stimulation was longer in the luteal phase though didn't reach statistical significance (standardized mean differences (SMD)=0.43, 95% CI= -0.44 to 1.31; RE Model) The total of eggs retrieved was significantly higher in luteal phase (SMD=0.46, 95% CI= 0.31 to 0.61; FE Model), as well as the number of MII oocytes (SMD=0.45, 95% CI= 0,3051 to 0,6078; FE Model) The number of embryos obtained was also higher in luteal phase (SMD=0,42, CI 95%=0,21 to 0,62; FE Model), as well as the number of good quality embryos (SMD=0.24, 95% CI=0.03 to 0.44; FE Model).

Limitations, reasons for caution: This meta-analysisi only contains 4 studies.

Wider implications of the findings: Although more prospective randomized studies are needed, it seems that this stimulation could be more effective than stimulation in two different cycles, since in luteal phase are obtained significantly more eggs and more embryos than in follicular phase.

Trial registration number: Not applicable

P-657 Oral dydrogesterone versus micronized vaginal progesterone for artificial frozen-thawed embryo transfer cycles

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Study question: What are clinical outcomes of artificial frozen-thawed embryo transfer (FET) cycles utilizing oral dydrogesterone (DYD) versus (vs.) micronized vaginal progesterone (MVP) for luteal phase support?

Summary answer: Oral dydrogesterone 30mg was associated with similar pregnancy rates, as well as a similar incidence of early pregnancy bleeding and D&C, as compared to MVP.

What is known already: The "luteal phase" of an artificial FET-cycle is vulnerable, as ovulation is suppressed, a corpus luteum therefore absent, and the establishment of endometrial receptivity and the early pregnancy support completely relying upon the orchestrated exogenous administration of sex steroids. DYD is a convenient oral progestogen that has recently been shown to be effective and safe in fresh IVF-cycles (Tournaye et al., 2017; Griesinger et al., 2018). So far only two small-sized RCTs have compared daily 20 mg (Zarei et al., 2016) or daily 40mg oral DYD (Rashidi et al., 2016) with daily 800mg MVP for FETs, however with conflicting results.

Study design, size, duration: Retrospective analysis, 4/2016-4/2018, all patients initiating a FET cycle with constant daily oral 6mg E2 and daily oral 30mg DYD or MVP (600mg capsules or 90mg gel). Each patient contributed one, the first, IVF/ICSI cycle of stimulation and fresh transfer with cryopreservation of surplus 2PN or freeze-all, and all subsequent FETs from that first treatment cycle. All FETs had to be on the same endometrial preparation protocol. Sex steroid administration continued until gestational week 10.

Participants/materials, setting, methods: In 5/2017, the FET routine at the department was gradually switched from MVP to oral DYD. Accordingly, approx. one year of oral DYD usage is compared with the previous year of MVP usage. Primary outcomes were pregnancy and birth rate (the latter still incomplete at the time of writing), secondary outcomes were early pregnancy bleeding and dilatation and curettage incidence.

Main results and the role of chance: 314 patients underwent a first IVF/ICSI cycle with cryopreservation (surplus or freeze-all) and later FET.

Age, BMI, parity, IVF:ICSI ratio, gonadotropin type, trigger type(hCG or GnRH-agonist), peak E2 and peak progesterone were similar in patients later on DYD or MVP for their FETs as was the mean cumulus-oocyte-complex number(DYD: 13.9±7.6; MVP: 14.3±7.4 [p=0.65]). The proportion of patients undergoing freeze-all and the pregnancy rate (PR) in patients with fresh transfer was similar in the two groups as well

109 (DYD) and 204 (MVP) patients underwent 160 and 295 FET cycles, respectively (mean FET cycles per patient: 1.47 versus (vs.) 1.45). The cumulative clinical PR was 40/109 (36.7%, 95%CI: 27.9-46.5) with DYD vs. 72/204 (35.3%, 95%CI: 28.8 – 42.3) with MVP(difference: 1.4%, 95% CI: -9.4 – 12.6, p= 0.80). The PR of the first FET cycle was 27/109 (24.8%, 95%CI: 17.6-33.6) with DYD and 57/204 (27.9%, 95%CI: 22.2 – 34.5) with MVP (difference: -3.2%, 95%CI: -12.8 – 7.4, p= 0.54)Vaginal bleeding after establishment of a clinical pregnancy occured in 26/40 (65%, 95%CI: 49.5-77.9%) vs. 42/72 (58%, 95%CI: 46.8-69.0) pregnancies in DYD and MVP patients (difference: 6.7%, 95%CI: -12.2 – 23.9, p=0.49). Incidence dilatation & curettage: 13/40 (32.5%) DYD vs. 20/72 (27.7%) MVP (p=0.60)

Limitations, reasons for caution: A retrospective study has inherent risks of bias. Sampling error is potentially high, illustrated by the wide CI for the primary outcome. Selection bias, however, is unlikely as the FET protocol was changed to DYD for all patients starting a cycle after 5/2017.

Wider implications of the findings: Because of ease of administration, oral DYD may be preferable to vaginally administered or injectable progestogens, especially in artificial FET cycles with prolonged administration up to the completed luteo-placental shift. DYD 30mg is a dose to be tested further for artificial FET in randomized controlled

Trial registration number: not applicable

P-658 Antichlamydial antibodies and poor response to ovarian stimulation in women with tubal factor infertility undergoing in vitro fertilization

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Study question: Does past or persistent chlamydial infection affect the response to controlled ovarian stimulation in women with tubal factor infertility undergoing *in vitro* fertilization (IVF)?

Summary answer: IgG-antibody positivity to *C.trachomatis*, as serological marker of past infection, is associated with both 'expected' and actual poor ovarian response (POR) to exogenous gonadotropin stimulation

What is known already: The absence of universal definition and standardized efficient treatment encourage further debate on the topic of POR in modern reproductive medicine. In some studies, pelvic inflammatory disease and its sequelae in the upper reproductive tract were suggested as factors, unequivocally associated with POR. Strong correlation between tubal factor infertility (TFI) and seropositivity for antichlamydial antibodies in women undergoing IVF was repeatedly established. However, knowledge about the prognostic value of chlamydia antibody testing for the definition of POR in this substantial subset of patients remains limited.

Study design, size, duration: A prospective comparative cohort study of 246 women with laparoscopically confirmed TFI was conducted at the IVF department of a state institution between February 2015 – December 2017. Patients with endometriosis or previous ovarian cystectomy were excluded.

Participants/materials, setting, methods: Blood serum (obtained at the start of stimulation) and follicular fluid (aspirated at oocyte retrieval) were analyzed using enzyme-linked immunosorbent assay for IgG, IgA to major chlamydial antigens and for IgG to chlamydial heat shock protein 60 kDa (cHSP60). According to the results of serum anti-*C.trachomatis* IgG test, 124 seropositive (main group) and 122 seronegative subjects (comparison) were defined. The number of pre-ovulatory follicles on the day of ovulation triggering was measured as primary outcome.

Main results and the role of chance: Maternal age, antral follicle count and serum anti-Müllerian hormone (AMH) levels did not differ between the studied groups. Nevertheless, the proportion of 'expected' poor responders (POSEIDON criteria, 2016; group 3 and 4) due to reduced ovarian reserve (AMH<1.1 ng/ml) was higher in women, positive for IgG to *C.trachomatis* in sera and follicular fluid (46.40% vs. 36.26%, p=0.023). Detection of this antibodies was also related to severity of peritoneal adhesions (R=0.46, p<0.001), repeated pelvic surgeries (OR: 1.93, 95%CI 1.12-3.29) and previously diagnosed fallopian tube pathology (OR: 4.51, 95%CI 2.60-7.84). The proportion of younger 'expected' hypo-responders (<35 years, POSEIDON group 3) was almost two times higher in the main group. Albeit the pre-ovulatory follicle count and the number of retrieved oocytes were comparable, anti-*C.trachomatis* IgG detection in sera was associated with actual POR (<4 retrieved oocytes; OR: 2.63, 95%CI 1.35-5.11) and oocyte maturity (R=-0.22, p=0.019). Follicular output, fertilization and blastulation rates did not vary between the groups. Seropositive and seronegative women demonstrated similar clinical pregnancy (20.49% vs. 25.00%, p=0.142) and life birth rates (13.1% vs. 20.0%, p=0.149). No significant correlations between ovarian response parameters and anti-cHSP60 IgG or anti-*C.trachomatis* IgA positivity of sera or follicular fluid were observed.

Limitations, reasons for caution: The proportion of patients with antibodies to cHSP60 (markers of persistent infection) was rather small. Exclusively women with endoscopically confirmed TFI were recruited for the study. Neither genetic polymorphisms, known to be linked to POR, nor cumulative pregnancy rates could be assessed. All this limits generalizability of the obtained results.

Wider implications of the findings: For the subpopulation of women with TFI undergoing IVF, chlamydia antibody testing should be employed in prediction, counseling and planning of management of 'low prognosis patients'. Further randomized trials may investigate advisability of empiric antimicrobial therapy prior an IVF cycle for women, diagnosed with TFI and/or positive for antichlamydial antibodies.

Trial registration number: Not available

P-659 A first description of a novel 20mg letrozole stimulated IVF cycle as compared to gonadotropins in GnRH-antagonist cycles, in extremely poor ovarian reserve

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Study question: To evaluate the outcome of IVF stimulated with 20mg letrozole as compared to high-dose gonadotropins in ultra poor responders

Summary answer: 20mg letrozole in women who previously stimulated 0-2 follicles and may yield lower cancellation rates, better pregnancy rates and at lower costs than high-dose gonadotropins

What is known already: Poor ovarian response presents a significant challenge in IVF. There is no consensus in the literature on the ideal controlled ovarian hyperstimulation protocol for these patients and pregnancy rates remain low. Many strategies have been studied in such patients, including increasing the gonadotropin dosage and administration of adjuvant therapies, such as clomiphene but have failed to improve pregnancy outcomes. Clomiphene while cheaper than gonadotropins may lead to stimulation without increases in pregnancies. Clomiphene is not available in our country; as such high dose letrozole was investigated, with expected rapid recovery of the endometrium, possibly prior to transfer unlike clomiphene.

Study design, size, duration: A retrospective cohort study conducted at a university hospital with data from January 01, 2015 to June 01, 2018. The analysis included women who were up to 42 years of age. All subjects had performed 1-2 previous IVF cycles in the year prior with at most 0 to 2 follicles stimulated with 300-450IU gonadotropins daily. Sample size calculations required a total population of >190 to detect an increase of 0.5 follicles/oocytes with SD=1.2 follicles.

Participants/materials, setting, methods: All were GnRH-antagonist cycles. Controls (N=247) were treated with 300-450 IU/daily of HMG. The study group (N=62) received 20mg/daily of letrozole orally, with the GnRH-antagonist and HMG(150IU) commencing when the lead follicle was 14mm. Statistics were analyzed with T or chi-squared tests. Logistic regression was used to control for confounding effects of any statistically different baseline parameters. Cycles were canceled if no follicles grew by 20-days stimulation or linings <6mm on day of the transfer

Main results and the role of chance: The two groups were comparable in terms of age, infertility duration, #failed transfers, AFC and basal-FSH. The letrozole group received less total gonadotropin with lower medication costs (645±175IU, $555±150) compared to controls (5360±1028IU, $4616±885) (p=0.0001 twice). The number of metaphase-II oocytes per cycle start (1.9±1.0 vs. 1.0±0.8) and per collection (2.1±1.0 vs. 1.6±0.8) were higher for letrozole than controls (p=0.0001 twice). Cancelation rates were lower with letrozole 7/62 vs. 95/247, p=0.0001. Max ET at triggering was thinner with letrozole 6.4±1.o vs. 7.4±1.7mm (p=0.0001). No subjects had transfer canceled for linings <6.0mm on transfer day. Ongoing pregnancy rate per cycle start (14.5 vs. 4.5 %, p=0.004) and per transfer (16 vs. 7%, p=0.05) were higher with letrozole

Limitations, reasons for caution: A moderate number and non-randomization of the study participants may result in undetected bias. The main outcome which is live birth is still to be determined, an ongoing pregnancy in the third trimester was used as a surrogate outcome

Wider implications of the findings: 20mg letrozole can be used in poor ovarian responders with a history of stimulating 0-2 follicles and may yield lower cancellation rates, better pregnancy rates and at lower costs than high-dose gonadotropins. A prospective randomized study should be performed to evaluate this protocol described for the first time here

Trial registration number: none

P-660 Consecutive minimal controlled ovarian stimulation (COMCOS) to increase the number of oocytes in patients with poor ovarian response or poor ovarian reserve.

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Study question: Investigate the impact of consecutive minimal controlled ovarian stimulation associated to oocyte vitrification on the reproductive outcome of women with poor ovarian response or reserve.

Summary answer: Consecutive minimal controlled ovarian stimulation associated to oocyte vitrification is a suitable alternative for patients with poor ovarian response or reserve

What is known already: Although different controlled ovarian stimulation protocols have been proposed for women with poor ovarian response or reserve there are still women who respond poorly to gonadotropins, thus resulting in few oocytes at retrieval, reduced number of embryos for transfer, and unsatisfactory pregnancy rates. An interesting alternative is to refer those patients for oocyte donation program. However, many patients still do not agree with this alternative. Also, no consensus has been reached for the best alternative to increase the number of oocytes and embryos available for transfer in this group of patients.

Study design, size, duration: We report an observational study of 84 patients submitted to assisted reproductive technologytreatment from April 2015 to November 2018.

Participants/materials, setting, methods: We evaluated 434 cycles of that were referred to receive donated oocytes and did not agree. Treatment started with Clomiphene Citrate and Letrozol. When follicles reached 13mm, patients received GnRH antagonist and rFSH/LH. GnRH agonists was administered for oocyte maturation when follicles reached 17mm. Oocyte retrieval was performed 36h later and oocytes were vitrified 2h later. A new stimulation was started on the following day, until reached 6 oocytes or a maximum of 6 retrievals.

Main results and the role of chance: The patients mean age was 38.3 ± 3.2 years (range 32-45). The number of oocyte retrievals ranged from 3 to 6 with a mean number of 5,1. A total of 444 Metaphase II oocytes were recovered (mean = 5.3 ± 2.4) ranging from 1 to 8. A total of 426 oocytes survived after warming (96%), 354 showed normal fertilization after ICSI (83%) and 278 reached cleavage stage (78% - mean=3.3). The number of transferred embryos ranged from 1 to 3. Overall clinical pregnancy rate was 27.3% (n=23) and miscarriage rate was 26% (n=6).

Limitations, reasons for caution: Additional controlled studies are necessary to confirm our results as this was an observational study and, to our knowledge, this is the first study to perform such analysis.

Wider implications of the findings: Consecutive minimal controlled ovarian stimulation might be an alternative to increase the number of oocytes and embryos and improve pregnancy rates, in patients with poor ovarian response or poor ovarian reserve. This alternative might be a suitable option for patient who do not agree to receive donated oocytes.

Trial registration number: not applicable

P-661 Anti-Müllerian Hormone (AMH) is an independent marker for oocyte survival post vitrification.

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Study question: Is Anti-Müllerian hormone related with oocyte survival and blastocyst formation after oocyte vitrification?

Summary answer: AMH shows a positive significant correlation with oocyte survival. Although blastocyst formation is lower after oocyte vitrification, no correlation was found with AMH.

What is known already: Oocyte cryopreservation is increasingly being used for medical and social reasons. Vitrification techniques led to improved survival rates for oocytes. Recent meta-analysis demonstrated that vitrified oocytes have a good survival, fertilization and cleavage rates. However, subgroup analysis indicated better results with donor oocytes, possibly explained by the likelihood of better-quality oocytes obtained from younger women.

Nonetheless, not only age, likewise ovarian reserve seems to be correlated with oocyte competence. AMH is one of the best quantitative predictors for ovarian reserve; however, it may also play an important role on prediction of survival rate after oocyte vitrification and embryo development.

Study design, size, duration: Retrospective observational analysis including 487 cycles for ICSI with devitrified oocytes and/or fresh plus devitrified oocytes, performed between August 2015 and August 2018.

Participants/materials, setting, methods: Patients with primary or secondary infertility undergoing ICSI treatment with previous stimulations for oocyte vitrification were included. Serum AMH was measured using a fully automated assay Elecsys® (Roche). Vitrification and warming were performed with the Cryotop method (Kitazato, Biopharma). Only cycles using ejaculatory sperm were considered for the secondary objectives.

Main results and the role of chance: We included 487 cycles with a total of 4530 devitrified oocytes. Patient median age was 36,2±6,08 years (CI.95 24,3-48,1), AMH 2,58±3,38 ng/mL and body mass index (BMI) of 26,5±4,59 Kg/m^2. Oocyte survival rate after vitrification was 85,9±20,4% (CI.95: 84,1-87,7%).

AMH showed a significant positive correlation (Tau´s Kendall=0,091, p=0,0055) with oocyte survival rate independently of the oocyte yield, using univariate correlation analysis. The correlation between AMH and oocyte survival was also significant (OR=1,017, p=0,0475) when a multivariant model was performed including AMH, age and BMI. A ROC curve was performed andAMH cut-off value to obtain at least 70% survival rate was 1.09 ng/mL with an AUC=0.669.

Regarding embryo development in cycles including fresh and thawed oocytes for the same patient, fertilization and cleavage rate were similar between embryos from fresh and thawed oocytes (OR=0,97, p=0,496; OR=0,99, p=0,997 respectively). However, blastocyst formation was better for embryos from fresh than thawed oocytes (OR=1,34, p<0,001). No significant correlation was seen between fertilization, cleavage or blastocyst rate with AMH, age or BMI.

Limitations, reasons for caution: The strength of the study is the number of included thawed oocytes (4,530) with AMH data for all cycles. Limitation is the retrospective design.

Wider implications of the findings: As AMH shows strong positive correlation with oocyte survival, clinicians should reconsider oocyte vitrification carefully for patients with AMH below 1,09 ng/mL, especially as blastocyst formation after oocyte vitrification also seems lower. Therefore, embryo accumulation should be recommended above oocyte accumulation. Further prospective studies should confirm these findings.

Trial registration number: NO

P-662 Two new compound heterozygous truncating mutations in NOBOX gene identified by Whole Exome sequencing (WES) in 2 sisters with premature ovarian insufficiency (POI).

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Study question: Can WES uncover genetic etiology of POI in 2 sisters presenting primary amenorrhea (PA) and delayed puberty?

Summary answer: WES identified two new compound heterozygous NOBOX truncating mutations. The familial segregation of the mutations strongly supported their implications in the development of POI.

What is known already: *NOBOX* is a homeobox gene playing a critical role in early folliculogenesis. Two chromosomal deletions involving NOBOX and fourteen *NOBOX* mutations have been reported so far in patients affected by POI. While the only 2 reported homozygous mutations were associated to PA, heterozygous mutations were mostly reported in patients with secondary amenorrhea although in some cases they were also identified in patients with PA with absent or incomplete pubertal development. Functional studies of the reported mutations gave more evidence of haploinsufficiency of *NOBOX* gene in human in contrary to data obtained from knockout *NOBOX* mice.

Study design, size, duration: WES was performed in trio in a POI Belgian patient and her two healthy non consanguineous parents on HiSeq 2000 Illumina sequencer at the BRIGHTcore platform. Identified mutations were confirmed by Sanger sequencing in the proband's POI sister. The proband and her family

members gave their informed consent to undergo genetic testing to uncover the etiology of POI in the affected sisters.
Participants/materials, setting, methods: The proband presented at our Fertility Clinic for oocyte donation. She had been diagnosed with idiopathic POI at 17 years old as she showed no pubertal development and PA. Laparoscopic investigation performed at adolescence showed a small uterus and hypoplastic ovaries. FSH and estradiol levels were respectively of 106 IU/L and <12ng/l. Her puberty was induced with hormonal treatment. Karyotype and array CGH were normal. Her sister presented the same POI phenotype.
Main results and the role of chance: WES identified 2 new compound heterozygous NOBOX truncating mutations in the patient: c.1421delG (p.Gly474fs) inherited from the mother (menopaused at 50 years) in exon 8 which induced a frame shift and c.826C>T (p.Arg276*) inherited from the father in exon 4 which induced a premature stop codon leading to a truncated protein devoid of its homeodomain. These 2 variants were not reported in different NGS databases including EXAC, 1000Genomes, ESP6500 and genomAD databases. The sister carried the same mutations. Both variants presented strong evidence of pathogenicity and are very likely responsible of the severe POI phenotype in our 2 sisters. Previously reported functional studies on NOBOX gene truncated at the level of the DNA binding homeodomain showed a defective transcriptional activity, an impairment of nuclear localization of the truncated protein, its intracellular aggregation suggestive of protein instability and subsequent cell toxicity as well as a potential partial sequestration of wild type protein.
Limitations, reasons for caution: The contribution of c.1421delG (Exon 8) mutation to the severity of the 2 sisters POI phenotype can't be assessed as functional study has not been performed.
Wider implications of the findings: NGS is a promising genetic tool that should be used for genetic diagnosis approach in idiopathic POI cases. We suggest using a specific panel of causal POI gene in the diagnosis management of all idiopathic POI cases, and restrict WES to families with a least two affected women.
Trial registration number: P2016/196/CCB B406201628264

P-663 Time of oocyte recruitment can affect their ploidy status

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Study question: To evaluate the efficacy of double stimulation in terms of embryo developmental potential and their genetic status.
Summary answer: The shorter time of oocyte recruitment can help increase the number of blastocysts and euploid blastocysts.
What is known already: Recent evidence indicates that folliculogenesis occurs in a wave-like fashion indicating that there are multiple follicular recruitment waves in the same menstrual cycle. This relatively new scientific concept provides new opportunities for the utilization of ovarian stimulation especially in women with diminished ovarian reserve, poor ovarian response or for patients who need to collect the highest number of oocytes in the shortest possible time. It is important to know if embryos resulting from this type of stimulation have similar developmental potential and euploidy rate when compared to embryos obtained by more traditional ovarian stimulation.
Study design, size, duration: This was a retrospective case-control study and consisted of 68 women (median age 38 years, range 28-43) with the diagnosis of infertility who were undergoing IVF treatment with PGT with double ovarian stimulation in the follicular (FPS) and luteal phase (LPS) between September 2017 and Juni 2018 at INVICTA Fertility Centre, Poland.
Participants/materials, setting, methods: A total of 932 MII were derived (428 FPS and 504 LPS) and 208 blastocyst (130 blastocysts FPS and 190 LPS) were evaluated with the NGS protocol. Ion Torrent Suite Software and Invicta Bioinformatics Team Script were used for chromosome copy number variation analysis.
Main results and the role of chance: No significant differences were observed in the mean number of metaphase II oocytes (6.5 ± 3.6; median 6; range 1-21 vs. 7.4 ± 3.7; median 6,5; range 2-17) and fertilized oocytes (4.5 ± 2.1, median 4; range 1-9 vs. 4.8 ± 2.6, median 4; range 0-11) between FPS and LPS. Blastulation rates were significantly higher ($p<0.01$) in LPS compared to FPS. The mean number of blastocyst in LPS was 2.8 ± 1.7, median 2; range 1-7 vs. 1.9 ± 1.1, median 2; range 1-5 in FPS. There was also a higher number of euploid embryos obtained after LPS ($p<0.01$). The mean fertilization rate calculated per number of MII oocytes collected from FPS and LPS was similar but the mean blastocyst and euploid blastocysts rate per MII were higher and were 35.7% vs. 41.1% and 17.6% vs. 25.2 respectively.
Limitations, reasons for caution: More data are required to confirm that better quality of oocytes is a result of shorter recruitment time of antral follicles.
Wider implications of the findings: The evidence of multiple follicular waves during a single menstrual cycle in women has important implications for the treatment of infertility. This strategy can maximize the number of oocytes obtained per menstrual cycle, in turn increasing the chance to obtain reproductively competent, euploid embryos in the shortest possible time.
Trial registration number: not applicable

P-664 Assessment of the feasibility and safety of a novel transvaginal ovarian drilling method in a bovine model: ovarian rebalancing.

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Study question: The objective was to evaluate the technical feasibility and safety of a new transvaginal ovarian drilling procedure using a dedicated device in a bovine model.
Summary answer: The procedure was successfully and safely completed in all animals with no incidents of inadvertent damage to other organs and without disruption of ovarian folliculogenesis.
What is known already: Polycystic Ovary Syndrome (PCOS), the most common endocrinopathy affecting women of reproductive age, is defined by at least two of the following features: ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology. In PCOS women with anovulatory infertility, the accepted first-line treatment is clomiphene citrate (CC). For patients resistant to CC, laparoscopic ovarian drilling (LOD) is recommended as a second-line therapy. Whilst very effective, LOD is an invasive technique requiring general anaesthesia and is associated with a significant risk of postoperative adhesions. To overcome these drawbacks, other techniques such as transvaginal ovarian drilling are being developed and tested in animal models.
Study design, size, duration: The study was conducted on thirteen female cattle aged 3-8 years, selected to have ovarian morphology and size similar to human PCOS ovaries. Most animals were in the follicular phase of the oestrus cycle. On days 0, 7, 36 or 69-72 (n=3, 4, 3, 3 respectively) following the procedure, the animals were slaughtered or subjected to ovariectomy to harvest the reproductive tract and ovaries and perform a macroscopic and histological analysis of the tissues.
Participants/materials, setting, methods: The procedure was performed under light epidural anaesthesia by an animal technician experienced in egg retrieval. A dedicated kit (*AblaCare*) was used comprising a device (ablation catheter deployed through a needle) and a radiofrequency energy generator. The device was mounted on a vaginal ultrasound probe, then introduced transvaginally and brought in close proximity to each ovary. The needle was inserted into the ovary and the catheter released to deliver 4-6 ablations/ovary (settings: 85° for 60s).
Main results and the role of chance: The procedure was successfully performed via transvaginal access and under ultrasound guidance in all animals. The animals did not manifest signs of pain or significant discomfort. The needle and catheter were clearly visualised on ultrasound and therefore easily located within the ovary ahead of performing the ablations. No adverse events or significant technical difficulties occurred during or after procedure, demonstrating good technical feasibility. From a safety standpoint, the macroscopic analysis of

harvested ovaries showed no damage to ovarian surface aside from the needle puncture. Thermal lesions, quantified using the ImageJ software, were confined to the ablation zones, were visible at days 0 and 7 - lesion volume amounting to 5.74% and 5.20% of the total ovary volume respectively, in line with what is known in terms of target volume reduction to achieve efficacy of ovarian drilling - and were no longer visible at days 36 and 69-72 ($p<0.001$). The ovarian structure was conserved, and no lesion secondary to ablation or puncture was found in surrounding organs, especially the tractus. All animals followed up for 7 days or longer presented no adhesions on ovarian surface and maintained a normal cycle with no disruption of folliculogenesis/corpus luteum development as monitored by ultrasound.

Limitations, reasons for caution: All animals followed up for 7 days or longer maintained a normal cycle with no disruption of folliculogenesis/corpus luteum development as monitored by ultrasound. However, the efficacy of the procedure in terms of ovulation induction was not evaluated in this protocol, as the chosen model was not a pathological model.

Wider implications of the findings: The technical feasibility and safety of this new procedure, ovarian rebalancing, using a dedicated device were successfully assessed in the bovine model. These preclinical results need to be validated by a clinical trial on anovulatory PCOS women, resistant to first-line treatment.

Trial registration number: NCT03760926

P-665 Recombinant LH (rLH) supplementation during GnRh-antagonist delayed-start OPCs-synchronized IVF cycles in an unselected population of infertile women

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Study question: May endocrine dynamics evaluation be used as a predictor of ovarian response and IVF outcome in an unselected population in GnRh-antagonist down-regulated cycles?

Summary answer: Endocrine dynamics may identify patients needing rLH supplementation,the threshold under which supplementation is mandatory and at what point of follicular stimulation rLH must be added.

What is known already: Oocyte quality is programmed well before ovulation and may be influenced with exogenous gonadotrophins earlier than oocyte collection,being amenable to endocrine/paracrine manipulation.Thus stimulation protocols may impact on oocytes quality other than quantity,and an inappropriate use of exogenous gonadotrophins might be deleterious.To date not clear picture emerges regarding the usefulness of rLH supplementation as a routinary part of an assisted reproductive technique:previous results do not support the hypothesis that rLH addiction increases pregnancy rate in unselected patients even if there may be a potential benefit when rLH is supplemented during ovarian stimulation in poor responders or women of an advanced age.

Study design, size, duration: 300 unselected women were enrolled.A delayed-start protocol with rhFSH, following daily 0,25 µg GnRh-antagonist administration was used.LH, E2, and Pg determinations were performed on day 3-4 of spontaneous menstruation (basal), at t0 (day of OCP stop),t1 (day of first GnRh-antagonist administration), t2 (first us scan),t3 (second us scan) to evaluate the need of rLH adding.An arbitrary cut off of 2,4 mIU/ml of circulating LH at t1 was considered as the threshold for rLH tailored supplementation

Participants/materials, setting, methods: 103 patients out of 300 (group I) received only tailored rFSH,whilst197 patients (group II) received tailored rFSH plus rLH starting from day six of rFSH administration.Primary outcomes were total number and MII retrieved oocytes,number of 2PN,top embryo quality rate.Secondary outcomes included total number of transferred embryos,BhcG/started cycle and BhcG/ET,implantation rate,clinical pregnancy rate/started cycle,clinical pregnancy rate/ET.Data were analyzed by using the SPSS version 20.0: Mann–Whitney U-test and Z-test were used.Significancewas set at $P<.05$.

Main results and the role of chance: Serum E2 and P level at t2 and t3 did not show any statistical significant difference between the two groups.LH values at basal time (t0)were similar in both groups(5,57 +2,80 vs 5,55 +2,65 mIU/ml),whereas LH serological values at t2(3,05+3,64 vs 1,85+1,69 mIU/ml)and at t3 (2,60 +2,10 vs 1,93+1,66 mIU/ml)were markedly reduced when COS was driven with rFSH alone respect to the stimulation supplemented with rLH,reaching a statistically significant difference between the two groups (*p*value <.05 and <.002 respectively)at any time.Notably Metaphase II oocytes (6,48+3,42 vs 4,70+2,63),fertilization rate (4,08+2,26 vs 2,91+1,46;) and top scoring embryos (3,07+1,84 vs 2,10+1,05) showed a statistical significance difference in favour of women receiving and add back of rLH (*p*values <.00001;<.00001 and <.00001 respectively).Total dosage of rFSH administration, total number of retrieved oocytes, total number of embryo transferred at day 2/3 were similar in the two groups.BhcG for started cycle and for embryo transfer,implantation rate and ongoing pregnancy rate for started cycle and for embryo transfer,whilst not reaching a statistically significant difference,showed a clear trend towards a better outcome in favour of the added rLH group (47,72% vs 37,86%; 50,81% vs 40,21%; 20,29% vs 16,1%; 38,50 vs 28,16%; 38,92 vs 29,9% respectively).

Limitations, reasons for caution: We are far from a clear comprehension of paracrine/endocrine signals regarding bidirectional cumulus-oocyte dialogue and more robust studies are needed to clarify who really needs of LH supplementation,which is the threshold under which supplementation with rLH is mandatory and at what point of follicular stimulation we need to add rLH.

Wider implications of the findings: As IVF main outcomes are all in favour of rLH-supplemented cycles,it may be argued that is not so much important the absolute value of a single LH snap-shot determination,rather the endocrine milieu throughout the entire length of follicular phase.It is possible that other iatrogenic interference may further impair the outcome.

Trial registration number: none

P-666 Effects of testosterone administration in patients poor ovarian responder undergoing IVF: a meta-analysis of randomized controlled trials

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Study question: To evaluate whether testosterone administration before or during controlled ovarian stimulation (COS) in patients defined poor ovarian responder can modify IVF outcomes

Summary answer: Compared to controls, women receiving testosterone showed significant higher live birth rate (LBR), clinical pregnancy rate (CPR), total M2 oocytes and total embryos.

What is known already: In-vitro and in-vivo studies demonstrated that testosterone is implied in the transition of follicles from the quiescent to the growing pool, it could increase the number of pre-antral and antral follicles and augment the expression of FSH receptors in granulosa cells potentially enhancing the ovarian responsiveness to gonadotropins. Over the last years, different observational studies and RCTs have evaluated the role of testosterone supplementation before or during controlled ovarian stimulation (COS) in POR, with variable results. Thus the debate on its effectiveness is still open.

Study design, size, duration: This is a systematic review and meta-analysis including 7 RCTs with 573 participants. In all except one trial (using oral route), testosterone was administered trans-dermally. Timing of administration was before starting COS for two-three weeks in four trials, for at least 40 days in one trial, for only five days preceding COS in one trial and until ovulation induction in one trial. The daily dose of testosterone varied among studies

Participants/materials, setting, methods: This is a systematic review and meta-analysis of RCTs evaluating the effectiveness of pre-treatment with testosterone on IVF outcomes in POR. Primary outcome: live birth rate(LBR); secondary outcome: clinical pregnancy rate(CPR), miscarriage rate(MR), total oocytes, MII oocytes, total embryos. All analyses were performed with the random effects model with an intention-to-treat approach. Variables were compared using the risk ratio(RR) or mean differences(MD), with a 95 % confidence interval (95%CI). A $p<0.05$ was considered statistically significant.

Main results and the role of chance: Those women who received testosterone treatment showed higher LBR than controls (RR=2.29;p=0.004); an advantage from intervention was also in terms of CPR (RR=2.32;P=0.0003), total number of oocytes,M2 oocytes and total embryos. No difference was found for MR.

We didn't found significant differences from the subgroup analysis according way of administration in terms of both LBR (transdermal RR=2.11; p=0.78,

oral RR=3.09; p=0.06) and CPR (transdermal RR=2.15; p=0.74, oral RR=3.15; p=0.03). Moreover. subgroup analysis according the days (≥21 days versus < 21 days) and the timing of testosterone administration (before the beginning of COS versus during COS), showed no statistical difference among the subgroups (p= ns).

No adverse effects were recorded.

Limitations, reasons for caution: Some limitations must be considered; firstly, different outcomes were calculated by pooling the results of a small number of studies, patients and events. Additionally, certain heterogeneity across studies was present in terms of POR definition and testosterone therapy.

Wider implications of the findings: Pre-treatment with testosterone seems promising. Our data support a positive impact of testosterone on different COS parameters. Further RCTs on larger populations, with rigorous inclusion criteria are still mandatory to finally confirm testosterone clinical effectiveness and to establish the best timing, dose and duration of its administration before IVF

Trial registration number: PROSPERO CRD42017067270

P-667 FSH requirements for follicle growth during controlled ovarian stimulation

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Study question: What is the relationship between recombinant FSH (rFSH) dose and follicular growth during controlled ovarian stimulation?

Summary answer: A sufficient weight-adjusted starting dose of rFSH reduces variability in follicle size by time of trigger and maximises the number of mature oocytes retrieved.

What is known already: A key factor determining success of IVF treatment is the induction of sufficient ovarian follicular growth in response to exogenous FSH administration. This must be balanced against the risk of excessive stimulation that could lead to an uncontrolled response, increasing the risk of the life-threatening complication 'ovarian hyperstimulation syndrome' (OHSS). Choosing an appropriate starting dose of rFSH can be challenged by inter-individual variability in response. A number of algorithms / nomograms have been developed to predict oocyte number to assist clinicians in choosing an appropriate starting dose, but limited data exists to directly relate rFSH dose to follicle growth.

Study design, size, duration: We performed a single centre retrospective cohort study of 1,034 GnRH antagonist co-treated and 103 GnRH agonist co-treated IVF treatment cycles (n=1,137) conducted between January 2012 and January 2016, who had ovarian stimulation by rFSH (Gonal F) alone.

Participants/materials, setting, methods: Women treated with rFSH alone for controlled ovarian stimulation during IVF treatment at Hammersmith Hospital, London, UK. Size of each individual follicle at every monitoring ultrasound scan and rFSH doses were analysed. The main outcome measure was median follicle size after five days of rFSH and the proportion of antral follicles recruited by the end of stimulation.

Main results and the role of chance: Serum FSH level correlated with rFSH dose adjusted for weight (r^2=0.352, p<0.0001). Starting rFSH dose (iU/kg) was associated with both median follicle size after five days, and the proportion of antral follicles recruited. Day 5 median follicle size predicted median follicle size on subsequent ultrasound scans (r^2=0.58-0.62; p<0.0001), and hence time to oocyte maturation trigger (r^2=0.22, P<0.0001). The dose-response relationship was lost in predicted poorer responders (age ≥35 years and antral follicle count ≤15) who were treated with doses greater than 2.25iU/kg. The proportion of follicles 12-19mm in diameter (which are most likely to yield mature oocytes) at the end of stimulation significantly increased with starting dose of rFSH and was associated with an increased number of mature oocytes retrieved. Insufficient starting dose of rFSH that required >5% dose-increase was associated with increased variability in follicle size on the day of oocyte maturation trigger, and negatively impacted the number of mature oocytes retrieved. Median follicle size after 5 days of rFSH was reduced in the 103 GnRH agonist co-treated cycles when compared to an equivalent subset of GnRH antagonist cycles (age 18-34 years, AFC 15-25).

Limitations, reasons for caution: This study was limited by its retrospective nature and variation in the day of monitoring ultrasound scans. Prospective studies to evaluate weight-adjusted doses of rFSH are required to investigate this relationship further.

Wider implications of the findings: These data inform the selection of rFSH dose to tailor follicular recruitment according to individual patient-need, for example to manage the risk of OHSS. Adjusting rFSH dose during the stimulation phase increases variability in follicle size by the end of stimulation and negatively impacts the number of mature oocytes.

Trial registration number: The trials were registered on the National Institutes of Health Clinical Trials database (NCT01667406).

P-668 Effects of follicular fluid and serum vitamin D levels on IVF outcome

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Study question: Is there any association between vitamin D levels in serum and follicular fluid (FF) with IVF outcomes in infertile women of Asian population?

Summary answer: Different concentrations of vitamin D in serum and FF have a significant correlation with the number of mature oocytes, fertilization rate and clinical pregnancy rate.

What is known already: According to current studies vitamin D in complex with its receptor, by regulating gene expression, endometrial immune response and stimulation of endometrium decidualization can be involved in implantation. The recent evidence indicates that the amount of vitamin D in follicular fluid may have the potential effects on ovarian function, endometrial receptivity, embryo maturation and embryo quality. The results regarding the effect of vitamin D on clinical pregnancy outcomes in assisted reproductive technologies are still now conflicting.

Study design, size, duration: A prospective cohort study was conducted in a private infertility center, Kolkata, India. In this study 76 infertile women were included who were all aged below 40 years and underwent In Vitro Fertilization (IVF) treatment after taking their written consent between November 2017 to November 2018.

Participants/materials, setting, methods: Stimulation protocol was same for all of them, long protocol of induction ovulation. Serum sample was collected for baseline endocrine profiling and vitamin D estimation. Serum Vitamin D was also checked on the day of HCG (human chorionic gonadotropin) triggering. FF was extracted from follicles over 14 mm. FF and blood samples were centrifuged and then level of 25-OH-vit D was measured by immunoassay method. Number of oocytes, fertilization rate and pregnancy outcome were noted.

Main results and the role of chance: A significant correlation was found between the levels of vitamin D in follicular fluid and serum (p = 0.001). The data regarding to baseline characteristics including age, endocrine profile, type and cause of infertility, endometrial thickness, and number of transferred embryos were similar between women with different serum and follicular fluid 25OH-D levels. Study population has been divided into three groups according to serum vitamin D status: 60% had vitamin D deficiency (group A), 20% had vitamin D insufficiency (group B) and 20% had replete vitamin D status (group C). The overall rates of clinical and ongoing pregnancy were 23.2% (n=19), 29.3% (n= 24) and 35.5% respectively. No significant difference was found in pregnancy rates and 25OH-D level in FF (p = 0.949, 0.985 and 0.614, respectively). The fertilization rate decreased significantly and the implantation rate increased (not significantly) with increasing 25OH-D level in FF. The fertilization rates associated with these three levels of vitamin D were 43.17%, 53.37%, and 58.77 %, respectively, (P = 0.055). No significant correlation was seen between the pregnancy rate and FF vitamin D level (P = 0.170), but the serum vitamin D levels showed a significant correlation (P = 0.000) with increasing pregnancy rate.

Limitations, reasons for caution: This study evaluated vitamin D levels from the follicles above 14mm only, but we haven't study the Vitamin D level in individual follicle of the same patients. We also did not measure vitamin D binding globulin level (VDBP) and oocyte quality. Also our study is based on Asian population only.
Wider implications of the findings: Vitamin D has an impact on IVF outcomes. As serum and FF vitamin D has a significant correlation, it is highly recommended to measure the vitamin D level before undergoing IVF procedure and if required vitamin D supplementation should be considered prior to the treatment to increase the success rate.
Trial registration number: Not applicable

P-669 Predicting accumulated pregnancy rates before IVF according to woman´s age and AMH.

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Study question: To determine whether the use of AMH and the age of the woman can predict the accumulative pregnancy probability before starting an IVF treatment.
Summary answer: IVF accumulative pregnancy outcomes are affected in a statistically significant way by the association between woman´s age and AMH.
What is known already: IVF outcomes will strongly depend on ovarian reserve, as the more eggs we obtain, the greater the chances will be. In the same way, accumulative pregnancy rates will increase depending on the number of good quality embryos obtained. Furthermore, chances of success will depend on woman´s age, as egg quality decreases with time.

Ovarian reserve will decrease with women´s age, but there is a great variability between each case, so it is very important to estimate ovarian reserve with a vaginal ultrasound to see the number of antral follicles and compare this with AMH blood value.
Study design, size, duration: Retrospective study of 80 patients who did an AMH and follicle count before IVF at our center during 2017-2018. The main aim was to study the association between AMH with age and the probability of accumulative pregnancy. Secondary outcome variables were antral follicle count, number of follicles equal or greater than 16mm measured before trigger, number of eggs obtained after retrieval, number of mature eggs microinjected, and number of good quality blastocysts obtained.
Participants/materials, setting, methods: IVF cycles included were performed with ICSI and blastocyst culture. 97% were single embryo transfers. Exclusion criteria were donor´s sperm, testicle biopsy, severe masculine factor, severe endometriosis and PGS cycles.

Logistic regression was performed to assess the association between AMH and age with probability of accumulated pregnancy. Lineal regression was performed to study the association between AMH and age with secondary outcome variables.

P below 0.005 was considered statistically significant and no significant above 0.05.
Main results and the role of chance: In our study group, the total accumulative pregnancy rate was 40.2%.

Global significance test shows that the association between AMH and age can predict the probability of pregnancy in a significant way. When we asses both parameters by themselves, we found out that each ng/ml more of AMH will multiply the probability of pregnancy by 1.36 in a significant way ($p<0.03$), however the older the women, the probability will decrease by multiplying it by 0.98 but in a non-significant way ($p=0.77$).

The association between AMH and age compared to secondary outcomes showed that either the antral follicle count, the number of follicles greater than 16mm before trigger, the number of eggs obtained after retrieval, the number of mature eggs microinjected, and the number of good quality blastocysts obtained increased in a significant way with higher AMH values ($p<0.001$). Nevertheless, this association was not significant when compared to woman´s age.
Limitations, reasons for caution: Many variables can influence pregnancy outcomes, therefore the prediction based in AMH and age has 30.3% sensibility but an 85.71% specificity.

Due to the statistical design and sample size, more studies are needed to obtain an accurate model to predict accumulative pregnancy rates before IVF.
Wider implications of the findings: Although age does not influence in a significant way regarding all the stimulation and laboratory parameters mentioned, the older the woman is, the lower the embryo quality will be. This means that despite an older woman has a good ovarian reserve, pregnancy outcomes will be worse than younger women.
Trial registration number: not applicable

P-670 Can cytochrome P450 2D6's polymorphism explain the variability in clinical response to clomiphene citrate in infertile women with ovulation disorders ?

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Study question: Is it a link between the cytochrome genotype and the response to clomiphene citrate?
Summary answer: The genetic polymorphism of cytochromes P450 2D6 does not appear to influence the clinical response to clomiphene citrate used in the induction of ovulation.
What is known already: Cytochrome P450 2D6 participates in the metabolism of many drugs. It has a genetic polymorphism responsible for four different metabolic phenotypes. The genotype of this protein is used to predict the efficacy and / or adjust the dosages of certain drugs, including tamoxifen, which is similar in structure to clomiphene citrate, used in the induction of ovulation. The response to this treatment, which is very often proposed in the management of dysovulation of polycystic ovarian syndrome (PCOS), is variable and no predictive factor has been identified at this time.
Study design, size, duration: This is a retrospective, observational, unicentric study performed in our institution, from July 2005 to January 2018, concerning 77 patients aged 20 to 39 years. All patients signed informed consent for genetic study and data analysis.
Participants/materials, setting, methods: Seventy-seven patients with ovulation disorders treated with clomiphene citrate benefited from the cytochrome P450 2D6, 3A4 and 3A5 genotype study. Response predictions have been established based on these genetic examinations. The ovarian response was assessed by ultrasound at the tenth day of the cycle and by the dosage of progesteronemia 9 days after the expected date of ovulation. The response predictions according to the genetic status of cytochromes were compared with the observed clinical responses.
Main results and the role of chance: Regarding their genotype, 4 patients with limited metabolizers were detected, including 1 patient with a homozygous deletion of the CYP2D6 gene. In contrast, 5 ultra-rapid, 41 normal and 27 intermediate metabolizers were included. For response predictions according to the combination of the three cytochromes, the concordance between the response predictions and the actual responses was 36.71%, with a negative Kappa coefficient ($K = -0.0240$), which corresponds to a big disagreement. Similarly, for predictions based on the genetic status of cytochrome P450 2D6 alone, only 39.24% of predictions were verified (coefficient $K = -0.0609$). Several mechanisms may explain our results. The metabolism of clomiphene citrate may be different than the one supposed. There are probably unknown mutations of the cytochroms, thus modifying the predicted phenotypes.
Limitations, reasons for caution: The strength of our population is the presence of "extreme" phenotypes. Nevertheless, the number of these phenotypes remains low and it follows a lack of power of our study. It is therefore a first study, the results of which need to be confirmed by other broader studies with higher numbers.
Wider implications of the findings: This study shows that the CYP2D6 phenotype is not correlate with the clinical response to clomiphene citrate in female dysovulatory infertile women. Broader studies are necessary to define the predictors of response to this treatment commonly used in gynecology. Our study brings elements on the metabolism of clomiphene citrate.
Trial registration number: not applicable

Table I The distribution of different genotypes association in low and normal ovarian reserve groups.

Variable	TP53/TT+TP73/GG	Other genotypes association	*P*	OR	95% CI
n	26	113			
Age (y)	33.9±3.5	33.5±2.8	0.56		
AMH (ng/mL)	0.95±1.3	2.62±3.6	0.0001		
AFC (2-9mm)	8.3±5.5	15.4±11.4	<0.0001		
Groups					
Low ovarian reserve (AMH<1+AFC≤9)	22 (84.6%)	58 (51.3%)	0.004	5.2	1.6-16.1
Normal ovarian reserve (AMH≥2+AFC≥15)	4 (15.4%)	55 (48.7%)			

P-671 Association between TP53 (rs1625895) and TP73 (rs3765730) polymorphisms can help in the prediction of low ovarian reserve.

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Study question: Is low ovarian reserve associated with TP53 and TP73 polymorphisms?

Summary answer: The association between TP53/TT (rs1625895) and TP73/GG (rs3765730) genotypes can help in the prediction of low ovarian reserve

What is known already: The ovarian reserve is a complex clinical phenomenon influenced not only by age but also by external pathogenic factors and genetic abnormalities. Research on new ovarian biomarkers is being developed to ensure optimal patient care and true personalization of ovarian stimulation. The p53 family members are powerful transcription factors and small variations in their DNA structure can modify gene expression patterns. The TP53 gene is involved in genomic stability and regulation of blastocyst implantation and TP73 gene controls the meiotic spindle, but the influence of these genes on the ovarian reserve is still unclear.

Study design, size, duration: All of the recruited women (n:139) met the following inclusion criteria: age ≤37years, normal karyotype, ultrasound evidence of the two ovaries and no ovarian surgery, endometriosis, hydrosalpinx, infections or endocrine problems. The women were divided into two groups according to their Anti-Müllerian hormone/AMH (ng/ml) levels and antral follicle count/AFC (2-9mm), evaluated during the early follicular phase:

-Low ovarian reserve (LOR/n=80): AMH<1+AFC≤9
-Normal ovarian reserve (NOR/=59): AMH≥2+AFC≥15

Participants/materials, setting, methods: DNA was extracted from peripheral blood samples taken from LOR and NOR groups. DNA was sequenced on MiSeq/Illumina to search for single nucleotide polymorphisms (SNPs) in the TP53 and TP73 genes. SNPs found by Next-Generation Sequencing (NGS) were analysed to find a possible association with LOR. Statistical analyses were performed to evaluate the power of the association between TP53 and TP73 polymorphisms and LOR. All statistical tests were considered significant at a level of $P<0.05$.

Main results and the role of chance: The TP53 T>C (rs1625895) and TP73 G>A (rs3765730) SNPs were identified by NGS associated to low ovarian reserve. The association of TP53/TT and TP73/GG genotypes was more frequent in the LOR group, leading to a 5.2-fold increase in the chance of women being included in this group when compared with women in the normal ovarian reserve group. Table I shows the results.

Limitations, reasons for caution: Additional validation of the SNPs analysed would be important to provide more information about the relationship of theses polymorphisms and ovarian reserve, once sample size was limited despite recruiting all eligible participants during the study period. Differences in the genetic backgrounds of various ethnic populations should also be considered.

Wider implications of the findings: The SNPs identified could provide an additional tool to test ovarian reserve, helping in the individualization of ovarian stimulation protocols. To the best of our knowledge this is the first study relating the association between TP53/TT (rs1625895) and TP73/GG (rs3765730) genotypes and low ovarian reserve.

Trial registration number: Not applicable. The local ethics committee authorised the study. Merck Grant for Fertility Innovation (GFI-2014-16) supported this study.

P-672 Baseline hyperandrogenemia and oocyte maturation in women undergoing in vitro fertilization

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Study question: Do women with baseline hyperandrogenemia have differential ovarian response, oocyte maturation, and/or embryo quality as compared to normoandrogenemic women?

Summary answer: Despite greater ovarian reserve, hyperandrogenemic patients had similar oocyte yield, blastulation, and aneuploidy compared to normoandrogenemic women. Hyperandrogenemia was associated with reduced oocyte maturity.

What is known already: While serum androgen levels have been proposed to have an important impact on early follicular development and granulosa cell proliferation, the relationship with ovarian stimulation outcome and on oocyte/embryo development remains unclear. In women with diminished ovarian reserve, treatment with exogenous androgens has been used to increase oocyte quantity and quality. Conversely, hyperandrogenemia has been proposed as a cause for reduced oocyte maturity and quality in patients with polycystic ovarian syndrome (PCOS). To clarify the relationship between endogenous androgens and oocyte/embryo maturity and quality, we examined the relationship between baseline hyperandrogenemia and the outcome of ovarian stimulation for IVF.

Study design, size, duration: This is a retrospective cohort study, including 97 hyperandrogenemic patients (69.4% PCOS; 4.7% congenital adrenal hyperplasia (CAH)) and 635 normoandrogenemic patients (72.9% PCOS; 5.0% CAH) who underwent 162 and 996 IVF cycles, respectively. Patients underwent testing of serum androgen levels as part of routine fertility work up prior to undergoing IVF. Approximately half of the cycles in both the subject and control group involved the utilization of preimplantation genetic screening (51.9% vs 51.4%, respectively).

Participants/materials, setting, methods: Patients, from 2010 to 2017, who underwent serum free testosterone and DHEAS testing and were categorized as hyperandrogenemic or normoandrogenemic. PCOS was diagnosed according to Rotterdam criteria. Baseline demographics, ovarian reserve, response to stimulation, oocyte yield, IVF laboratory outcomes, and embryo quality were compared using student's t-test and chi-square test. Multivariate linear and logistic regression analyses (controlling for age and ovarian

reserve) assessed whether baseline hyperandrogenemia associated with ovarian response, embryo development, and embryo ploidy.

Main results and the role of chance: Hyperandrogenemic patients with elevated serum free testosterone (95.8 ± 155.4 vs. 24.4 ± 17.7, p<0.0001) and DHEAS (523.4 ± 1066.9 vs. 171.3 ± 82.9, p<0.0001) were similar in age (34.3 ± 4.8 vs. 34.8 ± 5.0, p=0.3), compared with controls. Study patients had increased anti-mullerian hormone (AMH) levels (8.5 ± 13.1 vs. 5.0 ± 5.7, p=0.008) and basal antral follicle count (BAFC) (17.6 ± 11.3 vs. 15.4 ± 9.8, p=0.04). Groups significantly differed by stimulation protocol, with a higher proportion of GnRH antagonist protocol use in hyperandrogenemic patients (75.3% vs. 67.1%, p=0.04) and GnRH agonist downregulation in controls (18.1% vs. 9.9%, p=0.003). The cumulative gonadotropin dosage was similar (3249.3 ± 1854.1 vs. 3057.9 ± 1417.2, p=0.2) among groups. Controlling for age and ovarian reserve, study patients received more days of ovarian stimulation as compared to controls (β=0.5, p=0.0007). Hyperandrogenemia was not associated with the odds of cycle cancellation due to lack of response (OR 1.35 (95% CI 0.68-2.69), p=0.4), the number of eggs retrieved (β= -0.8, p=0.3), the degree of blastulation (β=0.0004, p=0.9), blastocysts amenable to trophectoderm biopsy (β=0.03, p=0.4), and the degree of embryonic aneuploidy (β= -0.009, p=0.8). Patients with baseline hyperandrogenemia had reduced oocyte maturity (β=-0.04, p=0.04).

Limitations, reasons for caution: This analysis is retrospective, and therefore vulnerable to confounding bias. While the multivariate model aimed to account for possible confounders, our findings should be supported by future studies that prospectively analyze the effect of hyperandrogenemia on ovarian stimulation in all patients, accounting for their ovulatory status and underlying diagnosis.

Wider implications of the findings: Baseline hyperandrogenemia was not correlated with egg/embryo yield or quality. However, there was an association with reduced oocyte maturity. Further studies should assess whether hyperandrogenemic patients with poor oocyte maturity in prior cycles could benefit from androgen suppression treatment prior to stimulation, and/or increased stimulation duration.

Trial registration number: This study was approved by the Western Institutional Review Board (Study Number: 1167398).

P-673 Follitropin delta in real-life clinical practice: short term AMH variation and treatment outcomes

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Study question: How often and to what extent does short term AMH variation affect follitropin-delta (FD) dosing and what is the treatment outcome in high AMH patients?

Summary answer: Short term serum AMH level variation affects dosing of FD in approximately 50% of patients by a magnitude of 1.6±1.3 µg/d dose change.

What is known already: Dosing of FD is based on a distinct algorithm utilizing one serum AMH value (measured within 12 months before treatment in one distinct ELISA) and body weight. Short-term intra-individual AMH and body weight variation could cause dosing inconsistencies.

The phase-III trial ESTHER-1 (Nyboe-Andersen et al., 2017) reported less patients with excessive ovarian response and OHSS/preventive interventions for patients receiving an individualized FD dose in a cohort of patients with a mean AMH level of only 2.28 ng/ml and a mean body weight of 64.7 kg. In the ESTHER-1 trial patients with cycle irregularities and BMI >32.0 kg/m^2 were excluded.

Study design, size, duration: Analysis of all patients receiving FD for ovarian stimulation for IVF/ICSI. At treatment planning, patients' most recent AMH and body weight were used for prescribing FD, and weight and AMH were determined again on treatment cycle-day 2/3 (and the dosage changed, if necessary). The target ovarian response was defined as 8-14 cumulus-oocyte-complexes (COCs) alike to the ESTHER-1 study. Weight and AMH are expressed as ±standard deviation (SD). The study is ongoing.

Participants/materials, setting, methods: AMH levels measured within 12 months and ad-hoc body weight was used for initial dosing. On the first day of follitropin delta injection, AMH and body weight were re-assessed and FD dose was adjusted accordingly. COC numbers in FD and previous and/or following controlled ovarian stimulation (COS) cycles using conventional FSH were analysed for intra-individual response comparison. AMH assessment was conducted using the Roche Elecsys ELISA AMH assay.

Main results and the role of chance: 74 patients (mean age 33.2±4.1 years) with 79 FD COS cycles and 61 cycles with complete bodyweight and AMH data were included in this analysis. 13 patients were diagnosed with cycle irregularities (oligomenorrhea). The mean body weight was 69.5±16.4 kg on first and 70.6±14.4 kg on second assessment. Mean AMH was 4.8±3.6 ng/ml on first and 3.9±2.5 ng/ml on second assessment, respectively. Accordingly, there was a dose change of, on average, 1.6±1.3 µg/d FD in 31 out of 61 cycles (50%, 95%CI: 37 - 62%).

A target response in COC numbers was achieved in 35 patient cycles (44%, 95% CI: 35-53%). Interestingly, 14 out of 67 (20.9%) patients with AMH levels >2 ng/ml showed a hyporesponse with less than eight COCs including three patients who had cancelation of the COS cycle. No predictor for hypo-response (age, body weight, size, daily or total FD dose, duration of stimulation, AMH levels or cycle irregularities) could be identified in this subgroup. A hyper-response with >14 COCs was observed in 25 patient cycles (31.6%, 95% CI: 21 - 41%). In 16 out of 50 (32% 95% CI: 18 - 45%) cycles having a fresh a fresh embryo transfer, a pregnancy was achieved.

Limitations, reasons for caution: The cohort is small and the study uncontrolled. Patients at risk of hyperresponse as well as patients with irregular cycles were included.

Wider implications of the findings: AMH and body weight changes which cause FD dose change occur frequently. A risk of underexposure to FSH and thereby hyporesponse for some patients with normal to high AMH needs to be explored by further investigations.

Trial registration number: not applicable

P-674 Initiation of ovarian stimulation independent of the menstrual cycle (random-start) in an egg donor program: a one-year single center experience.

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Study question: Are the cycle and reproductive outcomes after randomly initiating ovarian stimulation during the mid to late-follicular/luteal phase versus conventional protocols in egg-donors similar?

Summary answer: The oocyte yield and competence from random-start ovarian stimulation appears comparable to conventional protocols in egg-donors. Luteal-phase stimulation requires longer stimulation and higher FSH consumption.

What is known already: Recently, random-start ovarian stimulation protocols have emerged as an alternative to conventional ovarian stimulation allowing treatment to begin independently of the day of the cycle. Random-start protocols have been well studied in oncologic patients for fertility preservation and in women who desire "elective" cryopreservation of oocytes, resulting in optimal outcomes. A recent pilot study has also explored this protocol in oocyte donors (NCT02821819). In the context of an egg donation program, if ovarian stimulation can be initiated irrespective of the phase of the menstrual cycle without adversely impacting oocyte yield or quality, the approach may facilitate schedules.

Study design, size, duration: Retrospective analysis of egg-donation cycles (n=805) performed between January and November 2018. Egg-donor cycles starting stimulation on day 1-3 of the cycle were compared to egg-donor cycles initiating the ovarian stimulation independently of the menstrual cycle (day 4 onwards). The cycle, laboratory and clinical outcomes per embryo transfer in matched recipients (n=455) receiving a fresh embryo-transfer were also assessed.

Participants/materials, setting, methods: Egg-donors were stimulated following a conventional antagonist protocol and started stimulation with 150-300 IU/day uFSH (according to AFC/BMI) on day 1-3 of the menstrual cycle

(n=203) or alternatively in mid to late (Day 4-14) follicular phase (n=375) or luteal (Day 15-29) phase (n=227). A GnRH agonist was used for final triggering. Donors in the random-start group were not using OC pills the month before starting ovarian stimulation. Cycles were performed in a private IVF center.

Main results and the role of chance: Overall, baseline and cycle characteristics were similar between conventional vs random-start stimulation cycles with regards to: age, AFC, BMI, total dose of gonadotropins, duration of stimulation and days of GnRH-antagonist use. Additionally, the number of collected eggs were also similar: 17.49 ±8.9 vs 17.46 ±8.4, p=0.9, respectively.

A sub-group analysis showed an increased number of days of stimulation (10.4 ± 1.7 vs 9.8 ± 1.7 vs 10.2 ± 1.7, p=0.001) and gonadotrophin consumption (IU) (2171 ± 684 vs 2003 ± 647 vs 2037 ± 647, p=0.01) in the luteal phase group vs the mid/late follicular and conventional groups; respectively. Importantly, in matched recipients receiving a fresh embryo-transfer, the pregnancy rates (%) (62,7 vs 63,7 vs 63,4) and clinical pregnancy rates (%) (52.1 vs 55.7 vs 53.5) per embryo-transfer were similar between these sub-groups. Additionally, the mean number of transferred embryos 1.1 ± 0.3 vs 1.2 ± 0.4 vs 1.1 ± 0.3 and the number of surplus blastocyst stage embryos for freezing (3.1 ± 1.9 vs 3.1 ± 1.9 vs 2.7 ± 1.8) were also similar between sub-groups.

Limitations, reasons for caution: The inherent limitations of a retrospective design. Additionally, studies need to be conducted in the future including preimplantation genetic testing for aneuploidies (PGT-A) in embryos coming from the cohorts of oocytes obtained after random-start protocols and especially in terms of peri/post-natal outcomes to confirm the safety of random-start protocols.

Wider implications of the findings: These data, from a retrospective study design, shows that ovarian stimulation can be initiated regardless of the day of the menstrual cycle in egg donors without adversely impacting oocyte yield or competence; the approach may facilitate schedules in egg donation programs.

Trial registration number: Not applicable

P-675 TGF-β1 promotes hyaluronan synthesis by upregulating hyaluronan synthase 2 expression in human granulosa-lutein cells

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Study question: The aim of this study was to explore the effects and the underlying molecular mechanism of Transforming growth factor-β(TGF-β)1 on hyaluronan(HA)production in primary and immortalized human granulosa-lutein cells.

Summary answer: We demonstrated that TGF-β1 significantly increased the production of HA through up-regulation of HA synthase 2(HAS2) in human granulosa lutein cells.

What is known already: HA is the major component of extracellular matrix(ECM)of the cumulus oocyte complex (COC). HA-mediated ECM remodeling plays a pivotal role in antral formation and ovulation. TGF-β1 is an important cytokine in regulating follicular development including the ovulation processes. TGF-β1 participates in the process of extracellular matrix deposition in patients with chocolate cyst or PCOS. Moreover, also belonging to the TGF-β superfamily, our previous data showed that BMP4, BMP6, BMP7 are involved in the regulation of HA production. Taken together, we hypothesized that TGF-β1 may involve in the ECM synthesis in COC expansion via targeting HA synthesis in human granulosa cells.

Study design, size, duration: This is an experimental study which was performed over a 1 year period.

Participants/materials, setting, methods: Primary human granulosa-lutein cells obtained from women undergoing IVF and immortalized human granulosa cells were used as study models. HAS2 mRNA and protein expression were studied after treating with TGF-β1. An TGF-β1 type I receptor inhibitor SB505124,and small interfering RNAs of SMAD2, SMAD3,SMAD4,SNAIL,ALK4 and ALK5 were transfected to the cell models to explore the effects of TGF-β1 on HAS2 regulation. ELISA was used to evaluate the expression level of hyalurona.

Main results and the role of chance: For the first time, we demonstrated that TGF-β1 significantly increased the production of HA through up-regulation of HA synthase 2(HAS2), not through HA synthase 1 or 3, or HA degradases in human granulosa lutein cells. We confirmed TGF-β1 type II and type I receptors were associated with the TGF-β1-induced HA production by using the inhibitors of the receptors. Using small interfering RNA knockdown technique, we further demonstrated that the up-regulation of TGF-β1-induced expression of HAS2 and HA production was mediated by SMAD2, SAMD3 and SNAIL.

Limitations, reasons for caution: The results were conducted in the in vitro system and may not reflect the intra-ovarian microenviroment in vivo

Wider implications of the findings: Our study for the first time demonstrated that TGF-β1 induced HAS2 expression and enhanced HA synthesis. Both SMAD2 and SMAD3 independently contributed to TGF-β1-induced production of HAS2. Knocking down of SNAIL expression impaired HA generation. These results provides us new insights on HA production in human granulosa cells.

Trial registration number: NA

P-676 Poor ovarian response according to the Bologna criteria is associated with an increased aneuploidy rates in women undergoing preimplantation genetic testing for aneuploidy (PGT-A)

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Study question: Does poor ovarian response (POR) defined by Bologna criteria have an impact on the aneuploidy rate in cycles undergoing preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: Embryos of patients with poor ovarian response defined by Bologna criteria are found to associated with an increased aneuploidy rate when compared to normo-responders.

What is known already: It is well known that advancing maternal age negatively affects both oocyte quality and quantity. However, the impact of ovarian reserve on the oocyte aneuploidy, independently from the female age is still controversial. Moreover, defining "poor ovarian response" itself is still one of the unresolved issues of contemporary infertility management and treatment. Bologna criteria has recently been proposed and so far increasingly used internationally to define POR. Its main goal is to minimize these definition-related discrepancies and maximize the comparability of the published literature.

Study design, size, duration: This is a retrospective cohort study performed between January 2013 and September 2018. Patient records were analyzed and grouped according to normal or poor ovarian response defined by the Bologna criteria. 2123 ICSI cycles undergoing PGT-A for the indications of unexplained repeated pregnancy loss, unexplained repeated implantation failure and advanced maternal age were included. After evaluation, 318 of these cycles were found to be belonged to women with POR and 1805 were from normo-responders.

Participants/materials, setting, methods: A total of 5117 embryos were biopsied and trophectoderm biopsy materials were tested by either aCGH (between 2013 – 2016) or NGS (2016-2018) or aCGH for PGT-A. Demographic features, controlled ovarian stimulation characteristics, and embryology laboratory results were compared. The aneuploidy rates were also subjected to a subgroup analysis based on women's age.

Main results and the role of chance: Poor ovarian response group (POR) was significantly higher aneuploidy rate compared with normo-responders (NR) (74% vs. 63%, p=0.0001). Subgroup analysis according to the woman age also showed higher aneuploidy rates except in the most young age group [(POR vs. NR, p): <30 years: 27.5 % vs. 42.5, p>0.05; 30-34 years: 69.4% vs. 49.8, p=0.003; 35-37 years: 54.5 vs. 56%, p>0.05; 38-39 years: 71.7% vs.

67.7%, p>0.05; 40-41 years: 84% vs. 78.9%, p=0.022; 42 years: 93.2% vs. 89.8, p>0.05].

Limitations, reasons for caution: Its retrospective nature is the main limitation of the study.

Wider implications of the findings: The findings of this study indicate that higher embryonic aneuploidy exists in cases with poor ovarian response based on Bologna criteria. An additional contribution of diminished ovarian reserve on the age-related aneuploidy rate should be taken in consideration a potential negative effect on the reproductive outcome.

Trial registration number: N/A

P-677 Higher risk of gestational diabetes mellitus (GDM) after assisted reproductive technologies (ART) compared with spontaneous conception in singleton pregnancies: a systematic review and meta-analysis

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Study question: Are singleton pregnancies after ART associated with a higher risk of GDM compared with singleton pregnancies after spontaneous conception?

Summary answer: Singleton pregnancies after ART are associated with a higher risk of GDM compared with singleton pregnancies after spontaneous conception.

What is known already: Pregnancies resulting from ART have been associated with increased risks for obstetric and perinatal complications, such as preeclampsia, placental anomalies, preterm birth and increased caesarean sections, compared with spontaneous conception. However, it is not clear whether ART is associated with an increased risk of GDM. Several, recent, retrospective studies have evaluated the risk of GDM in singletons pregnancies resulting from ART compared with spontaneous conceptions. Thus it is feasible, by synthesizing the available evidence, to reach more solid conclusions.

Study design, size, duration: A systematic review and meta-analysis was performed aiming to identify studies comparing the risk of GDM in singleton pregnancies after ART and after spontaneous conception. For this purpose, a literature search was carried out until 20/01/2019.

Participants/materials, setting, methods: Matched and unmatched studies that compared the risk of GDM in singleton pregnancies following ART and spontaneous conception were included in the systematic review and meta-analysis. Studies were excluded if pregnancies were achieved after ovulation induction or intrauterine insemination. Meta-analysis of weighted data was performed using random effects model. Results were reported as risk ratio (RR) with 95% confidence interval (CI).

Main results and the role of chance: Thirty-two eligible studies (15 matched and 17 unmatched), published between 1995 and 2017, were eligible for the meta-analysis evaluating a total of 1,145,114 women. Diagnosis of GDM was made in 2,962 out of 36,367 women who underwent ART and in 61,267 out of 1,108,747 women who became pregnant spontaneously. In studies evaluating GDM after ART, *in vitro* fertilization (IVF) / intracytoplasmic sperm injection (ICSI) was performed in 16 studies, IVF in 13 studies and ICSI in three studies. Maternal age and parity were the most commonly used variables for matching pregnant women after ART with their counterparts after spontaneous conception. Additional matching variables included height, weight, ethnic origin and smoking.

Singleton pregnancies after ART were associated with higher risk of GDM compared with spontaneously conceived pregnancies (RR: 1.63, 95% CI: 1.43 to 1.85; heterogeneity: I^2=79%; random effects model; n=32 studies). A sensitivity analysis was performed using only the matched studies, without materially changing the direction or the magnitude of the effect (RR: 1.67, 95% CI: 1.33 to 2.01; heterogeneity: I^2=49.8%; random effects model; n=15 studies).

Limitations, reasons for caution: This systematic review and meta-analysis is based on observational studies and thus the presence of bias cannot be excluded. Moreover, the definition of GDM was not reported in the majority of studies. These limitations should be taken into account when interpreting the results obtained.

Wider implications of the findings: Women achieving pregnancy after ART should be monitored for GDM since the risk is increased compared with naturally conceived pregnancies. Whether this risk is attributed to the underlying infertility status of the couples undergoing ART as compared with those who conceived spontaneously, needs to be further explored.

Trial registration number: not applicable

P-678 A novel flexible progestin primed ovarian stimulation protocol for ART and its effect on oocyte yield compared to the flexible GnRH antagonist protocol

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Study question: Is pituitary suppression with medroxyprogesterone acetate (MPA) started on stimulation day seven or when the leading follicle is 14mm effective for ovarian stimulation for ART?

Summary answer: Flexible progestin primed ovarian stimulation(PPOS) with MPA effectively prevents premature ovulation and yields more oocytes than a flexible GnRH antagonist protocol with similar gonadotropin consumption.

What is known already: Currently, GnRH antagonists are the most commonly used agents for pituitary suppression in ovarian stimulation for ART. Progestins can also be used for preventing endogenous LH surge, when a fresh embryo transfer is not intended, e.g. freeze-all, oocyte cryopreservation, oocyte donation cycles. While various progestins have been used to this end, they have all been started at the beginning of stimulation simultaneously with gonadotropin. However, an LH surge is not imminent that early and whether progestins can effectively prevent a premature LH surge when started later in the cycle, like in a flexible GnRH antagonist protocol, is unknown.

Study design, size, duration: This is a retrospective analysis of 174 ovarian stimulation cycles of 87 oocyte donors between 2017 and 2018. Two stimulation cycles of the same donors, one with flexible GnRH antagonist and one with the new flexible PPOS, within a period of 6 months, were matched and compared. Thus, each woman acted as her own control, providing identical demographic and biological baseline characteristics, as would be in a randomized trial.

Participants/materials, setting, methods: Women were aged <35 years, had antral follicle count(AFC) of ≥20 and no contraindication to oocyte donation. 225IU/day rFSH was started on cycle day 2-3, 0.25mg/day GnRH antagonist or 10mg/day MPA was started on stimulation day 7 or when the leading follicle reached 14mm, whichever came first. 20IU leuprolide acetate was given when there were ≥3 follicles >17 mm. Data are defined with median (25^{th}– 75^{th}percentile). Wilcoxon signed rank test was used for comparisons.

Main results and the role of chance: There was a total of 87 donors who underwent both the flexible PPOS and GnRH antagonist cycles within six months during the study period. Mean age, body mass index and AFC were 25.46 years, 21.23 kg/$m^2$and 44, respectively. The duration of stimulation was 11 (10-11) days in both groups (p=0.13). Total gonadotropin consumption was similar with 2475 (2250 – 2475) IU in the flexible PPOS group and 2400 (2250 – 2475) IU in the GnRH antagonist group (p=0.35). Pituitary suppression was started similarly on day 7 (7-8), (p=0.37), and lasted similarly for 5 (5-6) days (p=0.37). There were no premature ovulations in flexible PPOS or GnRH antagonist cycles. The flexible PPOS cycles yielded a significantly higher number of cumulus oocyte complexes (COC) than GnRH antagonist cycles [33 (21-39) vs 26 (18-36), respectively; p=0.02]. Likewise, the flexible PPOS cycles generated significantly more metaphase-two oocytes than GnRH antagonist cycles [24 (17-34) vs 21 (15-28), respectively; p<0.01].

Limitations, reasons for caution: Retrospective design has limitations, however, participants acting as own controls ensures similar baseline characteristics between study groups. Although higher oocyte yield is associated with higher cumulative pregnancy rates and previous studies of progestin priming suggest comparable implantation, pregnancy and live birth rates, the unavailability of this information is a limitation.

Wider implications of the findings: The flexible PPOS can provide significantly more oocytes and an opportunity to choose pituitary suppression protocol later in the cycle depending on ovarian response and other factors,

e.g. switching to flexible PPOS from an intended flexible GnRH antagonist in case of unexpected excessive ovarian response requiring a freeze all policy.
Trial registration number: None

P-679 Effectiveness of Bariatric Surgery on Infertility in Morbidly Obese PCOS and non-PCOS Women

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Study question: What are the effects of bariatric surgery-induced weight loss on various endocrine parameters and pregnancy in infertile women with and without PCOS ?
Summary answer: Bariatric surgery showed favorable effects on certain parameters as well as in managing other metabolic co-morbidities like impaired OGTT, serum insulin levels, and successful pregnancy.
What is known already: Obesity has negative effects on fertility and IVF outcomes, and possibly also on AMH levels and other metabolic parameters.
Study design, size, duration: A retrospective cohort study of 28 women followed pre-bariatric surgery and followed postoperatively for 1 additional year between August 2007 and December 2015.
Participants/materials, setting, methods: This was a retrospective cohort study, comprising of 28 women (mean age=29.6±4.2 years). The study groups comprised of morbidly obese women (BMI > 37.5kg/ m^2)with (n=17) and without PCOS (n=11), with a prior history of infertility (average duration: 6.9±4.9 years), who underwent bariatric surgery. Retrospective analysis was performed for pregnancy rate, weight loss (in terms of body mass index), Anti-mullerian hormone (AMH) levels, OGTT value and serum insulin levels pre-surgeryand 1 year postoperatively.
Main results and the role of chance: At 1-year follow-up, the mean BMI among all the study participants decreased from 49.1 pre-surgery to 29.3 post-surgery. Similarly, there was substantial reduction in prevalence of impaired OGTT from 42.9% to 3.6% in the study groups. Prior to surgery, the mean AMH level was more than normal (3.5 ng/ml) in the PCOS group while it was 1.3 ng/ml in the non-PCOS group. After surgery, the AMH level for PCOS patients reduced to 3.1±0.6 and increased to 3.9±5.9 for non-PCOS patients. The total pregnancy rate was 64.3% (spontaneous pregnancy: n=14, IVF pregnancy: n=4).
Limitations, reasons for caution: This study is a retrospective analysis. Although the study period is long, sample size is still low and it should be enlarged.
Wider implications of the findings: Bariatric surgery may be a viable option for obese young women with infertility. More research is needed to evaluate the clinical effect of bariatric surgery prior to IVF treatment.
Trial registration number: NA

P-680 A model using a Comprehensive Algorithm of Reproductive Efficiency (CARE) system to predict outcome of IVF cycles

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Study question: Can male and female pre-treatment characteristics predict the laboratory performance of their gametes and embryos?
Summary answer: CARE system can predict number of cumulus-oocyte complexes retrieved and blastulation rates in patients undergoing IVF treatment
What is known already: Application of artificial intelligence (AI) in IVF has become a useful tool to boost treatment outcomes and improve patient counselling. McLernon et al. (2016) provided a predictive model for live birth for couples having up to six cycles but it was not adjusted for potential important predictors. Siristaidis et al. (2011) evaluated the potential use of artificial neural networks to predict clinical outcomes proposing several features for future algorithms. Recently, Correa et al. (2018) established an AI-based algorithm able to predict number of mature oocytes for first IVF cycles (unpublished).
Study design, size, duration: A total of 750 IVF cycles were included in this single centre retrospective cohort study from January 2018 to December 2018. Male and female pre-treatment characteristics included were: age, BMI, smoking/drinking habit, time of infertility, attempt, ovarian reserve test parameters (such as AMH, FSH, estradiol and antral follicle count) and sperm characteristics (ie. volume, concentration, motility and normal forms). Laboratory parameters included: oocytes collected, maturation, insemination protocol (IVF/ICSI), fertilisation rates and number of blastocysts developed.
Participants/materials, setting, methods: Sample was split into different sets for training the model (60%), cross-validate to tune hyperparameters (20%) and to test for accuracy (20%). A two-step strategy was designed and algorithms based on regression analysis were used for prediction analysis. CARE system was trained to predict number of cumulus-oocyte complexes (COCs) retrieved after stimulation and blastocyst formation after insemination.
Main results and the role of chance: A total of 7950 oocytes were collected, 4991 zygotes formed from which a total of 2682 developed blastocyst (mean 3.45 ± 3.2). Female and male age were on average 37.1 ± 4.1 years old (range 24-47) and 38.9 ± 5.8 (range 24-66), respectively. Ovarian response was categorised according to the number of COCs in sub-optimal (0-9), normal (10-14), and hyper response (>15). Moreover, blastocyst number was predicted with female and male pre-treatment variables in the following categories 0-1, 2-5 and >=6. In this first approach, CARE system algorithm could predict with a 63% accuracy the number of COCs obtained by a female patient only considering pre-treatment characteristics. Blastocyst formation was predicted with an accuracy of up to 61% for patients undergoing IVF treatment. The system could predict with more precision categories including 0 to 1 blastocysts precision=0.61, fscore=0.56) although accuracy decreased when blastocyst number was >6.
Limitations, reasons for caution: Larger sample studies are needed to increase accuracy and consolidate prospective application. Moreover, the inclusion of new variables will help to increase the discrimination power of the model.
Wider implications of the findings: Future models ought to provide not only predictive information on ovarian response but also information regarding blastulation and clinical outcomes. Such models would help clinicians to tailor treatment strategies for couples based only on their specific characteristics prior to ovarian stimulation.
Trial registration number: Not applicable

P-681 Increased primordial follicle retention by progesterone is counteracted by the concomitant application of Estradiol

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Study question: The aim of this study is to examine the effect of progesterone and estradiol alone or in combination on primordial follicles retention.
Summary answer: Progesterone significantly increases the number of primordial follicles. Estradiol alone decreased the number of primordial follicles and the combination counterbalanced the effects of progesterone.

What is known already: Progesterone suppresses the mTOR pathway (Lee et al, 2012) and preserves the primordial follicles (Zheng et al, 2018, Tsuyoshi et al, 2015). Estradiol stimulates the formation and development of primordial follicles in neonatal ovaries (Chen et al.2009, Kezele et al, 2003) although it is not clear the combined effect of the two hormones on the developed ovary.
Study design, size, duration: In a randomized preclinical study, 40 female immature Wistar rats were divided into four groups. All animals received the planned intervention from the 9 week and for 20 days in total. The combination of progesterone + estradiol was also used, to examine the effect on primordial follicles. Eight animals in each group were examined for the intervention effect and two animals from each group were allowed to mate.
Participants/materials, setting, methods: The control group received injections of corn-oil, subcutaneously. The other groups received progesterone (5mg/daily), estradiol (2mg/kg/daily) and progesterone together with estradiol (5mg + 2mg/kg/daily). Primary and secondary outcomes were measured at the left ovary after a treatment period of 20 days with histology. Serum AMH, estradiol and progesterone levels were assessed at the end of the intervention. Protein and RNA were extracted from the right ovary for further analysis.
Main results and the role of chance: Significantly more primordial follicles were observed in the Progesterone group (27. 75±12.8) whereas in the estradiol group they were less (13.12±6.4) (*P*=0.0114). The simultaneous administration of progesterone and estradiol reduced the primordial follicles (15±8.5).No difference was observed in the Atretic follicles between the groups (*P*=0.2107),or in the primary, secondary, pre-antral and Graafian follicles. All intervention groups displayed significantly less antral follicles (*P*=0.0024) than the Control group. The progesterone plus estradiol (11.37±4.65) and the Estradiol alone group (13.62±3.92) presented with significantly lower numbers of Corpora Lutea (*P*=0.0001) than the progesterone alone (35.75±4.39)and the control Group (34.37±8.97).

Gene expression analysis revealed that progesterone increased the mRNA levels of *SOHLH2* (a marker of the quiescent state of the follicles),whereas it decreased *GDF9* (present in the growing oocytes). Estradiol decreased the *FSHR* mRNA, possibly indicating a compensatory mechanism used by granulosa cells to reduce the FSH–driven production of estradiol. Both hormones decreased expression of *CYP17A1*, a gene important for steroid hormone biosynthesis. When progesterone and estradiol were administered together, the effect on *SOHLH2* and *GDF9* was similar to the Estradiol group, whereas the progesterone-mediated increases in *FSHR* and *CYP17A1* were smaller, suggesting that estradiol exerts a dominant effect on progesterone in the ovaries.
Limitations, reasons for caution: The time for hormonal intervention of 20 days, may be a limitation to fully understand the effect of progesterone on primordial follicle retention.
Wider implications of the findings: These findings may be used in with endometriotic cysts patients before undergoing surgery that may compromise ovarian reserve. The data might stimulate further research in the combined effect of progesterone and estradiol as well as on the mechanism by which estradiol overcomes the progesterone effect on primordial follicles retention.
Trial registration number: None

P-682 The cardiometabolic risk profile of middle-aged women with polycystic ovary syndrome (PCOS)

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Study question: To assess the cardiometabolic risk profile and prevalence of cardiovascular disease (CVD) in women with PCOS around the age of 50
Summary answer: Compared to age-matched controls from the general population, middle-aged women with PCOS exhibited metabolic disturbances, but no signs of premature atherosclerosis or increased CVD risk
What is known already: An increased prevalence and clustering of risk factors for CVD, such as metabolic syndrome, type II diabetes and hypertension, has been observed in women with PCOS. Contradicting results have been reported for the association of PCOS with surrogate markers for CVD and CVD end-points
Study design, size, duration: A cross-sectional study of 200 women diagnosed with PCOS during their reproductive years, and 200 age-matched control women from the general population
Participants/materials, setting, methods: We selected age-matched controls via propensity score matching (Greedy approach). We compared anthropomorphics, lipid levels and the prevalence of insulin resistance, type II diabetes, metabolic syndrome (NCEP definition) and cardiovascular events between both groups. We further compared carotid intima media thickness (IMT), 10-year CVD risk and cardiovascular health score between PCOS women and age-matched controls from the general population
Main results and the role of chance: The mean age was 50.5 (SD 5.5 years) in PCOS and 51.0 (SD 5.2 years) in controls. Increased waist circumference (93.0 versus 85.9cm), body mass index (28.4 versus 26.3) and prevalent hypertension (48.2% versus 26.5%) were more often found in PCOS women (P<0.001). The prevalence of type II diabetes (11.1% versus 6.5%) or metabolic syndrome (25.0% versus 17.0%) was not significantly increased and lipid levels were not different from controls. IMT was lower in women with PCOS (P<0.001). The estimated 10-year CVD risk (calculated according to the Framingham risk score) and cardiovascular health score (based on cholesterol and glucose levels, blood pressure, smoking status and BMI) in women with PCOS were similar to the controls
Limitations, reasons for caution: The results contradict with some previous reports and seem contra-intuitive but are nevertheless robust. Whether this relates to a more healthy population and environment needs confirmation in studies with similar demographic conditions
Wider implications of the findings: Although some unfavorable metabolic features were present in middle-aged women with PCOS, we found no evidence for premature atherosclerosis or an increased prevalence of metabolic syndrome, diabetes or CVD. Long term follow-up is necessary to provide a definitive answer on the long-term CVD risk in women with PCOS
Trial registration number: NCT02616510

P-683 Exploring outcomes with the use exogenous progesterone to replace the use of an antagonist in egg-donation cycles

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Study question: Is exogenous progesterone sufficient to block the LH surge and produce a similar oocyte yield in egg-donation cycles?
Summary answer: Exogenous progesterone seems sufficient to block the LH surge but a lower oocyte yield was detected in egg-donation cycles.
What is known already: Studies in own eggs IVF cycles shows that exogenous progesterone is capable to block the LH surge without compromising oocyte competence. The strategy may be also suitable for egg-donors (cost effective, patient-friendly, oral administration). However, very limited evidence is available with regards to the use of this strategy in the context of ovarian stimulation in egg-donors.
Study design, size, duration: Pilot, observational, prospective, (same-patient) comparative, cohort study in a private fertility center during year 2018. The pilot study planned to include 10 egg-donors in this exploratory phase.
Participants/materials, setting, methods: The study group started ovarian stimulation on day 2/3 of the cycle. Participants received urinary FSH 150-225 IU/d s.c. concomitantly with 100 mg micronized progesterone *per os*-.

Triptorelin 0.2mg induced the final follicular maturation. Controls: a previous cycle within 6 months performed by the same egg donor using the same type/dose of gonadotrophins and trigger agent but receiving cetrorelix 0.25 mg/d beginning with a leading follicle >14 mm. Egg-collection was performed 36 hours after triggering.

Main results and the role of chance: This exploratory trial was stopped prematurely after the inclusion of six egg-donation cycles in view of the suboptimal results in the study group. As expected, age was similar (23.6 ± 4.3 vs 24.6 ± 6.0 p=0.7) between control and study groups. The results were also similar with regards to: number of days of stimulation (9.2 ± 1.3 vs 10 ± 1.5 p=0.2) and gonadotrophin (IU) consumption (1641 ± 518 vs 1800 ± 653 p=0.7); however, a statistically significant difference was found in the number of collected eggs (23.6 ± 11.2 vs 8.4 ± 3.2 p=0.02) between control and study groups respectively. The average levels of LH (IU/L) and Progesterone (ng/ml) were: 3.7 ± 1.7 and 0.77 ± 0.2 on the trigger day in the study group. No premature LH peak (> 10 IU/L) was detected.

Limitations, reasons for caution: Although a statistical analysis is provided, the main limitation is the small number of cycles included which preclude strong conclusions. The LH/progesterone levels do not support a premature LH peak/luteinization as explanation for the significant difference in the number of collected eggs. Laboratory outcomes beyond fertilization were not analyzed.

Wider implications of the findings: Although the use of exogenous progesterone seems sufficient to block the LH surge, some concerns in relation with a potential suboptimal oocyte yield in egg-donor cycles warrants further investigations.

Trial registration number: No trial registration number

P-684 Aiding the decision-making process of subfertile couples by using a diagnostic test (ReceptIVFity) for recognition of embryo implantation failure in practice

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Study question: Does the ReceptIVFity-test influence a couples decision to postpone IVF or IVF-ICSI treatment?

Summary answer: Subfertile couples with a low chance to conceive, based on their individual profile of the ReceptIVFity-test, decide in >50% to postpone IVF or IVF-ICSI treatment.

What is known already: Research has shown that the bacterial composition within the vaginal microbiome can be used to predict the chance to conceive with an *in-vitro* fertilization without (IVF) or with intracytoplasmic sperm injection (IVF-ICSI). The ReceptIVFity test stratifies the vaginal microbiome into a low, medium or high microbiome profile, which correspond with the chance to conceive with IVF or IVF-ICSI treatment within two months, respectively low (6%), medium (24%) and high profile (53%). The predictive accuracy of the ReceptIVFity test with a low microbiome profile is 94% (with a sensitivity 26%, specificity 97%), indicating a chance of 94% to not conceive.

Study design, size, duration: In a randomised follow up trial, we intend to include 303 women prior to their first, second or third IVF/IVF-ICSI attempt. The women who are randomized in the 'intervention group' will receive a profile by the ReceptIVFity test and the women in the 'control group' will not. Inclusions started in September 2017 and is still ongoing.

Participants/materials, setting, methods: Women eligible for IVF/IVF-ICSI treatment and willing to perform a test prior to treatment are included. The ReceptIVFity test includes collecting a vaginal swab, which is subsequently analysed by the IS-pro technique. IS-pro is an eubacterial technique based on detection and categorisation of 16S-23S rRNA gene interspace regions with lengths specific for different microbial species. The resulting ReceptIVFity profile will be discussed with the couple by their physician using shared decision making before starting treatment.

Main results and the role of chance: So far, 168 women have been included of whom 161 are randomized in the intervention group, the other 7 women were randomized in the control group. In the intervention group, 26.1% (42/161) women had a low microbiome profile; subdivided in the first cycle 25.8% (32/124), second cycle 25.0% (8/32) and third cycle 21.4% (3/14). 54.8% (23/42) of the couples decided to temporarily refrain from initiating further treatment in case of a low profile. Moreover, couples who have a previous failed attempt seem more inclined to postpone treatment: first cycle 40.6% (13/32), second cycle 87.5% (7/8), third cycle 100% (3/3). None of the couples with a medium or high profile has discontinued treatment based on the result of the ReceptIVFity test.

Limitations, reasons for caution: Therapeutic interventions to improve a low vaginal microbiome profile are not yet available. Repeated empirical measurements of the effects of an intervention on local microbial communities are necessary to find a solution for patients with a low profile. Nevertheless, the vaginal microbiome is dynamic and can change spontaneously over time.

Wider implications of the findings: The ReceptIVFity acts as a timing tool and gives insight in the patients current (two months after sampling) success rate after IVF or IVF-ICSI treatment. Benefits include reducing unnecessary treatment burden, stress and disappointment, while increasing efficiency of treatment. ReceptIVFity might also benefit the development and monitoring of therapeutic solutions.

Trial registration number: Trial NL6442 (NTR6620)

P-685 Benefits of progesterone pituitary suppression stimulation protocol in comparison with a standard long protocol

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Study question: To compare stimulation protocol with progesterone given from first and 5th day of stimulation to standard long protocol.

Summary answer: The progesterone stimulation protocol could be good alternative for patients who do not plan a fresh embryo transfer.

What is known already: External supplementation of progesterone can act as a strong LH suppressor. This type of ovarian stimulation can be applicable for patients with any indication when fresh embryo transfer is not planned. Previously, this type of stimulation has been shown to significantly lengthen duration of stimulation and increase dosage of gonadotropins.

The use of progesterone to prevent premature LH surge has numerous advantages such as increased patient comfort level thanks to lower number of total injection during treatment, allows the use of GnRH agonist ovulation trigger instead of hCG and thus lowering the risk of OHSS.

Study design, size, duration: This was a multicentre prospective study at Invicta Fertility Clinics. The inclusion criteria were: age – 18-48 years, 1-2 previous IVF cycles. Statistical comparisons were performed using paired Wilcoxon test.

116 patients were included in the analysis. Median patient ages was 36 years old (IQR: 32 – 40). Two cycles were analyzed for each patient: first long protocol stimulation (long), then stimulation with progesterone (progesterone).

Participants/materials, setting, methods: First stimulation with long protocol had oral contraceptive (OC) priming, GnRH agonist (14 days after OC) and gonadotropin dosage based on patient's AMH level. HCG was used as ovulation trigger at 34-6 hours before retrieval.

Next stimulation also had OC priming. 7 days after the last pill stimulation started with daily gonadotropins. Progesterone was added starting from day 5 of stimulation – 100mg 3x daily vaginally. Trigger was 0.2 mg triptorelin 34-6 hours before retrieval.

Main results and the role of chance: Main outcome measures were duration of treatment, number of cumulus oocyte complexes and mature oocytes retrieved, number of top quality embryos on day 3 and final day of culture.

Duration of stimulation between long and progesterone stimulation was not significantly different. The difference in number of cumulus oocyte complexes (COC) retrieved was significant - more COC were obtained in the progesterone cycles (median: 10 (25th-75th: 6–14) than in the long protocol cycles (median: 8 (25th-75th: 4–14, p = 0.023). However, the number of matured oocytes was not significantly higher.

Embryo culture results showed statistically significant differences:

- number of correctly fertilized oocytes - progesterone: median:4 (25th-75th:2-6), long: median: 3 (25th-75th: 1-5), p = 0.006

- number of top quality embryos on day 3 - progesterone: median:2 (25th-75th:1-3), long: median:1 (25th-75th:0-2), p = 0.006

- number of top quality embryos on the final day of culture - progesterone: median:1 (25th-75th:0-2), long: median:1 (25th-75th:0-1), p = 0.008.

Limitations, reasons for caution: We found that short progesterone administration is sufficient to prevent premature LH surge. We did not find negative influence of progesterone on stimulation duration. No negative impact on embryo culture was found with higher numbers of TQ embryos obtained.

Wider implications of the findings: The progesterone stimulation protocol could be good alternative for patients who do not plan a fresh embryo transfer.

Trial registration number: not applicable

P-686 HMG does not improve the clinical pregnancy rate in poor responder patients undergoing IVF/ICSI: a meta-analysis.

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Study question: Is there a clinical advantage by using HMG for the ovarian stimulation in patients with poor ovarian response (POR) undergoing IVF/ICSI?

Summary answer: The stimulation treatment with HMG, compared with rFSH, does not improve the clinical pregnancy rate and it is associated with a longer duration of stimulation.

What is known already: Poor ovarian response to gonadotropins in IVF/ICSI cycles still today represents a challenge for reproductive physicians and a significant psychological burden for the patients. Different treatment regimens and gonadotropin combinations have been proposed during the years for improving the clinical pregnancy rates in these patients. Several studies suggest a positive effect of rLH in addition to rFSH in poor responders. HMG represents another source of LH activity and it is widely used for the ovarian stimulation in poor responders. Surprisingly, no systematic review or meta-analysis comparing HMG with rFSH, in poor responders, has been yet performed.

Study design, size, duration: A meta-analysis, based on PubMed, ISI-Web, Cochrane CENTRAL, EMBASE, was conducted to verify the effectiveness of the stimulation with HMG in poor responders. According to *PICO* format, inclusion criteria were: *Population*, poor responders; *Intervention*, treatment with HMG alone or in addition to rFSH or Corifollitropin alfa; *Control*, treatment with rFSH alone; *Outcome*, clinical pregnancy in patients undergoing IVF/ICSI. Secondary outcomes: duration of stimulation, total gonadotropins dose, cancelled cycles, retrieved oocytes, mature oocytes, fertilization rate.

Participants/materials, setting, methods: Electronic and hand search strategy, conducted from 1998 to 2018, yielded 211 studies. Two researches (A.M., S.G.) reviewed independently the studies, excluding 196 studies after the first screening (title and abstract) and 10 studies after the second screening. The Mantel-Haenszel method was used to calculate odds ratios (OR) and heterogeneity among studies (I^2). The results were expressed as OR with 95% confidence intervals (CI). Standardized mean differences (SMD) between groups were used for continuous outcomes.

Main results and the role of chance: Five studies with 1,076 participants were included. Two were RCTs; the other three were retrospective/prospective cohort studies.

In two studies (Eskandar et al., 2004; Youssef et al., 2017), HMG and rFSH treatments were directly compared; in the remaining three studies (Loutradis et al, 2004; Chung et al., 2005; Drakopoulos et al., 2017), HMG as treatment in addition to rFSH or Corifollitropin alfa was compared with rFSH treatment. The female age was similar among the studies.

Clinical pregnancy rate was not significantly different in women receiving HMG treatment (alone or combined) [93/550] compared with rFSH treatment [89/526] (OR 0.99, 95% CI 0.72 to 1.37, I^2=0%). No differences in number of retrieved oocytes (t= -1.46, p=0.15, SMD -0.93, 95%CI -2.17 to 0.32, I^2=86%), number of mature oocytes (t= 1.65, p=0.10, SMD 0.27, 95%CI -0.05 to 0.59, I^2=67%) and number of cancelled cycles [91/591 vs 106/580] (OR= 0.81, 95%CI 0.59 to 1.11, I^2=44%) were found between the two groups of treatment. The patients treated with HMG showed longer duration of stimulation (t=4.84, p=0.01, SMD 0.42, 95%CI 0.25 to 0.59, I^2=0%). The selected studies did not report sufficient data to analyze total dose of gonadotropins and fertilization rate.

Limitations, reasons for caution: In the selected studies, the definition of POR was not exactly the same but very similar. The couples' basal characteristics were not indicated in all the studies. Although the treatment with HMG was comparable among the studies, the dosages were different.

Wider implications of the findings: This meta-analysis indicates no improvement in IVF/ICSI success when the stimulation is performed with HMG alone or added to rFSH or Corifollitropin alfa, compared with rFSH.

The choice concerning the stimulation drug in poor responders should be mainly based on the costs of each treatment.

Well-designed cost-effectiveness studies are needed.

Trial registration number: Not applicable for non-clinical trials

P-687 Does Acupuncture Treatment Really Change the Outcome of IVF-ET in Poor Ovarian Response Population? –A Systematic Review and Meta-Analysis

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Study question: Does Acupuncture Treatment Really Change the Outcome of IVF-ET in Poor Ovarian Response Population?

Summary answer: Acupuncture treatment could improve the average number of retrieved oocytes and clinical pregnancy rate in POR patients. The deep mechanism still needs further consideration.

What is known already: Poor ovarian response (POR) remains a strict challenge for IVF-ET treatment. Due to a low number of retrieved oocytes, POR patients usually have significantly fewer transferable embryos. However, there is no consensus on the treatment of POR. Acupuncture was applied to females with infertility thousands of years ago in ancient China. According to the Traditional Chinese Medicine (TCM) theory, the insertion of microscopic needles at a specific point on human body will balance the inner Yin and Yang to lead a healthier situation. Multiple clinical approaches have been attempted to evaluate the effectiveness of Acupuncture in POR population.

Study design, size, duration: This article tried to summarise evidences from 7 RCT studies in recent 20years (1998-2018)by systematic review and meta-analysis to guide clinical decision.

Participants/materials, setting, methods: We started an RCT meta-analysis under Cochrane Systematic Review Guideline. After database searching and quality assessment, we enrolled 7 RCT studies (including 524 patients) focusing on acupuncture treatment in POR patients undergoing IVF-ET procedure. The intervention should be acupuncture and related techniques, with placebo or no treatment as control group. We set the outcomes as main endpoint characters in IVF-ET treatment.

Main results and the role of chance: After statistical analysis, our study found that, in POR population, although acupuncture improved average number of retrieved oocytes ($P<0.00001$). Other IVF-ET characters, like fertilization rate, cleavage rate and high-quality embryo rate did not show any difference($P>0.05$). Interestingly, the overall clinical pregnancy rate was markedly higher in the acupuncture group($P<0.0001$). This result might suggest that the more oocytes retrieved, the more transferable embryos could be gotten in POR patients.

Limitations, reasons for caution: The limitation of our study lies on the lack of original RCT studies, which would reduce the robustness of our result. We hope to make progress in this field.

Wider implications of the findings: Deep mechism of acupunture may still need further exploration.

Trial registration number: not applicable

P-688 Examining the effect of nicotine on a mouse model of the ovarian reserve

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Study question: Does nicotine have an effect on small follicles in the mouse ovary?
Summary answer: Neonatal ovaries express specific acetylcholine receptor sub-types and exposure to nicotine in vitro slightly inhibited growth of small, developing oocytes in comparison to controls.
What is known already: The substantial shift towards vaporiser and e-cigarette usage has led to the general assumption that these are safer alternatives to cigarettes. Although the negative effects of cigarette toxins and by products on the reproductive system are well established, very little is known about nicotine on its own; specifically, whether it has any impact on the ovarian reserve.
Study design, size, duration: Ovaries from 4-day old mice (n=6), 8-day old mice (n=5) and 16-day old mice (n=6) were initially used for gene expression analysis. To analyse effects of nicotine on small follicles, ovaries from 4-day old mice were maintained in culture for seven days exposed to high (45ng/ml; n=19), medium (15ng/ml; n=19), low (5ng/ml; n=19), or no nicotine (control; n=19) as determined by published plasma levels from e-cigarette users.
Participants/materials, setting, methods: RT-PCR was used to determine presence/absence of nicotinic acetylcholine receptor sub-types (alpha and beta nAChRs) in ovaries derived from C57Bl6 mice aged 4, 8 and 16 days. Expressed candidates were quantified between the same age groups using qPCR and proteins localised by immunofluorescence. Cultured ovaries were sectioned and stained for morphological assessment of follicle development, or qPCR to evaluate expression of genes associated with growth and viability.
Main results and the role of chance: Three specific alpha (*Chrna4*, *Chrna5* and *Chrna7*) and three beta (*Chrnb1*, *Chrnb2, Chrnb4*) nAChR subunits were detectable by RT-PCR in ovaries from immature mice. Expression of these subunits were relatively higher in ovaries from 4-day old mice (densely populated with primordial follicles) relative to older ages as determined by qPCR ($P<0.05$). Furthermore, CHRNA4, CHRNA5 and CHRNA7 proteins localised to granulosa cells and oocytes of small follicles, suggesting nicotine signalling in small follicles is plausible; therefore, day 4 ovaries were cultured in different concentrations of nicotine to evaluate the effects on gene expression and follicle development. After seven days, there were no differences in expression of *Amh, Gdf9, p63*or *Bcl2*between ovaries exposed to 0 (control), 5 (low), 15 (medium) or 45 (high) ng/ml nicotine ($P>0.05$). Interestingly, a slight decrease in the pro-apoptotic *Bax*gene transcript was detected in the high nicotine group relative to the low group ($P<0.05$). When morphological parameters were analysed, no differences were detectable between the proportion of primordial, transitional or early growing follicles between groups ($P>0.05$). However, the mean oocyte size was slightly reduced in transitional and early growing follicles of ovaries exposed to the high dose of nicotine relative to control ($P<0.05$).
Limitations, reasons for caution: This is a preliminary study using the immature mouse ovary as a developmental paradigm of the ovarian reserve. nAChR subunit expression may differ in humans and therefore the response to nicotine may not be extrapolatable. A single endpoint was evaluated after short-term culture and therefore longer-term effects are unknown.
Wider implications of the findings: This study raises the possibility that nicotine alone, when used at physiologically relevant levels, can cause subtle effects on the supply of small oocytes; however, the long-term consequence on ovarian function and fertility are yet to be determined.
Trial registration number: Not applicable.

P-689 Response to ovarian stimulation in IVF/ICSI cycles and its relationship with clinical pregnancy and live birth rates

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Study question: Which is the association between the number of oocytes retrieved and live birth rates in patients undergoing IVF/ICSI cycles?
Summary answer: The number of eggs in IVF/ICSI cycles is a predictor for clinical success. The number of oocytes to maximize live birth rate is 10-15.
What is known already: Several studies have shown that the number of retrieved oocytes in IVF/ICSI cycles is a possible predictor for live birth rate. It has been suggested that 6 to 15 oocytes is the optimal amount in order to increase the live birth rate in fresh embryo transfer IVF cycles.

The rate of newborns decrease if the number of oocytes obtained is lower to 6 or higher than 15.

Sunkara et al (2011) suggest that the number of eggs retrieved to maximize the live birth rate is 15.
Study design, size, duration: It was a retrospective cohort study including 1020 fresh IVF/ICSI cycles in the period from January 2012 to March 2017.

A binary logistic regression model was used to select the prognostic factors of clinical pregnancy and live newborn. In all hypothesis contrast a $p < 0.05$ was chosen as an alpha error.
Participants/materials, setting, methods: The data from all the fresh IVF/ICSI cycles of an Assisted Reproduction Unit of a public tertiary hospital were analyzed. 1186 cycles of controlled ovarian hyperstimulation of which 1020 reached the embryo transfer were included. The clinical pregnancy and live birth rate were estimated according to the ovarian response (poor response <4 oocytes, suboptimal response 4 to 9 oocytes, optimal response 10 to 15 oocytes and hyper response >15).
Main results and the role of chance: The probability of achieving a clinical pregnancy and the birth of a live newborn is greater when the number of retrieved oocytes increases reaching a maximum when 10 to 16 oocytes were obtained (optimal response) OR 1.79 IC 95% 1.33 - 2.49 and 1.62 IC 95% 1.17 – 2.24 respectively, decreasing the probability when more than 16 oocytes are captured OR 0.78 IC 95% 0.50 – 1.21

A significant difference was found in the rate of clinical pregnancy and of live newborns being lower in women in whom less than 4 oocytes were captured (poor response) since the probability of achieving a clinical pregnancy decreases up to 73% (OR 0.364 IC 95% 0.247- 0.536) and for a live newborn it decreases by 54.3% (OR 0.467 95% CI 0.306 – 0.712)
Limitations, reasons for caution: The cycles with frozen embryo transfer were not analyzed, its inclusion could increase the accumulated live birth rate.

The decrease in live birth rates in the hyper response group could be explained because the majority of fresh embryo transfer was avoided in order to reduce the risk of hyperstimulation ovarian syndrome.
Wider implications of the findings: The number of retrieved oocytes is a prognostic indicator of clinical success. The results showed a relationship between the number of eggs and the live birth rate in IVF cycles in agreement with some authors.
Trial registration number: Not Applicable

P-690 Myo-inositol may improve oocyte quality and fertilization rate in women with polycystic ovary syndrome undergoing assisted reproductive technology cycles

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Study question: Whether, if myo-inositol could improve the oocytes quality, fertilization rate and embryo quality in PCOS patients undergoing ART cycles based on molecular mechanisms?
Summary answer: Myo-inositol improved the percentage of MII oocytes, fertilization rate and ratio of good quality embryos by altering the expression of genes related to oocyte quality.

What is known already: Although some studies have shown the positive effects of Myo-Inositol supplementation among PCOS patients on the quality of oocytes and embryos but there are controversial opinions in this regard and the mechanism of Myo-Inositol effect on the quality of embryo and oocyte has not yet been fully elucidated.
Study design, size, duration: This placebo-controlled double-blind randomized clinical trial was conducted in Shahid Akbar Abadi hospital IVF center, Iran University of Medical Sciences, Tehran, Iran, Feb 2017- May 2018. Fifty infertile PCOS patients were randomly designated in two groups.
Participants/materials, setting, methods: Subjects received daily dose of 4g Myo-Inositol combined with 400mg folic acid from one month before starting the antagonist cycle until day of OPU. Oocytes and embryos quality were assessed according to ESHRE guidelines. The gene expression of PGK1, RGS2 and CDC42, as a factor of oocyte quality in granulosa cells, was analyzed using real-time RT-PCR. Levels of Total Antioxidant Capacity (TAC) and Reactive Oxygen Species (ROS) in follicular fluid were evaluated using chemiluminescence assay.
Main results and the role of chance: The number of retrieved oocytes and follicle count were not statistically different between the two groups, but the percentage of metaphase II oocyte, fertilization rate and embryo quality was significantly higher in the study group ($P < 0.05$). Furthermore, the gene expression of PGK1, RGS2 and CDC42 were significantly higher in the study group ($P < 0.05$), but no differences were found between two groups in terms of TAC and ROS levels. The cumulative pregnancy rate was not significantly improved in the case group either.
Limitations, reasons for caution: Our study provides some new molecular evidence about the possible mechanism of Myo-Inositol effect on the quality of oocyte and embryo based on improved granulose cell gene expression,but we did not observe an improved cumulative pregnancy rate which might be related to the limited number of subjects in this trial.
Wider implications of the findings: We observed that Myo-Inositol alters the gene expression in granulosa cells and improves oocyte and embryo quality. If the clinical efficacy of our findings on pregnancy rate is approved by further RCTs , the use of Myo-inositol as an adjuvant in ART cycle can be suggested for PCOS patients.
Trial registration number: IRCT20171208037790N1

P-691 Metformin versus the combined oral contraceptive pill for hirsutism, acne, and menstrual pattern in polycystic ovary syndrome

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Study question: To assess the effectiveness and safety of Metformin versus the Oral Contraceptive Pill (OCP) in improving clinical features of Polycystic Ovary Syndrome (PCOS) in adult women.
Summary answer: This updated systematic review shows an improvement in menstrual pattern and hirsutism with the OCP whilst Metformin shows an improvement in BMI.
What is known already: Hyperinsulinemia is one of the PCOS features and participates in hyperandrogenemia through the stimulation of both ovarian and adrenal androgen secretion and the suppression of liver sex hormone-binding globulin (SHBG). This excess of androgens is also responsible for irregular menses. It was thus advocated that Metformin could be a treatment for PCOS as effective as the OCP for improving hyperandrogenemia and menstrual cyclicity. In the previous systematic review, the OCP resulted in an improvement of menstrual patterns. However, there was insufficient evidence in favour of either Metformin or the OCP in treating hirsutism, acne (pimples) or BMI.
Study design, size, duration: For the update of this Cochrane systematic review we conducted a search according to the Cochrane Gynaecology and Fertility search strategy for randomized controlled trials (RCTs) in which Metformin was used versus OCP or OCP versus Metformin combined with OCP or Metformin versus Metformin combined with OCP from inception to October 2018. Eighty-three articles were identified (12 abstracts, 71 full texts). Thirty-three new RCTs met inclusion criteria added to the six original ones.
Participants/materials, setting, methods: Only randomized controlled trials (RCTs) comparing Metformin versus OCP, OCP versus Metformin combined with OCP, Metformin versus Metformin combined with OCP were considered for inclusion. Crossover trials were not included unless pre-crossover data were available, in which case only these data were used for the purpose of the review. The inclusion criteria were the presence of PCOS based on clinical (ovulatory dysfunction, hirsutism, acne, androgen dependent alopecia), biochemical (hyperandrogenemia), or ultrasound (polycystic ovaries) evidence.
Main results and the role of chance: The OCP resulted in an improvement in hirsutism (Ferriman and Gallwey score) compared to Metformin (Mean difference (MD) 1.08, 95% CI 0.57 to 1.59, 10 RCTs, n = 473, I^2= 50%). Metformin combined with the OCP resulted in an improvement in hirsutism (Ferriman and Gallwey score) compared to the OCP alone (MD 0.54, 95% CI 0.2 to 0.89, 6 RCTs, n = 389, I^2= 1%) and also compared to Metformin alone (MD 1.36, 95% CI 0.62 to 2.11, 3 RCTs, n = 135, I^2= 9%). The OCP resulted in an improvement of menstrual cyclicity compared to Metformin (MD 6.05, 95% CI 2.37 to 9.74, 2 RCTs, n = 153, I^2= 0%). The OCP alone resulted in an improvement in acne (clinical acne score) compared to Metformin combined with the OCP (MD -0.09, 95% CI -0.10 to -0.08, 1 RCT, n = 82). Metformin resulted in an improvement in BMI compared to the OCP (MD -0.63, 95% CI -0.97 to -0.29, 19 RCTs, n = 887, I^2 = 95%). Metformin resulted in an improvement in BMI compared to Metformin combined with the OCP (MD -1.47, 95% CI -2.27 to -0.66, 5 RCTs, n = 199, I^2= 25%).
Limitations, reasons for caution: There was heterogeneity between the trials and some results were made only on one trial. Therefore, the results must be interpreted with caution.
Wider implications of the findings: The OCP remains superior to Metformin in improving menstrual pattern. The OCP alone or combined with Metformin seems to improve hirsutism. Metformin is superior to the OCP regarding BMI. Concerning acne, further RCTs are required for any conclusions to be drawn.
Trial registration number: Not applicable

P-692 Vascular endothelial growth factor antagonist reduces the early onset and the severity of ovarian hyperstimulation syndrome

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Study question: This study aims' at evaluating the efficiency of prophylactic and therapeutic use of cabergoline in women with higher risk of developing ovarian hyperstimulation syndrome (OHSS).
Summary answer: Cabergoline use reduce the rate of early OHSS and its severity and does not affect ART outcomes in terms of pregnancy, implantation and miscarriage rates.
What is known already: OHSS is s the most serious complication of controlled ovarian stimulation(COH) during assisted reproductive technology protocols. This syndrome is a result of ovarian expression of vascular endothelial growth factor (VEGF), which increases vascular permeability. The rationale behind using cabergoline,a dopamine agonist is that it might counteract the increased production of VEGF by the follicles. It has been demonstrated in an animal model that cabergoline, partially inhibits the ovarian VEGF receptor-2 through dereasing its phosphorylation levels. in addition, several human studies reported that cabergoline might provide a new specific and non toxicapproch to the treatment of OHSS without altering angiogenesis
Study design, size, duration: A prospective randomized study including 146 couples undergoing ICSI cycles with agonist protocols and having higher risk of OHSS diagnosed during the hCG day injection .Two groups were identified

according to the administration or not of dopamine agonist (Dostinex©). A first group (n=78) women who received 0.5mg per day of cabergoline orally for 7 days starting from hCG administration day. The second group (n=68)who received no medication treatment.

Participants/materials, setting, methods: A total of 25 patients in each group developed OHSS and were admitted in our hospital. Subgroup 1, 25 cases of OHSS obtained in group 1received cabergoline and subgroup 2 where 25 cases of OHSS obtained in group 2 received no medication. Diagnosis of OHSS was made according to Golan criteria. According to Carriz et al, early OHSS was identified when the onset of OHSS was initiated during the first 9 days after hCG administration.

Main results and the role of chance: There was no evidence of a statistically significant reduction in the incidence of OHSS in cabergoline group (32.05%vs36.76%;p>0.05).Late OHSS was observed in 60.6% of cases in cabergoline group while 39.4% of cases in the other group(p=0.036).Early OHSS decreased significantly in the cabergoline group(p<0.05). Severe OHSS cases were more common within subgroup 2 than subgroup1 (32% vs 8%, P=0.000). There was no difference in clinical pregnancy rates and miscarriages rates between the two subgroups.

Limitations, reasons for caution: An important clinical question can still be raised concerning the best time for using Cabergoline to prevent and treat OHSS. Further studies can be carried out to evaluate the most effective time and dose of Cabergoline administration.

Wider implications of the findings: A low oral dose administration of dopamine agonist(Cabergoine) for patients with high risk of OHSS can reduce the rate of early OHSS and its severity in GnRH agonist IVF cycles, but can not prevent the incidence of OHSS.

Trial registration number: not applicable

P-693 Enhancing of human oocyte maturation by Kisspeptin-54

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Study question: Can Kisspeptin-54 be used to induce oocyte maturation in women undergoing in vitro fertilisation (IVF)?

Summary answer: Kisspeptin-54 is able to trigger oocyte maturation in women undergoing IVF

What is known already: Human chorionic gonadotropin (hCG) is currently used as the gold standard to trigger final oocyte maturation, despite its risk of developing ovarian hyperstimulation syndrome (OHSS). Animal studies showed that administration of Kisspeptin-54 could trigger LH surge and oocyte maturation through a GnRH-dependent mechanism.

Study design, size, duration: A systematic review was performed to related clinical trials published in Pubmed, Cochrane, and Medline

Participants/materials, setting, methods: Three clinical trials were analysed in this study with a total of 175 patients included

Main results and the role of chance: in normal women, oocyte maturation was observed in 75-85% of patients and increased with higher doses of kisspeptin-54. The fertilisation and embryo transfer rate were both 92%, the biochemical pregnancy was 40%, and the clinical pregnancy rate was 23%. In women at high risk of OHSS, oocyte maturation occurred in 95% of patients with 62-85% of pregnancy rates, 25-58% of implantation rates, and 25-62% of live birth rate. No patients developed moderate, severe, and critical OHSS whilst only 5% were having mild early OHSS and 2% were having mild late OHSS that needed no medical intervention. If Kisppeptin-54 was given twice, the proportions of ≥60% oocyte yield was greater (71%) than the single dose (45%) without significant difference in the occurrence of OHSS. Compared to single dose, the implantation rates and live birth rate were higher in patients received two doses of Kisspeptin (37.1% vs 23.3% and 39% vs 19.4%, respectively)

Limitations, reasons for caution: All trials were conducted by the same research group with limited samples. Some patients have had previous oocyte maturation trigger during previous cycle that might alter the results. Thus, further study with larger sample and comparison between current standard treatment (hCG and GnRH agonist) are recommended

Wider implications of the findings: This review revealed the efficacy of Kisspeptin-54 to trigger final oocyte maturation with less risk of developing OHSS, which was very important to develop a suitable IVF protocol for patient at risk of OHSS

Trial registration number: Not applicable

P-694 The correlation between serum progesterone level and clinical outcomes in hormone replacement prepared thawed embryo transfer cycle

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Study question:

- Is there any correlation between serum progesterone level and clinical outcomes in hormone replacement prepared thawed embryo transfer cycle?

Summary answer:

- No correlation was found between serum progesterone level on the day before embryo transfer and the clinical pregnancy rate in hormone replacement-thawed embryo transfer cycle.

What is known already:

- Artificial hormone replacement was widely used in thawed embryo transfer cycle to prepare endometrium, especially when exogenous vaginal administrated-progesterone was introduced. However, there was no consistent conclusion if there was an optimal serum progesterone level after exogenous progesterone administrated. A recent prospective study claimed that a minimum threshold of serum P values (>9.2 ng/ml) on the day of ET should be reached in artificial endometrial preparation cycles to optimize outcome.

Study design, size, duration:

- This is a retrospective cohort study. From January 2011 to January 2017, 395 patients who underwent their first HRT-TET cycle with one good blastocyst(>3BB) transferred were recruited.

Participants/materials, setting, methods: Patients who underwent their first HRT-TET cycle with single one good blastocyst(>3BB) transferred were recruited. To prepare endometrium, 4-6mg estradiol valerate was administrated for at least 10 days, when endometrial thickness reached 8 mm, vaginal micronized progesterone (200mg tid) and dydrogesterone (10mg tid) were administrated, and five days later, thawed blastocyst was transferred. Patients with severe adenomyosis, abnormal uterine cavity (intrauterine adhesions) or hydrosalpinx were excluded from this study.

Main results and the role of chance: No significant difference was observed in basic characteristics and serum estrogen and progesterone level on the day of progesterone supplementation (All P >0.05) . The cycle characteristics were listed in following table.

Logistic regression analysis was also performed and the result showed that only patients' age was negatively associated with clinical pregnancy rate, while progesterone level has no correlation with clinical pregnancy rate

Limitations, reasons for caution: Retrospective nature of this study might introduce some bias. After strictly screening, only patients with adequate endomentrial growth and good quality blastocyst were recruited to exclude interference from confounding factors, besides, the multivariate logistic regression analysis was also performed in this study to guarantee the reliability of our results.

Wider implications of the findings: Based on our results, serum progesterone level on the day before embryo transfer has no correlation with clinical pregnancy rate in HRT-TET cycles, therefore there was limited value to routinely check serum progesterone level before thawed embryo transfer . At last, large-scale multi-center RCT is needed to verify our results.

	Group1(n=)	Group2(n=)	Group3(n=)	Group4(n=)	P
Age	30.38±4.20	31.84±4.78	30.44±4.44	31.46±4.54	0.257
BMI(kg/m2)	21.96±3.01	21.91±3.14	21.46±2.79	21.46±2.81	0.629
endometrial thickness(mm)	9.73±1.47	9.59±1.32	9.91±1.57	10.24±1.91	0.853
Estradiol level on the day of progesterone supplementation(pg/ml)	220.25±296.43	185.74±104.26	228.45±176.69	287.58±267.24	0.184
Progesterone level on the day of progesterone supplementation(ng/ml)	0.29±0.18	0.39±0.58	0.36±0.21	0.63±0.54	0.000
Estradiol level on the day before embryo transfer(pg/ml)	221.13±236.03	237.23±380.96	197.80±112.19	302.50±366.44	0.306
Progesterone level on the day before embryo transfer(ng/ml)	5.51±1.39	8.00±0.76	10.80±0.92	19.93±8.31	0.003
Clinical pregnancy rate	62.60%	52.50%	53.50%	50.00%	0.305
Miscarriage rate	13.10%	10.10%	7.10%	5.10%	0.209

Trial registration number: -

P-695 characterization of circular RNA profiles in plasma from healthy women and women with primary ovarian insufficiency

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Study question: Are there expression differences in plasma circular RNA (circRNA) between healthy women and women with primary ovarian insufficiency (POI)?
Summary answer: Our study demonstrated that circRNA profiles in plasma from healthy women and POI patients differed significantly.
What is known already: The mechanisms of POI remain largely unknown, approximately 50% accordingly. More effective therapeutic measures might be taken in time if clinical diagnostic biomarkers are discovered. Circular RNAs (circRNAs) have recently been implicated in ovarian aging, but their potential as biomarkers was never investigated in POI. It is reasonable to sequence circRNAs in plasma from POI patients, which might predict their biological functions in the development of POI. Herein, this study aimed to identify the role of circRNAs as a potential biomarker in POI patients.
Study design, size, duration: A total of 25 POI patients and 25 age- and sex-matched controls were included in this study. Recruitment took place from July to November 2018.
Participants/materials, setting, methods: CircRNA expression screening was performed in plasma RNA from five pairs of POI patients and age- and sex-matched controls using circRNA microarray. In the validation phase, 18 circRNA candidates were validated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in a cohort of 15 patients diagnosed with POI and 15 age- and sample storage time-matched controls. For further verification, We performed qRT-PCR on samples from 10 POI patients and 10 controls.
Main results and the role of chance: A total of 6644 differentially expressed circRNAs, 12 significantly up-regulated and 23 significantly down-regulated circRNAs were identified in the POI patients compared with the control group, which showed as red points in the volcano plot analysis($p<0.05$, fold change>1.5). Based on the evaluation of the initial expression quantity and fold change values, 18 circRNAs candidates were selected for the validation phase. Hsa_circ_0000376 was significantly downregulated in POI patients compared with the control group in qRT-PCR ($P<0.05$). The area under the ROC curve (AUC) of hsa_circ_0000376 was 0.698(95% CI 0.499-0.897). The sensitivity and specificity at the cutoff value of 0.685 were 73.3% and 60%, respectively.
Limitations, reasons for caution: Primary ovarian insufficiency (POI) is a reproductive disorder occurs to women before 40 years old, which affects approximately 1%-3% of women of reproductive age. Due to the limitation of time, larger sample size is expected in our futher study.
Wider implications of the findings: Taken together, our study indicates that hsa_circ_0000376 may play potential roles in predicting POI.
Trial registration number: ChiCTR1800017312

P-696 The relationship between endogenous androgens levels and ovarian response to controlled ovarian stimulation for in vitro fertilization

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Study question: Are endogenous androgens serum levels related to ovarian response to controlled ovarian stimulation (COS) performed for in vitro fertilization (IVF)?
Summary answer: The serum level of the androgens seems to be related to the oocytes and zygotes number obtained after COS performed for IVF.
What is known already: The endogenous androgens have a central role in folliculogenesis and both low and high androgens levels were related with inadequate response to controlled ovarian stimulation in in vitro fertilization in particular conditions as poor ovarian responders or polycystic ovary syndrome. However, limited data are available about a possible relationship between endogenous androgens levels and ovarian response to stimulation in a population of infertile patients without a specific pathology affecting androgens production.
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 2017 and January 2018 with all causes of infertility were reviewed. Two hundred eighty patients were included in the study. Patients with conditions known to be associated with abnormal androgen production like polycystic ovary syndrome or advanced reproductive age (over 40 years old) were excluded from the study.
Participants/materials, setting, methods: The following were measured in serum at the first evaluation for infertility before IVF: total testosterone, dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG) and free androgen index (FAI) was calculated. The number of the oocytes obtained after COS, zygotes number and fertilization rate were also retrieved from the medical files of the patients.
Main results and the role of chance: We found a positive correlation between total testosterone and oocytes (beta=0.176, p=0.001) and zygotes number (beta=0.230, $p<0.0001$) which was maintained after adjustment for age. Patients in the lowest quartile of DHEAS serum level had a significantly lower number of oocytes as compared with patients in the highest three quartiles (6.92±5.6 versus 8.49±5.43, p=0.045). A value of FAI above median value was associated with higher oocytes (9.36±5.98 versus 6.96±4.88, p=0.001) and zygotes (6±4 versus 4.59±3.2, p=0.003) number in comparison with

patients with lower FAI level. No relationship between androgens level and fertilization rate was identified.

Limitations, reasons for caution: The main limitation of the study is the relatively small number of patients included in the study. Another limitation is the fact that the serum androgen level was measured before IVF was performed and were probably influenced by the COS protocol.

Wider implications of the findings: Since the oocytes and zygotes number was reported to be an important predictor of live birth, the association between higher androgen levels and these parameters in patients without obvious abnormalities of androgen production offers an interesting perspective of possible ways to influence therapeutic success.

Trial registration number: NA

P-697 Investigation and management of fertility problems: A systematic review of guidelines

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Study question: What is the variation in recommendations and the methodological quality of the different national and international guidelines on the investigation and management of infertility?

Summary answer: There is substantial variability on the scope as well as on the methodological quality of the guidelines.

What is known already: Infertility is a complex term including a plethora of etiologies and possible therapies. The infertile patients are also a unique category of patients, with high hopes for procreation that makes them potential easy targets for exploitation. For those reasons, the existence of good quality clinical guidelines on infertility could help towards universal, evidence-based, high-quality standards of care.

Study design, size, duration: Systematic review.

Participants/materials, setting, methods: A database search in EMBASE, MEDLINE, Pubmed and the International Guideline Library as well as an additional purposive search from leading national and international authorities and Google took place on 07.07.2018 in order to identify relevant guidelines on investigation and/or management of infertility. Their recommendations were mapped, a presence and a disagreement rate was calculated and their methodological quality was assessed using the AGREE-II instrument.

Main results and the role of chance: A total of 24 guidelines were included in the study. Only 2 of them addressed infertility as an entity, whereas the rest addressed only a specific etiology. Overall, substantial variability on the scope of the guidelines was observed, but individual recommendations appeared to be coherent. Methodologically there was substantial variability on the quality not only across guidelines but also in different methodological aspects within the same guideline.

The development of universal guidelines for complex medical entities is challenging. However, for infertility, the majority of the recommendations agreed among different guidelines. Methodologically, all the guideline had their pros and cons but the domains needing the biggest improvement are public involvement, financial transparency and suggestions for better applicability

Limitations, reasons for caution: Due to the language restrictions, 7 guidelines written in Spanish, Russian and Polish were excluded and the possibility that valuable information on variability, especially for the 4 Spanish-speaking countries of Central and Latin America that have clear ethnological, cultural and geographical differences from any other included guideline cannot be excluded.

Wider implications of the findings: The discrepancies of the present guidelines can potentially create confusion among patients with infertility but also health providers in that field. A consensus on the most important steps in the investigation and management of infertility would be advantageous for all parties.

Trial registration number: PROSPERO ID CRD42018111255

P-698 Follicular fluid composition impact on folliculogenesis - age and BMI as major factors contributing to oxidative/inflammatory biomarkers levels

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Study question: How does oxidative and inflammatory biomarkers levels correlate with patients' intrinsic factors?

Summary answer: Age and BMI appear the be major factors correlated with CRP, AOPPs and TAS levels on FF

What is known already: Follicular fluid (FF) composition results from the contribution of blood plasma constituents that cross the blood follicular barrier and granulosa cells (GC) secretory activity. Since it provides the microenvironment for oocytes development, it represents an optimal source of non-invasive biochemical predictors of reproductive potential. Any dysregulation in FF composition can alter ovarian follicular dynamics and, thus, impair oocyte quality and fertility. Although the research in this area has progressed towards a more complex type of molecular analysis, no reliable biochemical predictors of oocyte quality in FF has been determined so far, nor the main factors affecting FF composition.

Study design, size, duration: This study was conducted in 238 women undergoing IVF at Hospitalar Center of Vila Nova de Gaia/ Espinho, Portugal, from March to December 2018. This study was approved by the Ethical Committee of the Hospital and by the National Data Protection Commission (authorization number 526/2017). All patients provided written consent before entering the study. Cluster Analysis was carried so that groups with similar subjects, with respect to the study variables, could be defined.

Participants/materials, setting, methods: At oocyte pick-up, FF was collected, centrifuged and supernatants were stored at -80°C for further analysis. The quantification of C-reactive protein (CRP), Total Antioxidant Status (TAS), Superoxide Dismutase (SOD) and Glutathione were performed automatically using commercial kits; Advanced Oxidation Protein products (AOPPs) and Total Hydroperoxides (TH) were measured using in-house spectrophotometry methods. The different clusters were compared by an Analysis of Variance (ANOVA). Significance was assessed for $p<0.05$.

Main results and the role of chance: Four clusters were identified based on age and BMI (low BMI/older; low BMI/younger; high BMI/older; high BMI/younger). Significant differences (ANOVA $F=3.537$, $p=0.016$) in AOPPs levels between higher BMI/young and higher BMI/older; in TAS levels (ANOVA $F=4.023$, $p=0.009$) between low BMI/young and low BMI/older; and in CRP levels (ANOVA $F=10.089$, $p<0.001$) between low and high BMI patients. No significant differences were found for the other factors. Thus, age and BMI were the patients' intrinsic factors that mostly contribute for the clusters formation.

Limitations, reasons for caution: Since this study was performed using the pool of FF, our results indicate the global implications of FF composition for follicular development.

Wider implications of the findings: The use of FF as a source for fertility biomarkers is of great interest since it is non-invasive and easily available. In this study we suggest that age and BMI strongly affect FF composition and may contribute to an imbalance in the oxidative or inflammatory status, ultimately affecting reproductive potential.

Trial registration number: not applicable

P-699 free luteal phase support in mild ovarian stimulation with clomiphene citrate in IVF patients: a randomized prospective controlled trial

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Study question: Is there a need for a luteal phase support in mild ovarian stimulation with clomiphene citrate (CC) in IVF patients?
Summary answer: There is no need for luteal phase support with clomiphene citrate in IVF.
What is known already: The benefit of mild ovarian stimulation with clomiphene citrate with or without human menopausal gonadtropins (hMG) has been well established in previous studies (Benadiva 1995, Fauser 1999, Nargund 2007,Cohen 2012, Ferrareti 2015).

The endocrine profile of the luteal phase in ovarian stimulation with clomiphene citrate and hMG was analyzed and it has been demonstrated that LH concentrations were normal (Smitz 1988).

In a pilot study it has been demonstrated that there is no need for luteal phase support in IVF cycles after mild stimulation with clomifene citrate and hMG (Ferrareti 2016).
Study design, size, duration: It is a randomized prospective controlled trial including 61 patients.

Patients are divided in two groups: 31 patients are allocated in group 1 with luteal phase support. 30 patients are allocated in group 2 without luteal phase support.

Endocrine profile in the luteal phase was analyzed: progesterone, LH and estradiol were measured

The study was conducted from September 2017 to December 2018.

Chi^2 T-test , multivariate linear regression and curve Roc analysis were performed.
Participants/materials, setting, methods: 61 normo-responders patients <37 years old, BMI <29Kg/m^2, 1st or 2 nd attempt.

Patients were stimulated in antagonist protocol with 100mg of CC and 150IU of hMG. One blastocyst was replaced. Group 1 received 600mg of vaginal progesterone from the evening of egg retrieval, and in group 2 the luteal phase was not supported. Supernumerary blastocysts were vitrified, after thawing, one blastocyst was replaced in a natural cycle.

The study was performed in Tiziri IVF center.
Main results and the role of chance: The baseline characteristics were similar in the two groups. The total dose of gonadotrophins was 609.7±159.4 IU in group 1 and 655±234.6 IU in group 2 (P=.0.379)

The mean of metaphase-II oocytes was 3±1.8 in group1 and 3.2±2 in group 2 (P=0.739)

The 2PN rate was 90.3% in group 1 and 84.4% in group 2 (P=0.825)

The mean number of blastocyst obtained was 1.7±0.8 in group 1 and 1.8±0.9 in group 2 P=0.688)

The blastulation rate was 61.9% in group 1 and 62.2% in group 2. (P=0.880)

Clinical pregnancy rate was 35.4% in group 1 and 33.3% in group 2. (P= 0.850)

The Ongoing pregnancy rate per fresh transfer was .22.6% for group1 and 20% for group 2. (P=0.806)

In the first transfers of frozen blastocysts, 5 pregnancies were obtained out of 14 replacements (35.7%). 21 blastocysts are still vitrified.

In a multivariable analysis progesterone, LH and estradiol level at day 5 and day 7 ajusted to arm intervention with or without luteal phase support, only progesterone level at day 5 was a predictor of the ongoing pregnancy. Roc curve analysis identifed progesterone level ≥ 37.4 as the best threshold to ipredict of the ongoing pregnancy (AUC 0.795) OR 11.32 (95CI%, 1.33-96,13) P=0.026.
Limitations, reasons for caution: This protocol has been tested in patients with normal ovarian function i.e. with normal AMH , normal AFC and normal ovulatory cycle. It has not been tested in poor prognosis patients and high responders
Wider implications of the findings: The mild ovarian stimulation with clomiphene citrate without luteal phase support in IVF normal responders patients is a promising and realiable approach ,minimizing the burden and the cost .
Trial registration number: None

P-700 Gestational sympathetic stress decreases noradrenaline placental transporter an program the offspring fertility, a third generation study

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Study question: Could exposure to sympathetic stress during gestation changes placental NE transport to modify female offspring in their reproductive function when adults?
Summary answer: The exposure to gestational sympathetic stress program their postnatal development and decreased fertility capacity for three generations.
What is known already: . During pregnancy, the placenta plays an essential role in keeping low levels of monoamines in the uteroplacental circulation, the NE transporter (NET) generates an influx of NE both from the maternal and the fetal blood circulation to the placenta, which allows to maintain low concentrations of NE in the fetal circulation. If this system fails, NE clearance may be reduced, which could lead to increased fetal exposure to NE.
Study design, size, duration: The aim of this work was to determine the changes caused by gestational sympathetic stress (exposure to 4°C/3h/day during all gestation), in the functional capacity of the NE transport by placental NET and the reproductive capacity of the female offspring as adults. We studied if the next generation were also affected by these stress exposure of the first generation of pups. Morphological analysis of the ovaries was performed.
Participants/materials, setting, methods: Sprague Dawley rats were used. We determined: a.- at gestating mother level, the NE plasma concentration by high performance liquid chromatography (HPLC), placental NET functionality by the capacity to incorporate 3H-NA and protein by western blot analysis. To study the programming of reproductive capacity, we studied second and third generation female offspring (percentage of pregnancy, number and weight of female offspring) and preliminary the fourth-generation of rats.
Main results and the role of chance: : Gestational stress increases plasma NE concentrations in pregnant rats throughout gestation, which correlates with a decrease in the ability of the placenta to perform NE clearance from the fetus to the mother. This would indicate that the exposure of fetuses to high NE could program their postnatal development. In the first generation, we found a small number of secondary and antral follicles in the ovary of the female rats; delayed puberty and disrupted estrous cycling activity that conduct to a decreased fertility. A small number of pups per litter and a decrease of about 20% characterize the second generation in their fertility capacity. The third generation decreased even more their estrous cycling activity and have a 50% decrease in their fertility capacity.
Limitations, reasons for caution: The fourth generation is being studied but it seems to recover their estrous cycling activity and preliminary evidence shows that there is also a recovery of their reproductive capacity, thus it is important to study the possible epigenetic effect involved in these changes.
Wider implications of the findings: The results strongly suggest that changes in the environment in which the gestating mother maintain gestation, affect in a permanently form some reproductive capacities of the offspring
Trial registration number: This study was authorized the Bioethics committee of Universidad de Chile number CBE2012-18

P-701 Antimullerian Hormone predicts supernumerary blastocysts for cryopreservation in first IVF cycles

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Study question: Can antimullerian hormone (AMH) levels, independent of age, be used to predict the availability and number of supernumerary blastocysts for cryopreservation in the first IVF cycle?
Summary answer: AMH levels of <1 are associated with a lower likelihood of obtaining supernumerary blastocysts for cryopreservation across all age groups.
What is known already: Antimullerian hormone (AMH) has been established as a marker of ovarian reserve and as a predictor of response to controlled ovarian hyperstimulation. The ability of AMH to predict the availability and number of supernumerary blastocysts after a single in-vitro fertilization (IVF) cycle across age groups has not been well-delineated.

Study design, size, duration: This is a retrospective cohort study. A total of 2,561 patients who initiated an IVF cycle with the intention of fresh transfer and cryopreservation of excess blastocysts between January 2010 and December 2016 were analyzed.

Participants/materials, setting, methods: Patients who underwent their first IVF cycle with the intention of fresh transfer and cryopreservation of supernumerary blastocysts were included. Patients were divided into three age groups (Group A: age <35, Group B: age 35-39, and Group C: age ≥40), and then analysis was performed based on AMH levels of <1 or ≥1 ng/mL. Statistical analysis included student's t-test and chi-square test. $P<0.05$ was deemed statistically significant.

Main results and the role of chance: Patients were divided into three groups: Group A consisted of 860 patients (AMH<1 = 163, AMH≥1 = 697), Group B had 919 patients (AMH<1 = 424, AMH ≥1 = 495), and Group C included 782 patients (AMH<1 = 510, AMH ≥1 = 272). A total of 430 patients did not undergo embryo transfer due to cycle cancellation, no oocytes retrieved, no normal fertilization, no normal embryo development, or the decision to cryopreserve to avoid OHSS. The groups were similar for gravity, parity, BMI, and fertilization rates per oocyte retrieved. Peak estradiol levels, the number of oocytes harvested, and the number of mature oocytes decreased with older age and lower AMH. AMH levels of ≥1 were associated with an increased likelihood of having day-5 or day-6 blastocysts available for cryopreservation across all age groups (Group A: 34% vs. 64%, Group B: 19% vs. 52%, and Group C: 26.5% vs. 4%, for AMH<1 vs. ≥1, respectively; all $P<0.001$). For each age group, there was a significantly greater number of supernumerary blastocysts cryopreserved per cycle start in patients with an AMH of ≥1 compared to an AMH of <1.

Limitations, reasons for caution: This is a retrospective study. A multicenter study may help to prove the generalizability of our single-center data.

Wider implications of the findings: The use of AMH to predict the availability and number of supernumerary blastocysts for cryopreservation in the first IVF cycle regardless of age could help in counseling these patients regarding outcomes in the first IVF cycle.

Trial registration number: Not applicable

P-702 Use of oral micronized natural progesterone to prevent LH surge during controlled ovarian stimulation in oocyte donors with a previous donation cycle with antagonists.

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Study question: Can oral progesterone be as good as antagonist protocol when used to prevent LH surge in donors of our oocyte donation program?

Summary answer: The number of mature oocytes obtained, stimulation days, gonadotrophin consumption, as well as gestation, implantation and abortion rates were similar between both protocols**.**

What is known already: In order to reduce costs and avoid discomfort to patients, hyperstimulation protocols have recently been designed using progesterone primed ovarian stimulation (PPOS), instead of GnRH analogues, achieving good cycle control in patients undergoing In vitro fertilization. In these patients "freeze all" strategy is applied because progesterone during follicular phase produces non-receptive endometrium, with the need to transfer the embryos later in a substituted cycle with good results.

There is not much information about the use of PPOS in oocyte donors.

Study design, size, duration: Prospective case control study performed with 30 oocyte donors and 60 controlled ovarian stimulation (COS) cycles from July 2017 to September 2018. All donors had a previous cycle of COS with menotropins and GnRH antagonists. The donors served as their own control for the next cycle using PPOS. Oocytes retrieved could be used for one or more recipients. Results obtained in the recipients were also analyzed in order to verify embryo competence.

Participants/materials, setting, methods: 30 donors with an age between 18 and 30 years and normal ovarian reserve who had a previous COS cycle of oocyte donation with menotropins (Merapur 225 IU/day, Ferring) and GnRH antagonists (Cetrotide 0.25 mg/day, Merck) starting on day 7. PPOS started on day 3 with 225 IU of HMG (Merapur, Ferring) and with 100 mg of oral micronized natural progesterone (Utrogestan, CORNE) daily until the day of the triptorelin application for oocyte maturation.

Main results and the role of chance: There was no significant difference in the number of oocytes retrieved (20.3±12.1 for antagonists and 21.5±7.5 for PPOS, p=0.27) and MII oocytes obtained 17.3±5.2 and 18.1±6.1, respectively, p=0.48), with similar number of days required for stimulation (9.0±1.1 and 9.2±1.5, p=0.24) and HMG required. There was no premature elevation of LH in either of the two protocols and the mean value of LH on the day of the trigger was similar also (3.7±1.7 and 3.4±1.5, respectively). There was a profound suppression of LH values (<2mUI/ml) in three patients of the PPOS protocol (10%), while none was found in the antagonist protocol ($p\leq0.05$).

Pregnancy between both protocols was similar: (OR: 0.84, 95% CL 0.38-1.87, p=0.68): 64.5% for antagonists (33/51) and 68.3% for PPOS (41/60), as well as the implantation rate (OR: 1.07, 95% CL 0.62-1.83, p=0.80): 44.4% for antagonists and 42.7% for PPOS. No difference was found in abortion rates (OR: 1.29, 95% CL 0.37-4.47, p=0.68): 18.18% for antagonists and 14.6% for PPOS. The majority of pregnancies continue until the moment in course, without finding any congenital malformations in ultrasound or newborns. 28 of 30 (93.3%) donors expressed preference for the PPOS cycle due to a lower number of injections applied.

Limitations, reasons for caution: This is an initial study of our group and little has been written about this use of progesterone in PPOS in egg donation. It is a limited sample in size and more studies should be carried out to corroborate these findings.

Wider implications of the findings: PPOS is a very interesting option for COS not only in oocyte donation, but also in patients requiring IVF for any reason using the "freeze all" strategy and deferred embryo transfer.

Trial registration number: Not applicable

P-703 Letrozole versus Clomiphene Citrate for induction of ovulation in PCOS Infertile patients for IUI : a comparative study

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Study question: To compare the effects of letrozole and clomiphene citrate (CC) for ovulation induction in women with polycystic ovary syndrome (PCOS) IN IUI CYCLES ?

Summary answer: Letrozole has better ovulation induction than clomiphene citrate (CC) for in women with polycystic ovary syndrome (PCOS) IN IUI CYCLES .

What is known already: Clomiphene citrate (CC) is still the standard drug for inducing or augmenting ovulation. It is not, however, equally successful in all situations. Clomiphene resistance, which refers to the persistence of anovulation after standard CC therapy, occurs in 15–20% of the patients. In addition, CC may have a negative effect on the cervical mucus and endometrium. Treatment with CC is associated with a discrepancy between ovulation and conception rates, and the incidence of miscarriage has been claimed to be higher than in the general population.

Letrozole is an aromatase inhibitor used for ovulation induction and represent a real alternative to CC?

Study design, size, duration: This prospective clinical trial was conducted on 236 infertile Patients (2016-2018) who were diagnosed as having anovulation due to PCOS.

Participants/materials, setting, methods: This prospective clinical trial was conducted on 236 infertile Patients (2016-2018) who were diagnosed as having anovulation due to PCOS.

All patients were randomised by computer in two groups -Letrazole group (2.5-5mg) and CC group(50-100mg) given for 05 days.

All patients have documented at least one patent fallopian tube by either hysterosalpingogram or laparoscopy, and history of pelvic surgery with tubal blockage was excluded from the study. The male partners had a normal seminal analysis.

Main results and the role of chance: Ovulation occurred in 34% in the letrozole group and 18% in the CC group,

with a statistically significant difference between the two groups .

Limitations, reasons for caution: Our study found advantages to using letrozole rather than CC as a first-linetreatment for inducing ovulation in women with PCOS.

The strength of our study is that we have been able to preliminarily explore the effects of letrozole and CC for ovulation induction in PCOS in IUI.

Wider implications of the findings: Compared with CC, the use of letrozole led to a statistically significant increase in the number of developing and mature follicles .

The endometrium was, astoundingly, statistically significantly thicker in the letrozole group.

Trial registration number: not required

POSTER VIEWING
REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-704 The Impact of The Couple's Age on Fresh IVF/ICSI Outcomes

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Study question: Could the husband's age and the couple's age gap be correlated with IVF/ICSI clinical outcomes?

Summary answer: Both the husband's age and the couple's age gap might be important factors for predicting the outcomes of IVF/ICSI procedures.

What is known already: It is well-known that the female's age is closely related with the outcomes of assisted reproductive technology. While the influence of the couple's age gap or the husband's age remains unknown or less certain. The couple's age gap is an important impact factor in many social and health researches such as family violence, depression, cancer and so on. Age-related fertility decline and infertility treatment outcomes were discussed and reported mostly from single gender perspective.

Study design, size, duration: A retrospective cohort study of 4340 couples (non-donor gametes) in eighteen ART clinics representing the three regions of east, west, and east of China, including 3041 IVF and 969 ICSI fresh transfer cycles between January 2013 and December 2017.

Participants/materials, setting, methods: Participants enrolled criteria included heterosexual spouse, who undertook fresh IVF/ICSI embryos transfers with non-donor gametes. Clinical procedures were completed. The age gap between the couples were divided by five intervals (>=-5,-4~-1,0,1-4,>=5). Besides, the couple age combinations were analyzed by husband <40 or >=40 and wife<35 or >=35. Treatment outcomes were analyzed by 2PN embryos, clinical pregnancy rate, abnormal pregnancy rate (ectopic pregnancy and spontaneous abortion), live birth delivery rate.

Main results and the role of chance: Age gaps among 4340 couples range from: -16 to 26 in IVF cycles,-12 to 25 in ICSI cycles. The ratio of five gap intervals were 2.2%, 17.9%, 13.7%, 47.0%, 19.3% respectively. Wives who were >=5 years older than the husbands demonstrated significantly lower normal clinical pregnancy rate and live birth delivery rate.($P<0.05$), especially in ICSI cycles, even no clinical pregnancy happened. Wives who were 1-4 years younger than the husbands showed the highest normal clinical pregnancy rate(35.4%) and lowest abnormal pregnancy rate (3.1%), whereas no statistically difference were found in other age gaps groups.

The proportion of age combination groups (H<40+W<35; H<40+W>=35; H>=40+W<35; H>=40+W>=35) were 71.5%, 13.7%, 3.0% and 13.2%. The normal pregnancy and live birth demonstrated no doubtfully lower in husband ≧40+ wife≧35 group. Besides, data showed difference by wife's age, wife's age less than 35, no matter husband's age over 40 or not, normal pregnancy and live birth rates were nearly the same. While if the wife's age was over 35, different outcomes could be seen along with the husband's age, younger husband could lead to better clinical pregnancy rate and live birth rate. For IVF, husband age >=40, clinical pregnancy rate was 15.0%, while husband age<40, 24.6%.

Limitations, reasons for caution: The study was limited by its retrospective nature, population representative have drawback due to only fresh- IVF/ICSI were analyzed. Age gap and combination are social and biological interactive factors, and the findings need further study.

Wider implications of the findings: Big age gap couples had poorer IVF and ICSI outcomes, no matter these wives are young or not. These outcomes seems far more than biological reasons.

Age combination analysis, even though a woman missed the optimal reproductive age, with younger husband (less than 40), she might also have better chances.

Trial registration number: not applicable

P-705 Analysis of menstrual cycle data from a fertility awareness based mobile application

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Study question: Does analysis of menstrual cycles using a fertility app give us a new way to look at the menstrual cycle?

Summary answer: Cycle and phase lengths differed significantly from the conventional 14+14 day cycle model. Age, but not BMI, had a significant effect on cycle characteristics.

What is known already: Normal menstrual cycles are widely thought to be an average of 28 days with ovulation around day 14 and a 14 day luteal phase. The length is known to reduce with age but estimates vary as to how much. Most studies include a small number of women or restrict cycle length too much to be able to describe the general population with great accuracy.

Study design, size, duration: Prospective analysis of anonymised data collected from 155,345 users of the Natural Cycles app between August 2014 and December 2018. Users were aged 18 to 45, had BMI ranging from 15 to 40, had not recently used hormonal contraception and were not pregnant. Users had to have logged at least 30 entries in the app. Cycles between 10 and 90 days with a detected ovulation were included.

Participants/materials, setting, methods: Menstruation, basal body temperature (BBT) and luteinising hormone (LH) tests were recorded anonymously by users of the Natural Cycles app. Ovulation day was calculated by a proprietary algorithm using BBT measurements and optionally LH tests. Mean cycle and bleed lengths were calculated in cohorts of ovulatory cycles by cycle length, age and BMI. Mean follicular phase luteal phase lengths were calculated in a subsample of ovulatory cycles with LH tests meeting strict data quality criteria.

Main results and the role of chance: Data was analysed from 669,338 menstrual cycles with ovulation detected by the algorithm. The mean cycle length with 95% CI was 29.4 ± 5.7 days and the mean number of days menstruating was 4.2 ± 1.3 days per cycle. In 56,237 of the cycles ovulation was detected using BBT plus LH measurements. They had a mean follicular phase length of 16.2 ± 3.7 days and mean luteal phase length of 12.9 ± 2.0 days. Age had a statistically significant effect on mean follicular phase length, which decreased linearly by 2.4 ± 0.2 days from the age of 25 to 45, and mean cycle length, which decreased by 3.1 ± 0.1 days from the age of 25 to 45. BMI had no significant effect on the observed cycle characteristics.

The very large sample size reduces the likelihood that the results are observed by chance. All but one of the cohorts contained at least thousands of cycles. The possibility of finding false associations was eliminated by calculating confidence intervals instead of P values.

Limitations, reasons for caution: The sample was drawn from the Natural Cycles user base which is not fully representative of the general population. Medical diagnoses were self-reported. Ovulation detection by the algorithm is known to have a small bias relative to ultrasound detection. Only ovulatory cycles were included.
Wider implications of the findings: This is the largest study of menstrual cycle characteristics to date. Cycle, follicular phase and luteal phase lengths varied more than expected. The linear effect of age may have an impact on fertility research. Large scale real-world data collection from mobile applications is an invaluable tool in women's health research.
Trial registration number: not applicable

P-706 Economic analysis of preimplantation genetic testing for aneuploidy (PGT-A) by polar body biopsy in advanced maternal age

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Study question: What is the cost per live birth and the ICER for prevention of a miscarriage by PGT-A by polar body biopsy in advanced maternal age?
Summary answer: The cost per live birth is higher with PGT-A in all cost scenarios and the ICER for preventing one miscarriage is at least 44,047.95 $.
What is known already: A recently published randomized clinical trial (ESTEEM study) reported a lower number of embryo transfers with PGT-A necessary to achieve a similar cumulative live birth rate in patients of advanced maternal age as compared to IVF/ICSI without PGT-A and fewer miscarriages after PGT-A. Furthermore, the number of patients who had at least one embryo transfer was reduced, as was the total number of embryo transfers after oocyte selection by PGT-A.
Study design, size, duration: A decision tree model was constructed using the software TreeAge Pro Suite 2018 (TreeAge Software, Inc., Williamstown, MA) based on the interventions and outcomes of the ESTEEM trial (Verpoest et al. 2018). The ESTEEM trial is the largest RCT on the utility of PGT-A using polar body biopsy and array-based comprehensive genome hybridization (aCGH) and included women in advanced maternal age (AMA) (36 - 40 years).
Participants/materials, setting, methods: Costs and effects were analysed with this model for four different IVF/ICSI treatment cost scenarios: high cost, higher medium cost, lower medium cost and low cost. The cost of PGT-A was set as 5,000 USD ($) and was kept stable across different treatment cost scenarios. A base case, sensitivity and threshold analysis were used to examine the cost-effectiveness implications of PGT-A. Additionally, the cost necessary to prevent one miscarriage was estimated for all cost scenarios.
Main results and the role of chance: The cost per live birth was increased by approximately 11% in the high-cost scenario to approximately 61% in the low-cost scenario for patients undergoing IVF/ICSI with PGT-A. The cost per patient increased likewise, by approximately 14% to 62% in the high to low cost setting, respectively. Threshold analysis revealed that PGT-A would need to be associated with an increase in live birth rate of 28% to 32% or, alternatively, would need to be 2600 $ (high-cost scenario) to 4570 $ (low-cost scenario) less expensive. The ICER to prevent one miscarriage by PGT-A using the base case assumptions was calculated to be 44,047.95 $ (high-cost scenario) to 63,607.65 $ (low-cost scenario). A probabilistic sensitivity analysis showed a cost-effectiveness for PGT-A for 0.4% (high-cost scenario) to 0% (low-cost scenario) of calculated samples.
Limitations, reasons for caution: The model is based on polar body testing using array CGH; the findings may not be valid for other settings of PGT-A. Further simulations of cost and effects in different settings are still ongoing.
Wider implications of the findings: While avoiding unnecessary embryo transfers and miscarriages are important goals, patients and doctors need to be aware of the cost implications of applying PGT-A using polar bodies.
Trial registration number: not applicable

P-708 Physical activity and sedentary lifestyle associations with infertility treatment and pregnancy outcomes among couples entering assisted reproductive procedures

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Study question: Do physical activity (PA) and sedentary lifestyle influence infertility treatment and pregnancy outcomes among couples entering assisted reproductive procedures?
Summary answer: Couples undergoing ART procedures were less active and more sedentary than couples not requiring ART. However, PA and sedentary time did not affect treatment/pregnancy outcomes.
What is known already: Several factors affecting ART success, such as age and individual's genetic background are non-modifiable, while there is emerging evidence that different modifiable factors including nutrition, PA, sedentary lifestyle can influence ART outcomes. PA has been associated with reproductive outcomes in men and women, however it is not clear to what extent PA could help or harm infertility treatment outcomes. Further, previous studies have focused on assessing PA using self-reported questionnaires, which have several limitations and there is a need to assess PA and sedentarism with objective tools in order to clarify the impact of PA on infertility and its treatment.
Study design, size, duration: This study was carried out among 128 individuals, 64 women and 64 men, entering for the first-time infertility clinic for ART procedures. The data were collected from the Centre for Reproduction at Uppsala University Hospital, Sweden between February 2011 and January 2014.
Participants/materials, setting, methods: Baseline PA and sedentary time (before entering any treatment) were assessed using an objective method - accelerometry for seven consecutive days and additionally by self- reported questionnaires. For every couple the infertility treatment and pregnancy outcomes were recorded. The sedentary time and PA were compared with the general Swedish population group (n=1172 individuals) data. Pearson's correlation and logistic regression models were used for data analyses.
Main results and the role of chance: In general, the couples, entering infertility treatment were more active (moderate or high intensity PA in women was 47,0 ±16,8 min/day and men 48,0 ±16,8 min/day) compared to the general Swedish population (women 36 ±3 min/day and men 41,0 ±4 min/day respectively). However, they had recorded more sedentary time (women 683,5 ±80,8 min/day and men 709,1 ± 65,0 min/day) than the general Swedish

population (women 486 ±11 min/day and men 497 ±13 min/day respectively). Among our couples undergoing infertility treatment, the mean sedentary time of women was lower than that of men, while moderate or high-intensity PA was higher in men.

When comparing the couples, these couples who underwent ART procedures (IVF, ICSI, or IUI) were physically less active (less high-intensity PA) and were more sedentary (watched more TV) than these couples who did not need ART (after revisions became spontaneously pregnant or mild ovarian stimulation was performed) (p<0,05). Nevertheless, in the present study we did not detect any significant impact of couple's PA and sedentary time on infertility treatment and pregnancy outcomes.

Limitations, reasons for caution: The findings of the current study on the limited sample size should be confirmed in a bigger study.

Wider implications of the findings: Couples undergoing ART are likely to be less physically active and more sedentary. Nevertheless, our study results do not show any effect of PA and sedentary time on infertility treatment and pregnancy outcomes indicating that there is no need to change couple's activity habits before entering ART procedures.

Trial registration number: Not applicable

P-709 Wearable sensors reveal menses-driven changes in physiology and enable prediction of the fertile window: a prospective, observational study

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Study question: Can wearable technology predict the fertile window from changes in wrist-skin temperature, heart rate, heart rate variability, respiratory rate, and perfusion across the menstrual cycle?

Summary answer: Wearable technology revealed significant changes in physiological parameters across the menstrual cycle, enabling the development of a high accuracy algorithm for detecting the fertile window.

What is known already: Prior research examining physiological changes across the menstrual cycle has often considered biological responses to shifting hormones in isolation. Clinical studies, for example, have shown women's nightly basal body temperature increases 0.28-0.56°C following post-ovulation progesterone production. Women's resting pulse rate, respiratory rate, and heart rate variability (HRV) are similarly elevated in the luteal phase as compared to earlier in the menstrual cycle. In contrast, skin perfusion decreases significantly during the latter half of the menstrual cycle. Although initially designed for recreational purposes, wearable technology could enable more ambulatory studies of multiple, concurrent physiological changes across the menstrual cycle.

Study design, size, duration: We conducted a prospective longitudinal study, recruiting 237 Swiss women who were trying to conceive between August 2016 and June 2018. As determined a priori, we excluded individuals who: were currently undergoing hormone therapy; had health-related issues or were taking medications affecting menstruation; traveled frequently across time zones; had a sleeping disorder; and/or had been trying unsuccessfully to conceive for over a year. The final sample included 708 menstrual cycles spread across 193 participants.

Participants/materials, setting, methods: Participants wore a fertility tracking device on their wrist nightly for up to one year or until they became pregnant. They also completed an electronic daily diary about their activities in the past 24 hours and took a urinary luteinizing hormone (LH) test at several points throughout each cycle. We assessed phase-based changes in physiological parameters using mixed effects models and trained a machine learning algorithm to recognize the fertile window in real-time.

Main results and the role of chance: After removing women who failed to follow instructions and/or did not have any documented LH surges during the study, the final sample included 708 cycles spread across 193 participants (M=33.02 years old, SD=3.68). On average, participants recorded 3.57 cycles (SD=2.41), with a mean cycle length of 28.21 days (SD=2.87). Analyses using multi-level modeling demonstrated that wearable technology can detect significant, concurrent phase-based shifts in wrist-skin temperature, heart rate, and respiratory rate across the menstrual cycle (all *ps*<.001). Heart rate variability and skin perfusion similarly varied across the menstrual cycle (all *ps*<.05), although these effects only trended towards significance following a Bonferroni correction to maintain a family-wise alpha-level of 0.05. Suggesting a methodological improvement over more traditional methods of Natural Family Planning, our findings were robust to daily, individual, and cycle-level covariates. Furthermore, our machine learning algorithm detected the fertile window with an accuracy of 89% across all cycles, outperforming similar smartphone apps as reported in prior literature.

Limitations, reasons for caution: The models and significant differences detected between physiological parameters in the luteal phase, fertile window, and menstrual phase were based on data from Swiss women aged 18 to 40 years old. Future studies should recruit a larger cross-national sample to ensure culture- or location-specific variables did not bias our findings.

Wider implications of the findings: Our findings align with conclusions from prior research on physiological changes across the menstrual cycle and highlight the impact of machine learning's integration into healthcare. By monitoring numerous physiological parameters simultaneously, wearable technology uniquely improves upon retrospective methods for fertility awareness and enables the first real-time predictive model of ovulation.

Trial registration number: ClinicalTrials.gov NCT03161873

P-710 Distance evaluation of oocyte donation experience by French donors

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Study question: Evaluate the percentage of oocyte donors who regretted their donation.

Summary answer: Of the oocyte donors interviewed, none regretted their donation.

What is known already: Studies on this subject have already been conducted abroad, but in many cases oocyte donors are paid, which is not the case in France. Limited researches evaluated voluntary and non-compensated oocyte donation. These studies showed that all the volunteer donors made this donation in solidarity, altruistically, and that the majority of the donors had a parent on an infertility course. The majority was against the payment of the gift. Further, the studies showed that oocyte donors were generally satisfied with their donation.

Study design, size, duration: An observational, cross-sectional and monocentric study was conducted. All the women who gave their oocytes from 01 January 2010 to 31 December 2015 in our institution were contacted by telephone from December 2018 to January 2019.

Participants/materials, setting, methods: 105 women were contacted by telephone during the study. 45 women participated in the study by answering a questionnaire by telephone. Of those who did not respond, 14 had a number that was no longer assigned, 45 did not respond to phone calls despite several trials and one woman declined to participate in the study.

Main results and the role of chance: The response rate was 42.8%. None of the women interviewed regretted having donated oocytes. The average age of the donors at the time of the phone call was 37.7 years, with a minimum age of 30 and a maximum of 44. 98% of the surveyed women gave their oocytes once. 89% of donors would agree to do an other donation. 82% of donors regularly thought back to their gift and kept a positive experience. 64% of women were supported by their relatives and 73% by their partner. Further, 90% of the interviewed women did not have any medical problems (gynecological or not) after donation. Finally, the majority of questioned donors were to keep the oocyte donation free and anonymous.

Limitations, reasons for caution: Methodological bias related to the low response rate. This low rate was probably related to the fact that the donors were contacted remotely from their donation. A significant number of women have changed their coordinates since their egg donation. Moreover, it was a monocentric study.

Wider implications of the findings: In France, current donation principles seem to be suitable for oocyte donors. The satisfaction engendered by the altruistic act seems to be sufficient.
Trial registration number: Not applicable.

P-711 Mediterranean diet and outcomes of assisted reproduction: an Italian cohort study

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Study question: Is there any relation between outcomes of In Vitro Fertilization (IVF) cycles and Mediterranean Diet in women of infertile couples?
Summary answer: No clear association was observed between adherence to a Mediterranean Diet and outcomes of IVF cycles.
What is known already: Infertility is a medical condition that affects up to 25 million people in Europe and it has an idiopathic origin in 10-20% of subfertile couples.

Besides non modifiable conditions (parental age), detrimental lifestyle habits have been suggested as potential risk factors for reduced fertility. In particular, over the last decade literature on the relation between diet and fertility has expanded. Focusing on IVF outcomes, folic acid and isoflavones, for example, are suggested to increase live birth rates, as well as healthy diets. The role of specific dietary pattern and food groups is conversely under debate and evidence are still accumulating.
Study design, size, duration: From September 2014 to December 2016 infertile couples, presenting for evaluation to the Infertility Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, and eligible for IVF, were invited to participate to an ongoing prospective cohort study on the role of lifestyle habits and diet on ART outcomes. The present study reported on evidence obtained from female partners. We enrolled 474 women.
Participants/materials, setting, methods: Women of couples who agreed to participate to the study were interviewed on the day of oocyte retrieval to obtain information on personal and health history, lifestyle habits and diet. Information on diet were obtained using a validated food frequency questionnaire (FFQ) while adherence to Mediterranean Diet was evaluated using an a priori score, Mediterranean Diet Score (MDS). Relative risks (RR) and 95% confidence intervals (CI) for clinical pregnancy were the main outcome measures.
Main results and the role of chance: We distinguished 3 categories of adherence to a Mediterranean Diet: low adherence group (MDS 0-3); intermediate adherence group (MDS 4-5) and high adherence group (MDS 6-9). No significant association was observed between MDS and age, BMI, education, occupational physical activity, cause of infertility and previous ART cycles. MDS was related to daily calories intake and leisure physical activity (PA). Age was the main risk factor for IVF failure: the outcomes considered were embryo transfer, clinical pregnancy and live birth. After adjusting for age, presence of previous IVF cycles was associated to higher risk of missed embryos transfer (RR 1.79, 95% CI 1.05-3.03) and exercising for $\geq$ 5 hour weekly to higher risk of not achieving clinical pregnancy (RR 1.25, 95% CI 1.05-1.49) as compared to $<$ 2 hours per week. Adjusted RRs for IVF failure at each step (embryo transfer, clinical pregnancy, live birth) were calculated including age, leisure PA, daily calories intake, and previous ART cycles. Analyses were also performed in strata of age, previous IVF cycles and cause of infertility: findings consistently showed that MDS was not significantly associated with ART outcomes, although a slightly lower risk of adverse outcome was observed in the MDS 4-5 group.
Limitations, reasons for caution: Potential limitations should be considered: findings should be referred only to patients of infertile couples; information regarding dietary habits was self-reported.
Wider implications of the findings: No clear association was observed between adherence to a Mediterranean Diet and oocyte quality or successful IVF outcomes. However, at the light of previous research and of general benefits due to a healthy diet (including those on obstetrics outcomes), women candidates to ART should be counseled about their dietary habits.
Trial registration number: Not applicable

P-712 Bisphenol A affects the number of oocyte retrieval and embryo implantation in women undergoing in vitro fertilization

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Study question: Are urinary BPA concentrations associated with outcomes among women undergoing *in vitro* fertilization (IVF) treatment?
Summary answer: Bisphenol A affects the number of oocyte retrieval and embryo implantation in women from a fertility center.
What is known already: Bisphenol A (BPA) is ubiquitously present in the environment and the reproductive toxicity of BPA has been well characterized in animal models.
Study design, size, duration: In this study the associations between urinary BPA concentrations and the outcomes of *in vitro* fertilization and embryo transfer (IVF-ET) both in fresh(n=351) and frozen cycles(n=125) were analyzed in the same cohort.
Participants/materials, setting, methods: Between September 2013 and October 2016, 351 women undergoing IVF treatment at the Center for Reproductive Medicine in Women's Hospital School of Medicine Zhejiang University in China were recruited into this study. One-spot urine samples were collected on the day of oocyte retrieval to detect BPA. Multivariable generalized linear mixed model was used to evaluate the association between urinary BPA concentrations and IVF outcomes.
Main results and the role of chance: After adjustment for age, body mass index (BMI), baseline follicle stimulating hormone (FSH), baseline estradiol (E_2) and antral follicle count (AFC) levels, a significant decrease of the number of oocytes retrieval and the rates of clinical pregnancy and implantation were observed in patients with high urinary BPA concentrations. Urinary BPA concentrations were not associated with endometrial wall thickness, E_2 peak levels, cleavage rate, and the proportion of high quality embryos or fertilization rates. Furthermore, there were no associations between urinary BPA concentrations and biochemical pregnancy, abortion or live birth rates per transferred cycle.
Limitations, reasons for caution: Limitations to this study include difficulties in extrapolating the findings to the general population and non-differential misclassification of exposure which remains a concern given the single-spot urine collection and the short half-life of BPA, although such non-differential misclassification of exposures will likely bias the results towards the null.
Wider implications of the findings: Data on the effects of human female exposure to BPA have been inconsistent and further research on the plausible mechanisms of BPA's reproductive endocrine-disrupting effects is warranted.
Trial registration number: not applicable

P-713 Female intimate hygiene practice correlates with the vaginal microbiota; a comparative study between in vitro fertilization (IVF) patients with normal and abnormal vaginal microbiota

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Study question: What is the intimate hygiene practice of women undergoing IVF treatment and its possible correlations with abnormal vaginal microbiota (AVM)?
Summary answer: 36 % of IVF patients had AVM. AVM was correlated with usage of low-pH intimate soap. Surprisingly, a total of 19 % practiced vaginal douching.

What is known already: AVM is a dysbiosis dominated by anaerobic bacteria and has been linked to irritative vaginal symptoms as well as adverse reproductive outcomes, such as increased risk of early miscarriage, preterm birth, and reduced chances of successful IVF treatment. A definitive etiology of AVM is still debated; however, intimate hygiene practice has been suggested to be a possible etiological factor, as the practice of vaginal douching is linked to bacterial vaginosis. This emphasizes the importance of studies in hygiene practice. This is the first study to investigate intimate hygiene practice in IVF patients and its correlation to AVM.

Study design, size, duration: The present study was conducted as an observational cross-sectional study, including a total of 206 IVF patients from four Danish fertility centers. Patients were included during December 2017 to October 2018.

Participants/materials, setting, methods: Patients were eligible for inclusion if they met a list of criteria and received their first, second or third IVF cycle. Besides standard gynecological work-up, patients reported intimate hygiene practice (soap, douching, probiotics) in a questionnaire. Vaginal swabs were obtained for quantitative PCR, targeting DNA of dysbiotic bacteria. Significant differences between women with normal microbiota versus women with AVM were tested using STATA. All p-values were two-sided with a significance level of 0.05.

Main results and the role of chance: From the total population of 206 women, a total of 36 % (75/206) were diagnosed with AVM. Most women used intimate soap (37 %; 76/206), followed by use of water, exclusively (33 %; 67/206); the smallest group used only regular soap for intimate hygiene (27 %; 56/206). Only a few women reported the simultaneous use of regular and intimate soaps (3 %; 6/206). Interestingly, a total of 19 % (33/206) reported the practice of vaginal douching, but surprisingly, this practice was not associated with AVM. Women with AVM tended to use intimate soap more often than women with normal microbiota, 45 % (34/75) versus 36 % (47/131); (p 0.18). No associations were seen between AVM and the use of water or regular soap, respectively. AVM positive patients were significantly older, difference 1.37 years [95 % CI 0.03;2.71] (p 0.04), and they had a significantly higher prevalence of sexual intercourse during 24 hours prior to vaginal sampling, 16 % (12/75) versus 7 % (9/131); (p 0.04). Finally, a fishy vaginal odor was significantly more often reported in AVM positive patients, 13 % (10/75) in the AVM group as compared to 2 % (3/131) in the normal group; ($p < 0.01$).

Limitations, reasons for caution: To the best of our knowledge, this is the first study to investigate intimate hygiene practice in an IVF population. While these results describe the Scandinavian IVF population specifically, more studies are needed to investigate intimate hygiene practice in other populations. Furthermore, larger populations are necessary to draw firm conclusions.

Wider implications of the findings: Future exploration in the cause and effect of the association between intimate hygiene habits and AVM is needed, requiring intervention-based prospective studies. The novel finding of a high prevalence of douching in a Scandinavian population demands further investigation, as this practice is generally discouraged by health authorities worldwide.

Trial registration number: NCT03420859

P-714 Application of a clinical pregnancy prediction model in analysis of Day5 single blastocyst transfer

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Study question: Construction of a prediction model to enable the selection of patients for single blastocyst transfer (Day 5).

Summary answer: The established predictive model can effectively predict the outcome of single blastocyst transfer (Day 5).

What is known already: With the improvement of sequential embryo culture media and incubation system, the blastocyst culture and transfer technique has been progressively used in ART. It has been approved that the clinical pregnancy rate of blastocyst transfer is significantly higher than that of embryo transfer from cleavage stage. Though the single blastocyst transfer significantly reduces multiple pregnancy rate, not all patients are suitable for single blastocyst transfer. Presently, single blastocyst transfer is applied to the selected patients, who have a higher risk of multiple pregnancy. However, how to judge people with better prognosis outcome remains to be solved in single blastocyst transfer.

Study design, size, duration: A case-control study was used in collecting patient data .The patients with ART treatment and single fresh blastocyst (Day 5) transfer were enrolled in the reproductive medicine center from October 2015 to March 2017.A total of 387 cases of Day 5 fresh single blastocyst were collected in this study, including which from 207 pregnant patients, aged 28.78±3.95 years. There were 180 patients without pregnancy, aged 29.23±4.44 years old. The pregnancy rate was 53.49%.

Participants/materials, setting, methods: The inclusion criteria are as follow: (1) age≤37 years old (2) single embryo transfer on day 5 from fresh cycle. A case-control study was used in collecting patient data: demographic data, history of diseases, pre-treatment examinations, and embryo laboratory data etc. The clinical pregnancy was regarded as the dependent variable, and other variables were regarded as the independent variables, which were done multi-factor logistic regression analysis.

Main results and the role of chance: 1.Univariate analysis: the number of transplantable embryos, the number of high quality embryos, the number of transplantable blastocysts, the number of transplantable blastocysts of high quality embryo, the level of inner cell mass in blastocysts and the level of transplantable in blastocysts were statistically significant ($P<0.05$). 2. Multivariate logistic regression analysis: The level of inner cell mass and the number of transplantable embryo were the factors affecting the clinical pregnancy outcome. OR (level of inner cell mass) = 4.86 (95% CI: 2.56-9.91), OR (transplantable embryo) = 1.09 (95% CI: 1.01-1.18). The predictive model $P=1/1+e^{-(1.689X1+0.087X2-0.405)}$ was established (X1 represented the level of inner cells and X2 showed the number of transplanted embryos). Patients were classified into two groups: one is predicted to be pregnant (the probability P value ≥0.509) and the other is predicted to be non-pregnant ($P<0.509$) (cut off value = 0.509). The predicted AUC of the model was 0.723 (95% CI: 0.673-0.773). 3. The sensitivity of the model (cut off value = 0.509) is 66% (137/207), The specificity of the model is 71%(127/180), the positive predictive value is 72.11%(137/190), the negative predictive value was 64.47% (127/197).

Limitations, reasons for caution: Although the model can predict the clinical pregnancy rate of D5 fresh single blastocyst transfer, the predictive power is 0.723. because of small sample size. It is expected that the predictive power will be increased as the growing of sample size in following study.

Wider implications of the findings: This study analyzed the factors affecting the clinical pregnancy rate of single fresh blastocyst transfer on D5, and established a predictive model of clinical pregnancy rate. The model helps clinicians to select patients who are suitable for single fresh blastocyst transfer on D5.

Trial registration number: None

P-715 Postponing pregnancy seeking and anticipating infertility treatments: an unwise combination.

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Study question: Is the current recommendation to anticipate the infertility work-up after only 6 months of pregnancy seeking in older women justified?

Summary answer: Anticipating clinical management in older women seeking pregnancy is clinically and economically questionable and can unwisely boost over-diagnosis and over-treatment.

What is known already: Postponing motherhood exposes women to the risk of childlessness. Natural fecundity decreases with age and, unfortunately, Assisted Reproductive Techniques (ARTs) do not overcome the detrimental effects of aging. For this reason, in women older than 35 years, it is recommended to initiate the infertility work-up after only 6 months of attempts rather than after the usual one year period. In fact, it is paradoxically claimed to anticipate clinical management in a population of reduced fecundity, thus in a population who would conversely need a longer rather than a shorter duration of pregnancy seeking to reach a reliable diagnosis of infertility.
Study design, size, duration: The aim of this theoretical model was to explore the effects of starting the infertility work-up after 6 months of pregnancy seeking rather than the usual 12 months' period in women older than 35 years. We aimed at determining whether the detrimental impact of a 6 months' delay on the chances of success of ARTs could justify this position. The model was based on cost-beneficial considerations. The perspective was the one of the health provider.
Participants/materials, setting, methods: The assumptions of the model were: 1) infertile women >35 years are straightly treated with IVF for up to three cycles; 2) IVF success rate at first cycle linearly declines with age from 30% at 35 years to 0% at 45 years, 3) the drop-out rate after the 1st and 2nd cycle is 18% and 25%, respectively, 4) the relative reduction of the success rate at 2nd and 3rd cycle is 16% and 26%, respectively.
Main results and the role of chance: The increase in the success of the IVF program associated to an anticipation of 6 months changed with age, increasing from 2.0% at age 35 to 3.0% at age 43. The corresponding Number of women Needed to be Treated (NNTs) decreased from 49 at age 35 to 34 at age 43. Sensitivity analyses modifying the gradient of pregnancy loss per year (decline from 40% to 0% from age 35 to age 45, corresponding to an annual loss of 4%) did not markedly change the results (the increase in success remains <4% at all ages). In order to shed light on the incremental cost-beneficial ratio of the anticipation of treatment we also calculated the incremental success rate per cycle. In the basal situation (live birth rate at 35 years of 30%), it decreased from 1.4% to 1.3% from age 35 to age 43. When setting the live birth rate at 35 years at 40%, the incremental success rate per cycle decreased from 1.9% to 1.8% from age 35 to age 43. In all the scenarios, the estimated impact is well-below the thresholds of 4-10% of success used to define IVF as cost-beneficial (ESHRE Capri Workshop Group, 2015).
Limitations, reasons for caution: The model is theoretical and based on the still debated ideas that IVF cannot overcome the detrimental effects of age and that the available tests for the diagnosis of infertility are inaccurate (duration of infertility still plays a crucial role for a definite diagnosis).
Wider implications of the findings: The position of stakeholders and major International Societies should be less trenchant. More consideration should be given to the consistent risks of overdiagnosis and overtreatment associated to an anticipation of the infertility work-up.
Trial registration number: NA

P-716 Understanding gender differences in factors influencing family-building decisions

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Study question: Are the factors that drive family-building decision-making the same for men and women?
Summary answer: Financial stability is a key driver for family-building decisions across genders; however women were more likely than men to cite age, partner and societal expectations.
What is known already: With recent demographic changes towards delayed parenthood comes involuntary childlessness and smaller family sizes than desired. Global health policies have highlighted the importance of improving knowledge and awareness of fertility and reproductive health; and as the average age of first-time parents continues to rise, attention is being called to the need to improve fertility awareness. Understanding factors influencing family-building decision-making is therefore important. Although studies have shown that men desire parenthood as much as women do, fertility awareness studies have traditionally focussed on women and few exist on men's attitudes towards family-building. Additionally, gender differences family-building decision-making are not explored.
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. Interviews were conducted over a 5 month period and were completed in February 2017. 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: Across both genders, we found recurring themes towards education, career aspirations, affordability, money and financial stability as key factors that drive family-building decision-making. However, men viewed these from the perspective of being able to provide for the family generally and "put a roof over their family's head", while women discussed these themes in more practical terms such as maternity leave, childcare, finding a home with the right proximity to schools, as well as the impact of career breaks on job security.

Own family experiences, freedom and travel, religion, the environment and the current global situation were themes that also emerged across genders.

The women in our study were more likely than men to cite age and biological clock; finding the right partner, relationships, self-doubt, having support systems, health, cultural and societal expectations as factors that drive their family building decisions.

We found that men desired family-building and wanted to be engaged and involved in reproductive decision-making, but tended to defer to the woman's primacy as fertility was seen as the woman's territory. Compared to women, men had lower awareness of age related fertility decline and other factors affecting male fertility.
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. Additionally, the 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 gender differences that exist 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-717 women's anti-müllerian hormone levels predict female general health status: a cross-sectional study

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Study question: To determine whether fertility-related clinical and hormonal data might predict women's general health in terms of significant comorbidities.
Summary answer: Lower Anti-Müllerian Hormone (AMH) levels and parity are associated with decreased women's general health after adjusting for age.
What is known already: Infertility is increasingly being recognized as a predictor and a potential marker of general health, representing a unique opportunity for surveillance and potential risk reduction. In males, specific reproductive parameters such as sperm concentration, testosterone and follicle-stimulating hormone (FSH) values have indeed been found to be inversely related with

the rate of comorbidities expressed by the Charlson comorbidity index (CCI). Conversely, no study has so far explored whether female specific reproductive parameters might be associated with general health.

Study design, size, duration: We performed a cross-sectional study of 8613 consecutive women presenting at our academic center for couple's infertility between August 2005 and April 2018.

Participants/materials, setting, methods: All-comers infertile women presenting at our academic center. Comorbidities were assessed with a self-reported medical history collected by physician, classified by the International Classification of Diseases modified 9th version (ICD-9-CM) and scored with the Charlson Comorbidity Index (CCI). Infertile women's health and fertility status were assessed by clinical and hormonal data. Univariable/multivariable linear regression and logistic analyses were used to test the association between predictors and CCI.

Main results and the role of chance: We assigned CCI=0 to 8181 (95.0%) patients, CCI=1 to 277 (3.2%) patients and CCI≥2 to 155 (1.7%) patients. Of variables studied, only AMH and parity were found to be statistically significantly different among CCI groups, both showing a decrease as general health status decreased. No other parameter including age, BMI, gravidity and hormonal levels differed among CCI groups. At multivariable linear regression models age (-0.034, 0.004; *beta, p value*), parity (-0.025, p=0.027; *beta, p value*) and AMH (-0.038, p=0.001; *beta, p value*) significantly predicted continuously coded CCI. At logistic regression models, only parity (most informative cutoff value <1, p=0.038) and AMH (most informative cutoff value < 2.25 ng/ml, p=0.006) achieved independent predictor status of CCI > 0. Subgroup analyses showed that the association between AMH remained significant after exclusion of patients with cancer or previous cancer treatments.

Limitations, reasons for caution: The design of our study does not allow causal inferences. Also, we didn't include fertile women, which could be useful to assess the generalizability of our findings. Finally, comorbidities were assessed through self-reported medical history, possibly causing information bias.

Wider implications of the findings: Our results suggest the novel finding that AMH is a marker of general female health expressed by comorbidity burden – as observed for sperm concentration, FSH and testosterone levels in males. The directionality of the causal pathways between health and infertility thus deserves further study, both in females and males.

Trial registration number: n/a

P-718 International Natural Procreative Technology Evaluation and Surveillance of Treatment for Subfertility (iNEST): enrollment and methods

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Study question: What are the participation and characteristics of participants in a multi-national study of fertility treatment, including demographic and reproductive characteristics and pregnancy/birth outcomes?

Summary answer: Among 834 consenting couples, nearly 60% completed follow-up questionnaires. Unadjusted, 44% percent had a pregnancy within 3 years, and 32% had a live birth.

What is known already: While much clinical research of infertility focuses on assisted reproductive technologies (ART) such as in vitro fertilization (IVF), some couples do not wish to use ART, or cannot afford it. Natural procreative technology (NPT) is an approach to treating infertility that does not involve ART, and which incorporates medical and surgical treatments with women's fertility tracking to improve reproductive function. The goal of NPT is to optimize reproductive function for conception in vivo. However, to date, evaluation of NPT outcomes in medical practice is limited to a few studies based on single medical practices.

Study design, size, duration: iNEST was a prospective longitudinal cohort study of couples presenting for possible treatment with NPT at 10 participating practices. Characteristics of participating couples were assessed at entrance by questionnaire. Treatments (including NPT or ART), pregnancies, and birth outcomes were followed for up to three years based on data from both practices and participants.

Participants/materials, setting, methods: Couples presenting for possible treatment with NPT at 10 different study sites: USA (Utah, Massachusetts, New Jersey, Louisiana, Missouri, North Carolina, Virginia); Canada (Toronto region); the United Kingdom (Leamington Spa); and Poland (Lublin) were enrolled in the study. Practices periodically entered data about clinic visits, diagnoses, surgeries, pregnancies, and pregnancy outcomes. Yearly questionnaires to the couple (separate questionnaires to women and men) assessed treatments received (NPT, ART, and other), pregnancies, and pregnancy outcomes.

Main results and the role of chance: A total of 834 couples consented to participate in the iNEST study. Of these, 58% of women and 48% of men completed an entrance questionnaire, and nearly 60% of couples (the woman or the man or both) also completed a follow-up questionnaire. Most (60%) of participants had clinic visit data and 52% had clinic diagnosis data. Female participants were on average 34 years of age (range 19-47); 94% of reporting women had 12 or more years of education (43% missing). For time trying to conceive at entrance, 29% reported 1-2 years, 24% reported 3 or more years (33% missing). About half (54%) reported a prior live birth (18% missing). About a third (36%) reported prior ovulation drug use (33% missing), and 6% prior IVF/ICSI (45% missing), and 15% prior artificial insemination (34% missing). Among the 435 with diagnostic information, the most common diagnoses included 29% endometriosis, 49% limited cervical mucus, 24% PCOS, 24% male factor. Forty-four percent achieved a pregnancy during the study (unadjusted proportion), and 32% resulted in a live birth (unadjusted proportion). In a subset of the study, there was a very high correlation for couples' reporting of pregnancy outcomes (live birth, miscarriage) as compared to medical records.

Limitations, reasons for caution: Many of the data are self-reported and some types of data have missing responses for up to 50% of the sample.

Wider implications of the findings: The iNEST study will allow for a broader assessment of NPT treatment received and fertility outcomes based on participant characteristics, clinical diagnoses, and treatments received, in a multi-national cohort.

Trial registration number: NCT01363596 (clinicaltrials.gov)

P-719 Knowledge in Reproductive Health and degree of acceptance of Assisted Reproduction Techniques in students of the University of Castilla - La Mancha

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Study question: What is the degree of knowledge of the students of biosanitary degrees about the concept of reproductive health and the Assisted Reproduction Techniques?

Summary answer: Despite having studied related subjects, students of biosanitary degrees are unaware of fundamental concepts related to Reproductive Health and Reproductive Medicine.

What is known already: According to the Spanish Fertility Society, one out of every six couples belonging to Western countries have fertility problems, presenting this fact a clear growing evolution. One of the causes is the age of the patients and most of the population do not have knowledge of the most basic aspects of Reproductive Health.

Study design, size, duration: Cross-sectional descriptive study that determines the knowledge and attitudes related to Reproductive Health in the university population through the realization of a questionnaire of 53 questions. 915 surveys were conducted between May and July, 2017.

Participants/materials, setting, methods: The respondents had to meet the requirements of being students of the Health Degrees of the Campus of Albacete: Nursing, Pharmacy and Medicine, as well as being willing to participate voluntarily. Those who could not do the survey in person were discarded.

The questionnaire was distributed individually in classrooms of the university itself. The time needed to answer the questions ranged between 10 and 12 minutes.

Main results and the role of chance: Regarding the concept of Reproductive Health, it is noteworthy that 42.3% of respondents considered that female reproductive problems begin after 40 years. Another alarming fact is that out of the 65.4% who had not had medical check-ups, 41.46% considered that their reproductive health was good. Regarding lifestyles, 98.3% said that they condition the time of having the first child and 65% agreed that the job situation should be a priority. Despite this, 82.1% would like to have children at the average age of 30.18 years, while Spanish statistics indicate that the average age of first-time motherhood is 31.47 years. Regarding the Assisted Reproduction Techniques, 92.1% of respondents would use them in the future in case of presenting reproductive problems, 31.4% would rule them out by the alternative of adopting. The difference is more significant when using donation of gametes, since of the 76.4% that would resort to this practice, 31.4% would prefer adoption. It should be noted that 85.6% of respondents said that there is not enough information on the process of donation of gametes.

Limitations, reasons for caution: Possible occurrence of biases that indicate the refusal not to answer certain questions due to lack of knowledge, or of responses that the student can understand as "socially acceptable", without taking into account its true point of view.

Wider implications of the findings: The lack of information present in the respondents could be extrapolated to the clinics of Reproductive Medicine, where patients who decide to postpone the moment of being mothers ends with a treatment with donated gametes, which could be the most recommended option and never the most shuffled.

Trial registration number: ..

P-720 Different sexual-romantic orientations have the same reasons for desire to have children but different prevalence for the desire itself.

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Study question: The main question of this study was to assess if statements about the desire for children were rated the same by different sexual-romantic orientations.

Summary answer: Overall, people with desire for children did not rate statements significantly different. The biggest impact on desire for children were emotional motives.

What is known already: Previous studies mostly focused on hetero- and homosexuals and their reasons for a desire to have children. They demonstrated a generally lower scoring of statements within homosexual individuals. Both sexual orientations had the highest score for emotional aspects of having a child and not financial or personal constraints or social recognition. So far, there have been no studies about other sexual or sexual-romantic orientation.

Study design, size, duration: The study was a monocenter cross-sectional non-interventional cohort survey. During two months people anonymously rated in an online questionnaire statements that might influence their desire for child. Recruitment was performed via use of social media, personal contacts and queer organisations in Switzerland. The study protocol was approved by the Cantonal Ethical Committee.

Participants/materials, setting, methods: The online questionnaire applied comprised general questions about the participant's background, the validated questionnaire about the desire to have children, the "Leipziger Kinderwunschfragebogen", and additional non-validated questions addressing the impact of sexual-romantic orientation and the desire for children. Only adults without children were included. The general questions distinguished the five monogamous orientations: hetero-, homo-, bi-, pan- and asexual with the corresponding romantic orientations.

Main results and the role of chance: Of 837 participants, 642 were included into the study. There were four groups of sexual-romantic orientations that consisted of more than 35 participants: bisexual-biromantic (n=38), heterosexual-heteroromantic (n=230), homosexual-homoromantic (n=159) and pansexual-panromantic (n=55). The prevalence of desire for children varied between 53.46% (homosexual-homoromantic) and 84.55% (heterosexual-heteroromantic). Non-heterosexual-heteroromantic participants rated statements that supported a desire for having children lower and statements with possible problems higher than heterosexual-heteroromantics. However, the corresponding subgroups with participants with a desire to have children showed a very similar pattern. The emotional motives seemed more important than social recognition or financial or personal constraints. Plus, there were only significant difference between individual statements and not between whole motives.

The way people hoped to fulfil the desire for children highly depended on how they biologically could have a child. However, adoption was a valid alternative for homosexuals-homoromantics and pansexuals-panromantics with around 30% of each who voted this as their first choice.

In other words: The significant difference between the orientations in rating the questionnaire is sourced in the different rates of people with desire for children.

Limitations, reasons for caution: The main limitation was the size of the subgroups and the restriction for statistics. In addition, the total number of participants was not enough to represent smaller sexual-romantic orientations. For polygamous people the whole setting would have to be different.

Wider implications of the findings: In conclusion, all sexual-romantic orientations might have a desire for children and for the same reasons. This should be a topic approached in everyday practice and should enforce the politics to take the desire seriously and adjust laws where necessary. Further research about the realisation of the desire is recommended.

Trial registration number: not applicable

P-721 Assessing spousal concordance and its impact on outcome in couples undergoing 1st cycle of ICSI in Glasgow

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Study question: To what extent are heterosexual couples undergoing ICSI concordant with respect to baseline characteristics/behaviours and does this impact upon outcome?

Summary answer: Couples are most concordant with respect to age and education status. Couples with discordant exercise habits had increased rates of biochemical pregnancy.

What is known already: Assortative mating and cohabitation concordance cause married/cohabiting couples to share similar traits, with spousal concordance known to contribute to cardiovascular disease and be associated with worse treatment outcomes in other medical specialities. Maternal and paternal characteristics/behaviours are known to affect both natural fertility and success of IVF treatment but the contribution of concordance is unknown.

Study design, size, duration: Prospective cohort study of 306 heterosexual couples recruited between August 2016 and December 2019.

Participants/materials, setting, methods: Couples with complete baseline phenotypic and outcome date were included. All patients underwent NHS Scotland funded treatment in Glasgow, using AMH stratified ovarian stimulation. Pearson's correlation assessed spousal concordance for continuous variables, whilst kappa analysis was employed for categorical variables. Investigation of association between concordance and outcome was achieved using Mann-Whitney U tests, Chi Squared Analysis, independent t tests and logistic regression.

Main results and the role of chance: 264 underwent fresh embryo transfer, of which there were 125 ongoing pregnancies. Couples were most strongly concordant for age ($r=0.59$ $p<0.000$), alcohol consumption ($k=0.661$) and educational attainment ($k=0.655$). Exercise concordance was significantly associated with outcome, with exercise discordance a predictor of biochemical pregnancy (OR: 1.86; 95% CI 1.18-2.92 $p=0.008$). Furthermore, females in discordant couples are significantly less physically active than females in concordant couples (mean difference = 0.4527 times/week $p=0.003$). Notably, the significant association with discordance did not translate to clinical ongoing pregnancy rate. However, there was also no association between exercise concordance and early pregnancy loss.

Limitations, reasons for caution: Kappa analysis is designed for assessing concordance of inter-rater agreement when multiple investigators classify participants. Thus, its use in this study could be controversial. Additionally, NHS eligibility criteria enforces a strict selection bias, most notably with age, BMI and smoking status. Furthermore, categorical variables were self-reported by patients.
Wider implications of the findings: If replicated in further studies, shared education and public health initiatives to attain spousal concordance of lifestyle factors may be beneficial in improving IVF success rates.
Trial registration number: not applicable

P-722 Cervical mucus patterns and the fertile window in women without known subfertility: A pooled analysis of 3 cohorts

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Study question: What are cervical mucus patterns and number of days with high or low probability of fertility in women without known subfertility, by parity and age?
Summary answer: Parous and younger (<30 years) women had more days of high estrogenic quality mucus compared to nulliparous and older (≥ 30 years) women, respectively.
What is known already: The rise in estrogen prior to ovulation supports the secretion of increasing quantity and quality of cervical mucus, and the subsequent rise in progesterone causes an abrupt decrease in mucus secretion. Prior studies have demonstrated that women can identify ovulation and the fertile window via daily observations of cervical mucus quality, as compared to follicular ultrasound, or serial serum or urine hormones. Limited data, however, are available to assess the relationship between women's observed cervical mucus patterns and key factors known to influence reproductive capacity, including age and parity.
Study design, size, duration: This study is based on a secondary data analysis, combining data from three cohorts of women. We evaluated cervical mucus patterns and the fertile window in 2,359 apparently ovulatory cycles of 502 women with no known subfertility (18–40 years of age), followed for up to 1 year. Linear and generalized linear mixed models were used to assess cervical mucus patterns and characteristics of the fertile window, taking into account women's self-reported parity and age.
Participants/materials, setting, methods: Participants were US or Canadian women age 18–40 years; not pregnant, breastfeeding or taking exogenous hormones at entry; and without any known subfertility. Women used a standardized protocol for daily observation, description, and recording of cervical mucus vaginal discharge (the Creighton Model protocol). The mucus peak day was used as the estimated day of ovulation.
Main results and the role of chance: The majority of women were <30 years of age (76%), non-Hispanic white (89%) and nulliparous (72%). The mean days of peak type mucus per cycle (one indicator of the fertile window) was 6.4 ± 4.2 days (median 6; interquartile range (IQR) 4–8). The mean number of any potentially fertile days (a broader indicator of the fertile window), identified by the defined algorithms, was 12.2 ± 5.4 days (median 11; IQR 9–15). Nulliparous women age ≥30 years had fewer mean days of peak type mucus per cycle (5.4 vs. 6.3, p=.07) and potentially fertile days (10.7 vs. 12.8, p<.01) compared to parous women age < 30 years. Regardless of parity, women aged <30 compared to women ≥30 years had more potentially fertile days (12.8 days vs. 11.8 days, p=.04). Compared to non-parous women, parous women had higher mucus cycle scores (for estrogenic quality of mucus) (9.0 vs. 8.0, p<.01), more days of peak type mucus (7.3 days vs. 6.1 days, p<.01), and fewer dry days (15.9 days vs. 18.9 days, p<.01). Among parous women, there was little difference in mucus parameters by age.
Limitations, reasons for caution: We cannot exclude the possibility that some women had unknown subfertility or undiagnosed gynecologic disorders. We did not have data on some factors that may impact ovulation, hormone levels, and mucus secretion, such as physical activity and body mass index. Study participants were geographically dispersed but relatively homogeneous demographically.
Wider implications of the findings: Patterns of cervical mucus secretion observed by women are an indicator of fecundity and the fertile window. Women's reproductive health can be enhanced by the assessment of a woman's mucus secretion patterns. Future work should investigate the distribution of similar cycle parameters in women with various reproductive or gynecologic pathologies.
Trial registration number: Not applicable

P-723 Levels and sources of education about the female human reproduction and sexuality in women at their first attempt of fertility treatment

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Study question: What is the level of knowledge and understanding of infertile women of the female reproductive anatomy physiology and sexuality ?
Summary answer: There is a real lack of knowledge of infertile women about their genitals' anatomy and physiology in addition to their sexual function.
What is known already: Some studies have reported a distortion in the cognition of the female genital tract in non reproductive women, especially after the menopause. Some research have been made on the level and quality of knowledge of infertile women about the functions of their reproductive system with contradictory results but very few have evaluated the education of infertile women about the female reproductive and sexual functions.
Study design, size, duration: We conducted a cross sectional study including 500 infertile women that were referred to our reproductive medicine unit for their first fertility treatment. The study took place from January 2018 to December 2018. We included two groups of patients : 250 women with feminine infertility (G1) and 250 women in couple with an infertile partner (G2).
Participants/materials, setting, methods: All the participants were nulliparus women at the time of their first attempt of fertility treatment. To evaluate the level of knowledge of the patients, we used a 5 sections questionnaire : socio-econimic characteristics and fertility history, anatomy of the female human reproductive system, physiology of the female human reproductive system, the female sexual function and the sources of information about the reproductive and sexual health.
Main results and the role of chance: The two groups were comparable in terms of age, academic level, age of the first sexual intercourse and the duration of infertility. The knowledge about the anatomy of the female human reproductive system was low and there was no significant difference between the two groups regarding the mean correct response rate for the external female genitalia and vulva pattern (5% in G1 versus 3% in G2 , p = 0.15) and for the female internal genitalia pattern (21% versus 17%). The correct answers rate about the physiology and function of the female genitals and the physiology of the menstrual cycle of was very low with no significant difference between the groups (4% in G1 versus 6% in G2). Regarding the physiology of sexual intercourse, the rates of correct answers were also very low in both groups (4%). There was a high rate of incorrect responses regarding the erogenous organs in both groups (82% in G1 versus 89% in G2, p= 0.32). The first source of information on sexuality was the Internet (65% in G1 and 58% in G2) during the last year. All patients reported some discomfort when asking information about the reproductive and sexual health from their physician.
Limitations, reasons for caution: The questionnaire used is not a standardized one. It was adapted for our local population.
Wider implications of the findings: Reproductive and sexual education have to be an important part of the fertility consultation prior to any counselling or treatment of the infertility of the couple. It may help the patients to

fully understand the procedures and treatment sequences in order to give consent.

Trial registration number: not applicable

P-724 Risk of breast cancer in women treated with ovarian stimulation drugs for infertility: a systematic review and meta-analysis

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Study question: Does ovarian stimulation increase the risk of breast cancer in women undergoing treatment for infertility?

Summary answer: Ovarian stimulation did not increase the risk of breast cancer in women undergoing treatment for infertility.

What is known already: Infertility treatment chiefly involves the use of ovary-stimulating agents such as selective oestrogen receptor modulators (e.g Clomiphene citrate, (CC)), aromatase inhibitors (e.gLetrozole) and gonadotropins. These drugs potentiate ovarian oestrogen production. While nulliparity and prolonged exposure to oestrogen are recognised risk factors for breast cancer, the association of ovarian stimulation drugs with breast cancer has not been thoroughly investigated. Past studies were inconclusive, reported inconsistent findings and differed in terms of control population (fertile and infertile women and with or without a past history of breast cancer).

Study design, size, duration: We performed a literature search using MEDLINE, COCHRANE Library, and Scopus, up to December 2018, using a predefined search algorithm. Randomised controlled trials (RCTs), non-randomised controlled studies, case-control studies and cohort studies reporting on any association between exposure to ovary-stimulating drugs and breast cancer were found to be eligible and were included.

Participants/materials, setting, methods: Study characteristics such as study design, sample size, stimulation drug, breast cancer incidence, and follow-up time from these studies were extracted and analysed through Comprehensive Meta-Analysis (CMA) Software. Subgroup analysis for each stimulating agent was performed, comparing treated infertile women versus general population and/or unexposed infertile women, to question the superimposition of subfertility and ovarian induction as an independent risk factor for breast cancer.

Main results and the role of chance: Twenty-two studies (nine case-control and thirteen cohort studies) were eligible for inclusion with a total of 1,597,233 participants.

Twelve studies compared CC treatment to unexposed infertile women (86,710 participants). Overall, the use of CC was not associated with an increased incidence of breast cancer (risk ratio (RR) = 0.69, 95% CI = 0.261; 0.96, p – value = 0.037). However, subgroup analysis found evidence of increasing risk for those who received ≥12 cycles [RR =1.65, 95%CI=1.08; 2.51, p – value = 0.02].

Four studies (66,555 participants) compared the sole use of gonadotrophins, to unexposed infertile women. Its use was also not associated with any increased risk [RR = 0.68, 95% CI = 0.24; 2.01, p – value = 0.493]. Furthermore, when compared to treatment naïve infertile women as controls, the use of Aromatase inhibitor such as Letrozole , was also not seen to be associated with increased risk of breast cancer (RR = 1.02, 95% CI: 0.88–1.18, p-value=0.06) as did the combined use of GnRHa with gonadotrophins (RR = 1.28, 95% CI = 0.75; 2.18, p – value = 0.36].

Limitations, reasons for caution: In some studies, there was limited information given about reasons for infertility in the women included which made it impossible to separate the treatment effects from hormonal imbalances that themselves may increase the risk of breast cancer. Potential confounders such as family history or genetic predisposition were also not clear.

Wider implications of the findings: Based on current review findings, clinicians should be aware of the associated risks of breast cancer with each of the type of ovarian stimulation drugs. The findings of this review will be helpful to guide patients, especially those at risk of breast cancer.

Trial registration number: Not applicable.

POSTER VIEWING
REPRODUCTIVE SURGERY

P-725 Fertility outcomes following laparoscopic reversal of tubal ligation - a retrospective single-centre experience

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Study question: What are the fertility outcomes associated with laparoscopic reversal of tubal ligation?

Summary answer: Our study found that laparoscopic reversal of tubal ligation offers good pregnancy and live birth rates, and is a feasible alternative to in-vitro fertilization (IVF).

What is known already: Tubal sterilization is a common form of contraception which can be associated with regret and requests to restore fertility. Options for such patients include surgical reversal and in vitro fertilization (IVF). The gold standard for surgical reversal has traditionally been laparotomy and microsurgery. However, recent advances in laparoscopy have offered a minimally invasive option for patients with a desire for fertility after undergoing previous tubal sterilization.

Study design, size, duration: We performed a retrospective review on all cases of laparoscopic tubal reanastomosis surgery conducted in our tertiary centre over a period of six years. Inpatient and outpatient clinical records were traced for data analysis.

Participants/materials, setting, methods: A total of 12 cases of laparoscopic tubal reanastomosis were performed in our tertiary centre between January 2011 to December 2016.

Basic fertility investigations and counselling were performed for all patients. Bilateral tubal reanastomosis was performed with microsurgical equipment following routine laparoscopic entry. Methylene blue hydrotubation confirmed tubal patency intraoperatively.

Demographic details of the participants are as follows:

	n = 12, mean value
Age	34
BMI	23.4
Parity	2.6
AMH	5.7

Main results and the role of chance:

Outcome measures

	n = 12
Pregnancy rate	75.0% (9/12)
Miscarriage rate	8.3% (1/12)
Ectopic rate	8.3% (1/12)
Live birth rate	58.3% (7/12)
Multiple pregnancy rate	0% (0/12)

Characteristics of hospitalization, surgery and interval to pregnancy

Limitations, reasons for caution: The main limitation of our study was the small sample size, although the results are reflective of other studies in this

Length of stay, days (median)	1
Duration of surgery, minutes (mean +/- SD)	156 +/- 20
Interval to conception, months (mean +/- SD)	5 +/- 6.6

field. We aim to use this pilot study to ascertain the positive fertility outcomes associated with laparoscopic tubal reanastomosis, and as a basis for larger prospective studies.
Wider implications of the findings: Based on our experience, reversal of tubal ligation has comparable subsequent intrauterine pregnancy rates to IVF treatment. In addition, laparoscopic microsurgery offers the advantage of faster recovery with less postoperative discomfort, less complications and a smaller surgical scar. More high quality studies should be encouraged in this field.
Trial registration number: Not applicable

P-726 endometrium transplantation facilitates successful fertilization and pregnancy in rats: a preliminary study of autologous orthotopic endometrium transplantation

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Study question: To explore the feasibility of endometrium transplantation for severe endometrial loss such as refractory Asherman's syndrome.
Summary answer: Endometrium transplantation could regenerate competent endometrium and result in successful pregnancy in rats.
What is known already: Although uterine transplantation has led to live births in some cases, there are much more patients with refractory Asherman's syndrome whose real need is the specific reconstruction of endometrium rather than uterus. In these women, the regeneration of endometrium is difficult by stem cell therapy because of a severe loss of endometrial epithelium and stroma, and for them, endometrium transplantation could be an alternative.
Study design, size, duration: The endometrium of adult Sprague Dawley rats was harvested by a procedure of endometrial stripping technique on one uterine horn randomly. A totoal length of 1 cm endometrium was removed and washed in cold phosphate buffer system, and the residual myometrium was left as recipient. In the transplantation group, stripped endometrium was inserted immediately to the eutopic space of residual myometrium, while no tissue was transplanted in control group.
Participants/materials, setting, methods: At 1 week, 2 weeks and 4 weeks after operation, the uteri were collected and endometrial regeneration was assessed by gross morphology, hematoxylin-eosin staining and Masson's trichrome stain. Immunohistochemistry was taken with antibodies against cluster of differentiation 31, cytokeratin-pan, smooth muscle actin-α.The expression of cytokeratin 7 was measure by western blot. 6 weeks after operation, rats were mated and fetuses in pregnant rats were evaluated on 20th day post coitus.
Main results and the role of chance: Endometrial stripping could remove endometrium completely while preserve myometrium, resulting in trace expression of cytokeratin 7. In the control group, uterine occlusion and hydrops occurred in all the horns of endometrial stripping (15/15), while all the grafts in the transplantation group (15/15) survived without uterine cavity occlusion or hydrops. In controls, endometrium was absent at the stripped site and replaced by fibrotic tissue; However, in transplantation group, good endometrial regeneration was revealed by intact epithelium and stroma with fine revascularization. The expression of cytokeratin 7 in transplanted site was significantly higher than that in stripped site. There were 9 viable fetuses and 1 retarded fetus implanted at the transplantation site in 5 pregnant rats with a total of 29 fetuses in the transplantation horns. The average number of fetuses on the transplantation horns was 5.8 ±1.4, which was significantly higher than that on the stripped horns in the control group (0).
Limitations, reasons for caution: The feasibility of endometrium transplantation were only tested with autologous orthotopic transplant in rats. Whether this procedure could be achieved in allogeneic endometrium transplantation and ultimately applied to the patients, and the mechanism underlying endometrium transplantation and regeneration, remain to be further explored.
Wider implications of the findings: Allogeneic endometrium transplantation has potential in the regeneration of endometrium in refractory Asherman's syndrome, and autologous endometrium transplantation could be applied to repairing cesarean scar defects.
Trial registration number: not applicable

P-727 Preoperative Magnetic Resonance Imaging Predictor for Fertility Preservation of Robotic Myomectomy

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Study question: The goal is to identify preoperative Magnetic Resonance Imaging(MRI) predictor for fertility preservation of robotic myomectomy for optimal selection of surgical technique: single-site(SS) or multi-site(MS).
Summary answer: Preoperative MRI predictor could be useful to select SS or MS robotic myomectomy for successful fertility preservation and assume the operative process.
What is known already: Myomas are clinically apparent in about 25% of women and become symptomatic in their reproductive age. Myomectomy is the choice of treatment for women desiring uterine preservation. Although proper minimal invasive surgical technique is requested, laparoscopic myomectomy has some limitations. Adoption of the da Vinci system made it possible to overcome the weaknesses of laparoscopic myomectomy. Owing to the ability of single-port laparoscopy to reduce pain and improve patient satisfaction, robotic SS surgery was developed and introduced. MRI was superior to transvaginal ultrasonography in ascertaining both the correct uterine wall embedment and the position of myomas.
Study design, size, duration: A total of 98 patients who underwent robotic myomectomy after MRI at Robot Surgery Center of Ewha Womans University Mokdong Hospital from July 2015 to March 2018, were evaluated. All patients underwent robotic myomectomy through SS or MS. Final pathology of leiomyoma was confirmed. The correlation was analysed between preoperative MRI predictor and operative or postoperative findings for fertility preservation of robotic myomectomy.
Participants/materials, setting, methods: We assessed FIGO classification, number(>3cm of diameter), size, signal intensity(SI), enhancement of myoma, endometrial distorsion, the thickness of endometrium and junctional zone on MRI. The operation time, estimated blood loss(EBL), chopping time for myoma removal, the number and total weight of removed myoma were measured. After two months, pelvic ultrasonography was performed to define the shape of uterus, endometrial thickness and pattern. The reproductive outcome was investigated when the patient wants to be pregnant.
Main results and the role of chance: Mean age was 35.68±5.04(range 23~45) years old and 80 patients(81.6%) were nullipara. Total diameter of myoma on MRI was 106.75±54.52(range 32.43~273.24) mm and the maximum diameter of myoma was 72.51±25.77(range 32.43~151.03) mm. The operation time was 157.89±61.22(range 60~355) minute and the chopping time was 13.83±17.31(range 1~90) minute. The number of removed myoma was 4.31±4.39(range 1~27) and total weight of removed myoma was 293.11±281.13(range 30~1260) g. The myoma with high SI could be chopped faster and EBL was increased for removal of myoma with high enhancement. Compared to SS, conventional MS robotic myomectomy was performed when preoperative number and total diameter of myoma were increased or deep-seated myoma was diagnosed by Figo classification. After

robotic myomectomy, all patients resumed normal menstruation and showed normal shape of uterus(91.8%), endometrial thickness and pattern(98.0%) by pelvic ultrasonography though 70.4% of patients had the compressed or distorted endometrium. The negative correlation between maximum diameter of myoma and postoperative endometrial thickness using Pearson correlation coefficient(R=-0.139, P=0.195). There is no significant difference of postoperative outcome according to preoperative MRI predictor or robotic surgical technique(SS versus MS). Among 12 patients who wanted to be pregnant, seven women(58.3%) got pregnant naturally(Cesarean section 2, on going 5).

Limitations, reasons for caution: All surgeries underwent using the da Vinci Si system by two gynecologic surgeons who were skilled robotic surgery at one institute and the follow-up period was not enough to conclude the outcome including reproduction.

Wider implications of the findings: The SS or MS robotic myomectomy could be recommended for patient who planned future pregnancy. However, the optimal surgical technique should be selected based on preoperative MRI predictor for effective operative process and successful fertility preservation.

Trial registration number: This study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (No. 05-038-003).

P-728 Does endometrial injury with bipolar electrode during office hysteroscopy improve cycle outcomes in the following or subsequent frozen embryo transfer(FET) cycles?

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Study question: Does endometrial injury with bipolar electrode during office hysteroscopy improve pregnancy and implantation rates in following or subsequent FET cycles?

Summary answer: Endometrial injury by using bipolar electrode has no effect on cycle outcomes neither in first or in subsequent FET cycles.

What is known already: Endometrial injury done by mechanical techniques such as pipelle, curettage or hysteroscopic scissor is supposed to promote the secretion of cytokines, growth factors and interleukines, and in this way, increase the chance of implantation in the following cycle. Although its use is widespread, the benefit of endometrial injury still remains controversial.

Hysterescopy by using bipolar electrode is a minimally invasive procedure that allows the treatment of uterine lesions in the outpatient setting without general anesthesia. In comparison with monopolar electrode, thermal injury and fluid overload are less likely. However, little is known about the effect of endometrial injury by bipolar electrode.

Study design, size, duration: This retrospective study was conducted at İstanbul Memorial Hospital ART Center between 2011 and 2018. A total of 673 patients who underwent single frozen thawed blastocyst transfer were analyzed. Control group without a history endometrial injury and subjects with endometrial injury by using bipolar electrode were separated into three groups; Group A(n=150): FET in following first cycle after endometrial injury, Group B(n=523): FET in second or subsequent menstrual cycles after endometrial injury, Group C(n=1413): controls.

Participants/materials, setting, methods: Patients <37 years old with good and top quality blastocysts were included and elective single blastocyst transfer (eSET) was performed. Exclusion criteria were: repeated pregnancy losses, Mullerian abnormalities, intra-uterine adhesions, endometrial thickness <7mm during FET cycle. Endometrial injury was performed with a bipolar electrode (Versapoint ®, through a 5Fr working channel from anterior to posterior uterine walls and uterine fundus on different areas at a depth of 3mm.

Main results and the role of chance: There was no significant difference in patient characteristics such as age, body-mass index, infertility duration, endometrial thickness on embryo transfer day, and Anti Mullerian Hormone levels between the three groups. Clinical pregnancy rate(CPR) was the main outcome 69.3% (n=104), 60.0% (n=314) and 65.3% (n=923), from group A to C respectively, (p=0.11). Ongoing pregnancy rate was 58.0%(n=87),50.0%(n=262) and 54.3%(n=768) from group A to C respectively,(p=0.2) . Clinical miscarriage rate(CM) was calculated as 16.3% (n=17), 16.5% (n=52) and 16.8% (n=155) from group A to C respectively, (p=0.8).

Comparing the hysteroscopic endometrial injury groups (Group A and B), CPR and OPR was higher in group A when compared to group B. Our results indicate that, endometrial injury effect with bipolar electrode was higher in the following first FET cycle compared to second and subsequent cycles, but this difference was not statistically significant.

Limitations, reasons for caution: The limitation of the study is its retrospective nature and prospective studies are needed to elucidate the exact impact of hysteroscopic bipolar electrode endometrial injury on cycle outcomes.

Wider implications of the findings: In conclusion, performing endometrial injury with bipolar electrode during office hysteroscopy in young patients does not improve clinical pregnancy rates after frozen-thawed single blastocyst transfers when compared with control patients without endometrial injury.

Trial registration number: None

P-729 Negotiating a difficult cervix during hysteroscopy using epidural catheter as guide: a novel approach

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Study question: To evaluate the role Epidural catheter as a guide to gain entry in the uterine cavity during extremely difficult hysteroscopy procedures due to a tightly stenotic/fibrosed or tortuos cervical canal.

Summary answer: Epidural catheter can facilitate hysteroscopy in difficult cases due to cervical canal fibrosis, stenosis or extreme tortuosity.

What is known already: Under experienced hands, hysteroscopy is relatively a safe procedure, technical difficulties are rare but may include inability to pass hysteroscope due to extremely tight or totuous internal os or fibrosis in the cervical canal. Uterine perforation is the most common complication (0.13% for diagnostic; 0.76% for operative). Several studies have shown that most uterine perforations occur at the start of the case during early manipulation of the uterus and cervix and commonly occur during uterine sounding, cervical dilation, or hysteroscopic entry.

Study design, size, duration: Case report of 25 cases of extremely difficult hysteroscopies due to stenosed, tortuous or fibrotic cervical canal or internal os, performed over a period of one year (October'17- November'18).

Participants/materials, setting, methods: Hysteroscopy was carried out in an operating theatre using a rigid BETTOCHI microhysteroscope with continuous-flow surgical sheath with a diameter 2.9mm and 30 degree telescope. In each of these difficult cases the scope could not be advanced through the cervical canal despite ultrasound guidance due to extreme stenosis or fibrosis. 18Gauge epidural catheter was used as guide in each of these cases.

Main results and the role of chance: 18G Epidural catheter passed through the operating channel of hysteroscope was used as a guide to delineate the track for hysteroscope. The passage of catheter inside uterine cavity was confirmed ultrasonologically. The scope was then advanced towards the cavity with axial pressure, keeping epidural catheter always under vision. Hysteroscopy could be completed in all these cases without any complications. No episode of perforation, false tract creation, laceration or bleeding was reported. Embryo transfer was subsequently done in all these cases without any difficulty. 3 out of first 5 cases have been positive for pregnancy either after IVF-ET. The result is awaited for rest of the cases.

Limitations, reasons for caution: The role of epidural catheter as a guide in difficult hysteroscopy procedures needs to be further evaluated in larger study setting.

Wider implications of the findings: Epidural catheter by virtue of being of thin caliber, easily malleable, firm but atraumatic can be passed through tight passages also and helps in delineation of correct passage for the hysteroscope. It may be an effective and safe alternative to blind dilation or Laminaria tent insertions.

Trial registration number: not applicable

P-730 Novel use of hysteroscopy in assessment of tubal patency in difficult cannulation

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Study question: To assess the method of chromopertubation in cases of difficult cannulation to minimize the false negative cases of tubal block.

Summary answer: Introduction of hysteroscope with visualization bypasses cervical factor and reduces false negative results of tubal patency that is added use of hysteroscope.

What is known already: Hysteroscopy is the procedure in which the uterine cavity is inspected by endoscopy with access through the cervix and it also serves as a method for surgical intervention (operative hysteroscopy). It has been known that 15 – 20% infertile women have uterine factor. So, hysteroscopy plays an important role in the preparation of patient for IVF. In the hysteroscopy, various intrauterine pathologies like polyp, fibroids, uterine deformities and adhesions can be detected and treated. All these pathologies can lead to implantation failure.

Study design, size, duration: Study design - Retrospective analysis
Size – 66 infertile females
Duration – 1 year

Participants/materials, setting, methods: We had performed the laparoscopy and hysteroscopy in 66 females as infertility work up in 1 year in which cannulation through cervical canal was difficult and tubal patency test showed tubal block with Leech Wilkinson Cannula. Then through the inlet of hysteroscope, methylene blue dye was injected and patency of tubes was assessed again.

Main results and the role of chance: In 59 out of 66 females, we observed that when cannuation and dilation of cervix was difficult, then the chromopertubation with hysteroscope showed positive tubal patency test.

Limitations, reasons for caution: In this study infertile females with difficult cannulation of cervical canal are included only

Wider implications of the findings: It reduces false negative results of tubal patency that is added use of hysteroscope that has not been reported earlier.

Trial registration number: Not applicable

P-731 Intrauterine infusion of platelet-rich plasma after hysteroscopic adhesiolysis for patients with recurrent intrauterine adhesions is a new strategy and effective therapy

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Study question: To evaluate the effectiveness of intrauterine infusion of platelet-rich plasma (PRP) in patients with recurrent intrauterine adhesions (r-IUA).

Summary answer: Intrauterine infusion of PRP is a new and effective strategy in the treatment of r-IUA in preventing r-IUA and promoting endometrial repair.

What is known already: Prevention of r-IUA and promotion of endometrial repair have been the key objectives for IUA cases after hysteroscopic adhesiolysis. Autologous PRP can promote endometrial growth, and improve endometrial regeneration in women with infertility and thin endometrium

Study design, size, duration: This is a retrospective study, and eleven patients with intrauterine infusion of PRP (group A) and thirteen patients with intrauterine infusion of barrier gels and balloon (group B) in the first hysteroscopy were included in the study.

Participants/materials, setting, methods: Eleven patients with intrauterine infusion of PRP and thirteen patients with intrauterine infusion of barrier gels and balloon in the first hysteroscopy were included. The second-look hysteroscopy and the third-look hysteroscopy were performed one week after the first operation and in the next menstrual cycle respectively. The fourth-look hysteroscopy was performed if necessary. AFS scoring system was used in the judgement of IUA, and the thickness of endometrium covered by endometrium was evaluated.

Main results and the role of chance: Among the 13 patients with the third-look hysteroscopy in group B, 5 patients showed no improvement. Among the 9 patients with the third-look hysteroscopy in group A, 3 patients recovered from severe or moderate IUA to slight IUA, and the remaining 6 patients showed no r-IUA with satisfying endometrial repair. The mean AFS score decreased from 8.22±1.47 in the first hysteroscopy to 2.22±0.63 in the third hysteroscopy in group A, while it decreased from 8.08±1.77 to 4.54±2.90 in group B respectively. There was significantly statistical difference comparing the AFS scores between the two groups in the third-look hysteroscopy ($P<0.05$) instead of the first hysteroscopy ($P>0.05$).

Limitations, reasons for caution: This a retrospective study instead of randomized controlled trials with larger samples. This is the first time that our study reported the application of intrauterine infusion of PRP in the treatment for patients with r-IUA. Intrauterine of autologous PRP is a new strategy in the management of r-IUA.

Wider implications of the findings: Intrauterine of autologous PRP is a safe and new strategy, as well as an effective adjuvant therapy in the management in preventing the recurrence of IUA and promoting endometrial repair in patient with r-IUA.

Trial registration number: not applicable

P-732 Fertility and clinical pregnancy outcome of modified adenomyomectomy in infertile women with uterine adenomyosis

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Study question: Does reduction surgery of adenomyosis have clinical efficacy in infertile women?

Summary answer: Modified adenomyomectomy as a uterus-sparing surgery could be an effective method for increasing pregnancy rate and conservation of fertility potential in infertile women with adenomyosis.

What is known already: The relation between uterine adenomyosis and infertility is controversial, but it appears to affect endometrial receptivity and increase abortion rate. In infertility where uterine conservation is paramount, the treatment of adenomyosis is often complicated, that is to say, medical treatment is often transient and hysterectomy for eradication could not preserve their fertility. At this stage, there is no agreement on the most appropriate therapeutic methods on fertility outcome in infertility patients with adenomyosis. Regarding surgical removal of adenomyosis, including laparoscopic reduction, uterus-sparing surgery appears to be satisfactory and reduced the need for hysterectomy, but needs well designed prospective study.

Study design, size, duration: Prospective clinical trial was conducted. The subjects consisted of 50 infertile patients with adenomyosis and were enrolled after the failure of In Vitro Fertilization (IVF) for pregnancy from December 2007 to September 2017.

Participants/materials, setting, methods: All cases were classified as having unexplained infertility, adenomyosis with severe periodic dysmenorrhea and occasional menorrhagia. This newly designed operative procedure included pediatric foley insertion into the uterine cavity, injection of diluted vasopressin along the uterine incision, T- or transverse H-incision on the adenomyotic wall, careful excision of adenomyosis tissue using argon laser under intraoperative ultrasonography. After debulking surgery, patients underwent follow up examination for symptom relief, reduction of adenomyosis by MRI and pregnancy rate.

Main results and the role of chance: The mean age and the duration of infertility were 35.60 ± 3.37 years and 55.48 ± 48.24 months, respectively. The mean volume of excised specimens of adenomyosis was 94.15 ± 56.63g. The relief of dysmenorrhea was observed clearly in all patients at 6 months after operation (NRS; 7.28 ± 2.29 vs. 1.56 ± 1.29, $p < 0.001$). The amount of menstrual blood was also significantly decreased (140.44 ± 91.68 vs. 66.33 ± 65.85, $p = 0.009$). The CA 125 level was significantly decreased at the time of 6 months after operation (187.75 ± 229.52 vs. 20.36 ± 19.19, $p = 0.026$). Post-operational complication occurred in four patients (subfascial hematoma, ureter fistula, shrinkage of uterus and premature ovarian insufficiency). Five patients were lost in the follow-up. Of 33 patients who attempted pregnancy, 18 patients conceived by natural or IVF or thawing ET after the operation (18 of 33; 54.5%). However, miscarriage occurred in five patients, ectopic pregnancy in three patients, preterm delivery in two patients and eight patients (8 of 33; 24.2%) delivered by cesarean section at term. The rest of the patients have been trying to conceive by IVF-ET or natural course.

Limitations, reasons for caution: The sample size is small, so further study with larger number of patients will be helpful to investigate the possibility of this result.

Wider implications of the findings: This reduction surgery was related to symptom relief of dysmenorrhea, menorrhagia and increasing pregnancy rate, implying that it could be considered as a successful method for infertile women with adenomyosis who need fertility preservation. This is one of few reports on the clinical pregnancy outcome of uterus-sparing surgery in adenomyosis.
Trial registration number: N/A

P-733 Ovarian reserve in women after second surgery for recurrent ovarian endometriosis.

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Study question: The aim of this study was to evaluate ovarian reserve in women with recurrent ovarian endometriomas compared with newly diagnosed follow-up in 6 months after surgery.
Summary answer: The laparoscopic stripping of recurrent ovarian endometriomas is associated with a high risk of ovarian reserve damage and ovarian failure.
What is known already: Surgery is frequently selected for the treatment of endometriosis. Although surgical excision of endometriosis both improves pain and enhances fertility, recurrence and repeated surgery can further exacerbate pain and reduce fertility, which in turn impacts quality of life. Current guidelines on the management of endometriosis do not give specific indications for treatment of the recurrence as opposed to first diagnosis, except for a more careful consideration of the decision to proceed with surgery on case of previous interventions.
Study design, size, duration: Prospective study was conducted in the reproduction and genetics center «NOVA CLINIC» during the last year.
Participants/materials, setting, methods: A total of 128 reproductive aged women, between 18 and 40 years underwent unilateral laparoscopic cystectomy by stripping method of ovarian endometriosis. I group included 88 women with recurrent ovarian endometriosis, the II enrolled 40 women with the newly diagnosed. The primary outcome of the study was to assess the changes in antimüllerian hormone (AMH) levels and in antral follicle count (AFC), and ovarian volume in each study group.
Main results and the role of chance: Patients in both groups were comparable in age - 29,94±4,96 years and 31,5±4,93 (p>0,05). Before surgery the differences in the ultrasound indicators of ovarian reserve (OR) were significant: mean ovarian volume was 5,6±2,1 cm^3 in I group, 7,4±2,1 cm^3 before primary surgery, AFC in I - 4,9±2,2; in II - 6,1±2,7. The serum concentration of AMH in I group – 2,7±1,3; II - 3,5±0,4 ng/ml. In 6 months the preoperative level of AFC decreased by 1.4 and 1.2 times, respectively (I - 3,4±1,8; II - 5,4±2,1) (p <0,05). Ovarian volume in group I (5,0±1,6 cm^3) has decreased by 1.2 times, in II (6,8±2,6 cm^3) - 1.3 times in comparison to that before surgery. AMH levels in postoperative patients in the group after second surgery, was 1.5-fold lower (1,6±0,9 ng/ml) than in the group where the primary surgery was applied (2,6±1,2 ng/ml).
Limitations, reasons for caution: Laparoscopic excision is considered the gold standard for the surgical of ovarian endometriomas. Excisional techniques are associated with lower recurrence rates. In the same time, surgery is associated with a high risk of reducing the ovarian reserve.
Wider implications of the findings: The ultrasonographic and serum data on the ovarian reserve from the present study suggest that surgery for a recurrent endometrioma may be more harmful to the healthy ovarian tissue than primary surgery. This is extremely important to consider when choosing a treatment plan for women with infertility.
Trial registration number: Not applicable.

P-734 Natural and assisted reproductive outcomes following salpingostomy for hydrosalpinx

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Study question: What are the reproductive outcomes of infertile women with hydrosalpinx who undergo salpingostomy at Birmingham Women's and Children's Foundation Hospital Trust?
Summary answer: Women who underwent salpingostomy had a 10.3% chance of spontaneous conception and 33.3% chance of assisted conception, with all pregnancies proceeding to live birth.
What is known already: A quarter of all female infertility is caused by tubal factor disease. Pelvic inflammatory disease accounts for the majority of this, which if left untreated can cause inflammation of the fallopian tubes leading to the development of hydrosalpinx. Historically, reproductive surgeons used salpingostomy, in an attempt to correct tubal pathology and achieve spontaneous conception. Since the advent of IVF/ICSI, this is rarely performed, as sterilising surgery which prevents hydrosalpingeal fluid from contaminating the endometrium is preferred. In the UK, there is limited government funding for IVF cycles and so salpingostomy is more commonly employed to allow for natural conception.
Study design, size, duration: A 5 year retrospective cohort study of all salpingostomy procedures was performed at Birmingham Women's & Children's Foundation Hospital Trust from 2013-2018. In total, 39 women underwent laparoscopic salpingostomy procedures.
Participants/materials, setting, methods: The 39 women in this study had a mean age of 31 years (range 23-40 years). All procedures were performed by two experienced tubal surgeons using a cuffed stitched technique. Data were collected by two independent researchers from March 2013 to September 2018 through hospital records and a targeted telephone survey using a standardised data collection proforma.
Main results and the role of chance: Overall, 28 (71.8%) unilateral and 11 (28.2%) bilateral procedures were performed. Indications for unilateral salpingostomy were previous salpingectomy/ clipping 10 (35.7%), unilateral tubal pathology (8; 28.6%), concomitant salpingectomy (6; 21.4%) and inability to access the contralateral tube (5; 17.9%). Significant pelvic pathology (endometriosis, adenomyosis, tubo-ovarian mass, adhesions) was identified in 23 (59.0%) cases.

Post procedural patency was assessed intra-operatively in 35 (89.7%) cases, with patency achieved in 33 (84.6%). Patency was reassessed by post-operative hysterosalpingogram (HSG) in 18 (46.1%) women. Of these, patency was confirmed unilaterally in 12 (66.7%) at 3-6 months, resulting in a re-blockage rate of 33.3% in those assessed.

In total, 4 women achieved spontaneous pregnancy giving a 10.3% chance of natural conception. The mean duration to natural conception was 8 months; range 4–10m). 29 women were offered IVF/ICSI, of which 12 (30.8%) proceeded to treatment and 17 declined / were ineligible for NHS-funded cycles. The successful assisted conception rate was 33.3% (n=4). In total, 4 (10%) procedural complications were identified. All conceptions resulted in a live birth, resulting in a live birth rate of 20.6%. No ectopic pregnancies were reported.
Limitations, reasons for caution: The results of this study are based on a small population size and so must be interpreted with caution. The cases included are clinically heterogeneous in terms of patient characteristics, additional pelvic pathology and duration of follow-up after salpingostomy.
Wider implications of the findings: Whilst reported natural conception rates are lower than expected, additional pelvic pathology was encountered. We suggest the importance of postoperative HSG in order to assess tubal patency to determine if assisted conception should be sought sooner in the event of tubal re-closure.
Trial registration number: N/A

P-735 Prevalance of dysmorphic uterus among parous women

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Study question: What is the prevalance of dysmorphic uterus (DU) among parous women?
Summary answer: Parous women who conceived spontaneously have a low prevalance of DU.
What is known already: Neither the prevalance of dysmorphic uterus in parous women nor its effect on reproductive potential is known
Study design, size, duration: Prospective cohort study including 133 women who conceived spontaneously and had at least one live birth. Participants were recruited from a contraception clinic between January 2017 and December 2018.
Participants/materials, setting, methods: Participants underwent 3D TVUS in the late luteal phase of menstrual cycle. The ESHRE/ESGE classification of congenital uterine anomalies was used. The following measurements were taken In the coronal view; 1)fundal cavity width (W1) (the distance between the two internal tubal ostia),2) width of uterine cavity at corpus-isthmic level (W2), 3) the lateral angle between the corpus-isthmic cavity and the two fundal endometrial layers (A right; A left). Uterine cavity volume was measured by VOCAL^{TM} program.
Main results and the role of chance: The mean (± standard deviation) age was 35.54±5.9 years. Mean parity was 1.88 (min 1 max 7) and time to pregnancy (TTP) was between 1- 12 months. Mean antral follicle count was 12.1±9.5 and 4.5% had at least one ultrasound feauture of adenomyosis. Four patients (3%) were diagnosed with congenital uterine abnormality of which two (1.5%) had partial septate uterus, one (0.75%) hemiuterus uterus and only one (0.75%) dysmorphic uterus. Overall mean uterine volume, W1, W2, W1/W2 ratio and mean lateral angles was 4.2 ±2.1 ml, 28.2+5.5mm, 11.8±3.3mm, 2.48±0.6 and 155.9+10.5°respectively. The women with DU had uterine volume, W1/W2 ratio and mean lateral angle of 3.62 ml, 2.26, and 108.03°, respectively. Her TTP was 7 months and she had given birth to a 3450 gr child at 39 weeks.
Limitations, reasons for caution: While the presence of only one case of dysmorhpic uterus supports a possible association between DU and fertility, the low event rate of only one case can also be regarded a limitation of the study at the current stage.
Wider implications of the findings: Low prevalance of dysmorphic uterus among fertile women may indirectly indicate poor reproductive performance of dysmorphic uterus anomaly. If women with infertility are shown to have higher prevalance of DU, a causal relationship between DU and fertility can be claimed.
Trial registration number: 2017.052.IRB1.008 Koc University Committee on Human Research

P-736 Post-surgical recurrence of benign ovarian disease: a cohort study of women in reproductive age

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Study question: Which factors are associated to recurrence of benign ovarian pathology after surgery in women in reproductive age?
Summary answer: Recurrence rate: cystadenomas, 4.2 per 100 patient/years; endometriomas, 3.4; rest: 0.7. Histology and size >5 cm are independently associated to recurrence of benign ovarian pathology.
What is known already: Many of the low risk cystic tumours resolve spontaneously or with medical treatment. Nevertheless, some are treated surgically because of size, symptoms, complexity or bilateral affection. A special care must be adopted for women in reproductive age, where preservation of healthy ovarian tissue must be sought.

After surgery, the exact time recommended for follow-up is difficult to define. Medical literature on recurrence of benign ovarian pathology after surgery is limited to case series and small cohorts of single histological type of benign cysts. How different factors independently influence on its overall recurrence is not well known.
Study design, size, duration: This retrospective cohort included consecutive women that had surgery of benign ovarian pathology in the period between 01/01/2012 and 31/07/2017. We analysed crude and specific recurrence rates for cystadenomas, endometriomas, and other conditions (simple cysts, dermoid, haemorrhagic and others). We considered different independent variables: age, type of surgery (laparoscopic/laparotomic), urgent/scheduled surgery, surgical technique, previous ovarian surgery, histology, size, and medical (hormonal) treatment after surgery.
Participants/materials, setting, methods: Inclusion criteria: women in reproductive age that had a surgical procedure for a benign ovarian disease and a follow-up of at least 6 months. Exclusion criteria: malignant ovarian neoplasm. Setting: university hospital.

We calculated crude incidence density for recurrence of different types of disease per 100 patient/years of follow-up. After a univariate analysis for recurrence of ovarian disease, we built a Cox-regression model to adjust for significant independent variables.
Main results and the role of chance: In the recruitment period, 472 women had surgical procedures for benign ovarian conditions. Out of these, 397 met inclusion criteria. Follow-up time range was from 187 to 2387 days. We had a total of 1294 person/years of follow-up and 28 recurrences (7.1%). Median age: 34 years (IQR 28-40). Histopathology: endometriomas, 41.1%; dermoid cysts, 24.7%; simple cysts, 12.1%; cystadenomas, 10.8%; haemorrhagic, 4.8%; others, 6.6%.

Median size: 5 cm (IQR 4-7). Bilateral location: 24.9%. Previous ovarian surgery: 13.1%. Medical treatment after surgery: 24.2%.

Overall crude rate of recurrence: 2.2 (95%CI 1.5-3.1) per 100 person/years of follow-up. Specific rates: cystadenomas, 4.2 (95%CI 1.7-10.1); endometriomas, 3.4 (95%CI 2.2-5.3); others, 0.7 (95%CI 0.2-1.7). The median days after surgery where recurrence was detected were 1455 (5th percentile, 265; 95th, 2346).

In univariate analysis for recurrence, the only variables significantly associated were: histopathology, size and posterior medical treatment. In the Cox-regression model, hazard ratios (95%CI; p value) were: histopathology (compared to basal hazard of simple, dermoid and haemorrhagic cysts): cystadenomas, 8.29 (2.08-33.01; p 0.003); endometriomas, 4.30 (1.30-14.18; p 0.017); size (compared to cysts of 5cm or lower): >5 to 7cm: 2.93 (1.14-7.58; p 0.026); >7cm: 2.69 (1.06-6.83; p 0.037); medical treatment: 2.10 (0.87-5.08; p 0.100, non-statistically significant).
Limitations, reasons for caution: It is a retrospective cohort. There might be other known or unknown variables not included in the analyses. Although the total number of included patients is significant, some analyses have wide confidence intervals. Other small studies show similar results
Wider implications of the findings: Benign recurrences can be detected as early as 260 days after surgery or as late as 6.5 years in women in reproductive age. Cystadenomas, endometriomas, and size > 5cm are associated with higher risk of recurrence. These specific rates and hazards can be used for shared decision making with patients.
Trial registration number: -

P-737 Do fibroid size and number of large fibroids removed at laparoscopic myomectomy impact on clinical outcomes?

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Study question: Do fibroid size and number of large fibroids removed at laparoscopic myomectomy impact on clinical outcomes?
Summary answer: Surgical outcomes and complication rates are comparable irrespective of number and size of fibroids removed.

What is known already: Laparoscopic myomectomy has multiple advantages compared to an open approach. Yet, its use in the setting of complex fibroids is contentious with the prevailing consensus being that this surgical approach should be reserved for smaller lesions. There is no clear consensus in the literature as to what size and number of fibroids can be safely removed laparoscopically nor is it clear how increasing fibroid size and number impact on clinical outcomes.

Study design, size, duration: This was a retrospective observational study conducted in a tertiary referral centre for gynaecological laparoscopic surgery. The study examined data from 305 women of reproductive age undergoing laparoscopic myomectomy between September 2004 and December 2017 for symptomatic large (≥6cm) uterine fibroids.

Participants/materials, setting, methods: Participants were identified through the unit's theatre logs and their clinical data was collected from their medical records. Comparisons were made between women with single fibroids measuring 6-8cm and those with either single fibroids >8cm or multiple fibroids ≥6cm. Main outcome measures were operative time, blood loss, blood transfusion rate, complication rates, rate of conversion to laparotomy.

Main results and the role of chance: Sixty-two women had multiple large fibroids resected, 117 had one fibroid measuring 6-8cm resected and 126 had a single myoma >8cm removed. The median fibroid size was 8cm (range, 6-20cm) with a median blood loss of 100mls (range, 20-2000mls) and operating time of 110minutes (range, 40-320). There was a significant correlation between fibroid size and operating time (Pearson's R: 0.313 p<0.001), and fibroid size and blood loss (Pearson's R: 0.195 p=0.003). There was no significant difference in overall complication rate (6.84% versus 5.85% p=0.81), blood transfusion rate (1.71% versus 3.19% p=0.72), conversion to laparotomy rate (3.42% versus 1.06% p=0.21) or hospital stay (median: 2 days; range: 1-19days vs. median: 2 days; range: 1-9 days p=0.34) when comparing women with fibroids measuring 6-8cm to those with either larger or multiple fibroids.

Limitations, reasons for caution: Our study is limited by its retrospective design. Whilst we reported only on large fibroids, 62% of women had multiple fibroids removed. This may introduce some heterogeneity in the data. Furthermore, operative times need to be interpreted with caution as over 53% of patients had a concurrent procedure.

Wider implications of the findings: Our study suggests that with appropriate operator experience, large and multiple fibroids may be safely removed laparoscopically with no adverse impact on clinical outcomes.

Trial registration number: Not applicable

POSTER VIEWING
SAFETY AND QUALITY OF ART THERAPIES

P-738 Impact of Intracytoplasmic Morphologically Selected Sperm Injection (IMSI) on birth defects. A systematic review and meta-analysis

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Study question: Could the use of Intracytoplasmic Morphologically Selected Sperm Injection (IMSI) decrease the incidence of birth defects (structural defects/chromosomal abnormalities) in ART cycles?

Summary answer: IMSI seems to be an effective tool in decreasing the incidence of structural-defects compared to ICSI. However, IMSI does not change the incidence of chromosomal-abnormalities.

What is known already: It is generally accepted that the incidence of birth defects in spontaneous conception ranges between 2.0% and 4.0%. However, several studies have shown that babies born with the aid of ART tend to present more congenital malformations than naturally conceived children, with as many as 6.5% of the babies conceived from ICSI presenting birth defects. Introduced in the early 2000s, high magnification sperm selection before ICSI allowed the identification of spermatozoa at low risk of sperm DNA damage. Therefore, IMSI might change the incidence of congenital malformations. Nevertheless, data on birth defects in children conceived after IMSI are still scarce

Study design, size, duration: A systematic review based on searches on electronic databases (PubMed, EMBASE, Web of Science, SCOPUS, and Cochrane Central Register of Controlled Trails) up to January 2019 was carried out to identify trails comparing the neonatal outcomes of ICSI versus IMSI. The outcome measured was birth defects rates in children born after ICSI or IMSI.

Participants/materials, setting, methods: Three trials were included as targets for data extraction and meta-analysis. Data were combined for purposes of meta-analysis with the StatsDirect statistical software package. Dichotomous data were expressed as Relative Risk (RR) with a 95% confidence interval (CI). The degree of heterogeneity was evaluated using Cochran's Q and I^2. Study data were combined using a random-effects model. *P*-values <0.05 were considered statistically significant.

Main results and the role of chance: The meta-analysis included 3907 children conceived after IMSI(1280) and ICSI(2627), and the incidence of total birth defects was 2.5%(32/1280) when IMSI was used versus 4.5%(119/2627) when ICSI was employed, showing a statistically significant difference (RR=0.60; 95%CI = 0.41-0.88; *P*<0.01)(Table 1).

Structural defects and chromosomal abnormalities were analysed separately. The results showed that IMSI significantly decreased the incidence of structural defects when compared to ICSI [2.2%(18/830) vs. 3.8%(78/2049](RR=0.58; 95%CI =0.35-0.97; *P*=0.04). No significant difference was found for chromosomal abnormalities(Trisomy 13/18/21/ and Triple X) between the children conceived after IMSI(8/830) and ICSI(19/2049)(RR=1.08; 95%CI=0.47-2.45;*P*=0.8)(Table 2).

Limitations, reasons for caution: Randomised controlled trials on IMSI effectiveness and safety of ART cycles do not include birth defects as an endpoint. Thus, studies with larger populations are needed to estimate the actual risk of birth defects associated with IMSI.

Wider implications of the findings: The present meta-analysis demonstrated that IMSI seems to decrease the incidence of structural defects in offspring compared to ICSI. It is critical that the comparative trials involving IMSI include the health statuses of the children after birth.

Trial registration number: Not applicable. The local ethics committee authorised the study.

P-739 Extreme mitochondrial DNA sequence divergence is tolerated in primate offspring produced by mitochondrial replacement therapy

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Table 1 IMSI vs. ICSI: Total birth defects(structural-defects/chromosomal-abnormalities).

Total birth defects	ICSI(n/N)	IMSI(n/N)	RR	95% CI
Cassuto *et al.*,2014	22/578	6/450	0.35	0.15-0.83
Hershko-Klement *et al.*,2016	71/1394	18/498	0.71	0.43-1.17
Gaspard *et al.*,2018	26/655	8/332	0.61	0.28-1.30
Total	**119/2627**	**32/1280**	**0.60**	**0.41-0.88**
Chi^2=6.7;*P*<0.01				
Cochran's Q=1.8;*P*=0.4				
I^2=0%				

Table 2 IMSI vs. ICSI: Structural defects and chromosomal abnormalities.

	ICSI(n/N)	IMSI(n/N)	RR	95% CI
Structural defects				
Hershko-Klement *et al*.,2016	57/1394	12/498	0.59	0.32-1.08
Gaspard *et al*.,2018	21/655	6/332	0.57	0.24-1.36
Total	**78/2049**	**18/830**	**0.58**	**0.35-0.97**
Chi^2=4.3;*P*=0.04				
Cochran's Q=0.002;*P*=0.9				
Chromosomal abnormalities				
Hershko-Klement *et al*.,2016	14/1394	6/498	1.20	0.48-3.0
Gaspard *et al*.,2018	5/655	2/332	0.83	0.19-3.69
Total	**19/2049**	**8/830**	**1.08**	**0.47-2.45**
Chi^2=0.03;*P*=0.8				
Cochran's Q=0.19;*P*=0.7				

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Study question: Does donor mitochondrial DNA (mtDNA) sequence divergence compromise the health and fitness of nonhuman primate offspring achieved through mitochondrial replacement therapy (MRT)?
Summary answer: Healthy rhesus macaque MRT offspring demonstrate tolerance to extreme sequence divergence of donor mtDNA.
What is known already: Proteins encoded by mtDNA must interact with proteins encoded by nuclear genome to form multisubunit complexes of mitochondrial oxidative phosphorylation (OXPHOS), therefore, sequence divergences in these genomes could result in poor subunit interaction. Indeed, nuclear and mitochondrial genome incompatibility is one of the mechanisms responsible for reproductive barrier between species. Trans-mitochondrial infants from distinct Indian and Chinese Macaca mulatta sub-populations were successfully produced by MRT between 2009 and 2012. However, total mtDNA sequence divergence was not known.
Study design, size, duration: We performed whole mtDNA sequencing (Illumina Miseq) on blood DNA libraries from rhesus females used as oocyte donors, for maternal (n=4) and donor mtDNA (n=4), to create MRT offspring. We analyzed DNA sequences to identify single nuclear polymorphisms (SNPs) between maternal and donor mtDNA. Offspring (n=5) body weight, blood chemistry at adulthood (5-7 years), and male fertility were compared to non-manipulated controls (n=10). Three MRT adults were bred and males trained to provide semen samples.
Participants/materials, setting, methods: We used NextGene software to perform whole mtDNA genome assembly and SNP searching, the MITOS webserver for annotation and Mesquite for pairwise alignments. MRT offspring bodyweight was monitored over 6 years. Blood analyses included complete blood count (CBC) and chemistry. Sperm were counted and assessed for motility, agglutination and morphology. Adult MRT offspring reproduced naturally following a time-mated breeding program.
Main results and the role of chance: Sequence divergence between maternal and donor mtDNA pairs ranged from 8 to 755 total SNPs, or 5 x 10^{-4} to 5% of the full mtDNA genome. Sequence differences in protein coding genes ranged from 4 to 542 SNPs (50% and 74% total SNPs, respectively) and translated to a range of 2 to 113 amino acid differences. Genes that code for subunits of OXPHOS complex I, the largest enzyme of the mitochondrial electron transport chain, differed at as many as 300 sites, or 61 amino acid changes. RNA-coding gene sequence divergence ranged from 0 to 93 SNPs. In the displacement loop (D loop), a noncoding region implicated in mtDNA transcription and replication, sequence divergence ranged from 4 to 93 SNPs. MRT monkeys were developmentally normal compared to non-manipulated controls in body weight and within normal range for blood chemistry. Male MRT monkeys produced morphologically normal sperm. Natural breeding of MRT offspring (two males, one female) resulted in three healthy births (two females, one male).
Limitations, reasons for caution: Study is limited to 5 MRT offspring (four males and one female). Although the results suggest safety of "unmatched" donor mtDNA within species, there is possibility of different mtDNA replicative capacity leading to reversal after MRT.
Wider implications of the findings: The staggering number of SNP differences between maternal and donor mtDNA coupled with health of MRT monkeys suggests that mtDNA haplotypes within species are compatible. Historically, mtDNA haplotype matching has been recommended to circumvent issues relating to mtDNA donor mismatch. Evidence here suggests this may be needlessly cautious.
Trial registration number: not applicable

P-740 The incidence of monozygonic twins in assisted reproduction technology

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Study question: Whether assisted reproductive technology effect the incidence of monozygonic twins
Summary answer: In assisted reproductive technology, blastocyst culture significantly increased the risk of monozygonic twins. However, the parental age, ICSI or assisted hatching had no signifcant impact.
What is known already: it is known that the incidence of monozygonic twin (MZT) in natural conception is about 0.4%. Previous report has shown that the risk of MZT has doubled after assisted reproductive technology. MZT can lead to miscarrige, increase the incidence of preterm birth and other adverse neonatal outcomes. Therefore, researchers have been take much attention to the incidence and reasons of MZT in assisted reproduction and found that maternal age, ICSI and blastocyst tranplantion may be associated with the happening of MZT.
Study design, size, duration: This study included 24777 fresh and frozen tranfer cycles in Peking University Third Hospital from 2015 to 2017.
Participants/materials, setting, methods: Maternal age, fertilization strategy and the embryos they transfered were analyzed between the MZT and non-MZT groups. All pregnant patients were confirmed by detection of fetal heart activity on the use of transvaginal ultrasound by early first-trimester. In all cases, a P-value was considered significant if <0.05. For the statistical analysis, SPSS software was used.
Main results and the role of chance: In fresh cycyles, the ratio of MZT in D3 cleavage stage was 0.49%(59/12060) while it was 1.52% (9/590) in blastocyst tranfer cycles. It is significantly higher in patients accepted blastocyst transfer. This trend is the same in frozen-thawn cycles of 0.63%(32/5042) with cleavage embryo transfer vs 1.96%(145/7085) with blastocyst transfer. In addition, maternal age seem to have no impact on MZT incidence. Moreover, for conventional IVF, the ratio of MZT was 0.52% and it was 0.57% in ICSI group with no significant difference. For assisted hatching, we found no significant difference in those with blastocyst hatching(2.24%, 59/2632) and those without hatching(1.83%, 16/872).
Limitations, reasons for caution: This is a retrospective research and more data from randomized trial are required.
Wider implications of the findings: This research indicated the risk of blastocyst transfer and give us more thinking about whether blastocyst transfer should be widely used.
Trial registration number: No

P-741 Predictive factors for dizygotic twin pregnancies after single embryo transfer: a retrospective analysis of a large-scale nationwide database study

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Study question: What are the risk factors for dizygotic twin pregnancies after single embryo transfer (SET)?
Summary answer: There were three independent risk factors as following: unexplained infertility, the frozen-thawed embryo transfer (FET) cycle, and the type of luteal support.
What is known already: The dizygotic twin pregnancies after SET might be the simultaneous pregnancies by assisted reproductive and natural conception. It remains unknown the predictive factors for dizygotic twin pregnancies after SET.
Study design, size, duration: A retrospective cohort study was performed, based on 1404648 treatment cycles in registered assisted reproductive technology (ART) data from JSOG between 2007 and 2012.
Participants/materials, setting, methods: A total of 1509 twin pregnancies after 595226 SET cycles were included in the analysis. The sex-discordant twin pregnancies are always dizygotic. Then, we analyzed the predictive factors for dizygotic twin pregnancies after SET based on the data of sex-discordant twin by multivariate analysis.
Main results and the role of chance: The numbers of sex-concordant and discordant twin pregnancies were 1391 and 118, respectively. The rate of sex-discordant twin pregnancies was 7.8% (118/1509). According to Weinberg's differential rule, the rate of dizygotic twin pregnancies accounted for 15.6% of the total twin pregnancies after SET. In the multivariate analysis, there were three independent predictive factors for dizygotic twin after SET as following: unexplained infertility (adjusted odds ratio (aOR), 1.47; 1.01-2.15, p=0.04), the FET cycle (aOR, 1.73; 95%CI, 1.07-2.79; p=0.03), and the luteal support with estrogen and progesterone (aOR 0.29; 95% CI, 0.14-0.62, p=0.001).
Limitations, reasons for caution: In the FET cycles, information about ovarian stimulation and fertilization methods did not include.
Wider implications of the findings: Clinicians should explain to the patients the possibility of causing the spontaneous conception during ART. Mainly, in the case of unexplained infertility, the FET cycle, and the specific type of luteal support, we had better explain the necessity of contraception during ART in the point of avoiding multiple pregnancies.
Trial registration number: not applicable

P-742 Safety of originator follitropin alfa (GONAL-f) for fertility treatment – Frequency of OHSS and thromboembolism in the scientific literature and the Merck KGaA safety database

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Study question: Based on systematic review of published data and the Merck KGaA Safety Database, what is the frequency of OHSS and thromboembolism with GONAL-f?
Summary answer: Reporting rates of OHSS and thromboembolism demonstrate that the risk after ovarian stimulation treatment with GONAL-f (Merck KGaA, Darmstadt, Germany) is low.
What is known already: Recombinant-human follicle-stimulating hormone (r-hFSH) is used for ovarian stimulation as part of treatment with assisted reproductive technologies (ART), with dosing (both starting dose and during treatment) individualized to optimize safety and efficacy. Owing to its mechanism of action there is a risk for OHSS with r-hFSH treatment, and there is also a risk for thromboembolic events, which is increased in the presence of pregnancy and OHSS.
Study design, size, duration: MEDLINE and Embase were systematically searched from inception to 19-Oct-2018 for clinical studies using GONAL-f. Keywords were 'GONAL-f' and 'r-hFSH' and their variants.

Reports of OHSS and thromboembolism were obtained from the Merck KGaA Global Safety Database from 29-Nov-2000 to 19-Oct-2018. This database includes reports from healthcare professionals, patients, health authorities, clinical trials, non-interventional studies and literature. Number of treatment-cycles were estimated from sales data based on an average 1875 IU administered per treatment-cycle.
Participants/materials, setting, methods: There were no exclusion criteria for the Global Safety Database search. The systematic review included clinical studies of patients with infertility receiving GONAL-f for ovulation induction or ART, with a starting dose within the range included in the SmPC. Case reports/series were excluded from the systematic review as they are included in the Global Safety Database. Data extracted were: number of patients exposed to GONAL-f, number of treatment cycles, incidence of OHSS, incidence of thromboembolism.
Main results and the role of chance: Data are presented separately for the systematic review and Global Safety Database search.

The systematic review identified 45 studies, including 5186 patients exposed to GONAL-f and 5240 treatment-cycles. Overall 272 reported cases of OHSS (reporting rate: 5190 per 100,000 treatment cycles; 5.19%) and 10 cases of severe OHSS (reporting rate: 191 per 100,000 treatment cycles; 0.19%) were identified; no fatal cases. No reports of thromboembolism were identified. The OHSS reporting rate may be high because of enhanced monitoring for adverse events in clinical trials and the characteristics of the patients included, which may not fully reflect clinical practice.

In the Global Safety Database, there were 1110 reported cases of OHSS (using MedDRA preferred term=OHSS) and 80 reported cases of thromboembolic events (MedDRA SMQ = Embolic and thromboembolic events). Overall, there have been an estimated 16,525,975 treatment cycles since 29-Nov-2000. This resulted in reporting rates for OHSS and thromboembolism of 6.7 per 100,000 treatment cycles (0.007%) and 0.48 per 100,000 treatment cycles (0.0005%), respectively. Three fatal cases were recorded; two fatal cases of OHSS and one fatal case of thromboembolism. The OHSS frequency reported from the Safety Database may be lower than the systematic review as it reflects spontaneous reporting.
Limitations, reasons for caution: The cases reported in the Global Safety Database were either submitted voluntarily or obtained from the literature. There may therefore be some duplication of cases between the Global Safety Database and the systematic review. In addition, the number of treatment-cycles in the post-marketing setting was estimated from sales data.
Wider implications of the findings: GONAL-f has been used for more than 18 years, with low rates of OHSS and thromboembolism. This provides reassurance about using GONAL-f for ovarian stimulation.
Trial registration number: not applicable

P-743 Lack of consistency in the execution and reporting of sperm FISH poses potential problems for clinical interpretation and patient counselling

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Study question: To what extent are strategies for sperm aneuploidy testing and the reporting of results standardized?
Summary answer: Comparison of FISH reports from multiple laboratories in nine different countries confirms a remarkable lack of consistency in technical, analytical and reporting methods.
What is known already: Improper segregation of chromosomes during meiosis results in the generation of genetically unbalanced sperm or oocytes. If these gametes participate in fertilization, the resulting embryo will be aneuploid and will consequently be at greatly elevated risk of implantation failure, miscarriage or the birth of a chromosomally abnormal child. Multiple studies have indicated that some infertile men with normal karyotypes,

and in many cases normal semen parameters, display increased sperm aneuploidy.

Fluorescence *in situ* hybridization (FISH) analysis with chromosome-specific DNA probes is capable of evaluating aneuploidy rates in human sperm and has been employed for this purpose by many laboratories.

Study design, size, duration: This is a descriptive study comparing a large number of FISH sperm reports issued by 46 different laboratories, located in nine countries. The patients that provided the sperm samples attended 11 ART clinics (part of a single network) between 2017 and 2018. Sperm-FISH was undertaken for specific couples when physicians wished to obtain a more complete picture of the male factor, using molecular methods to supplement routine semen analysis.

Participants/materials, setting, methods: The reports analysed were generated in laboratories that offer sperm FISH, located in Spain (n=28), France (n=6), Italy (n=6), Argentina (n=1), Norway (n=1), Portugal (n=1), Switzerland (n=1), Australia (n=1) and Turkey (n=1). Information from the reports was extracted, and a data sheet was created to analyse all the variables.

Main results and the role of chance: The data reported varied between laboratories. Reported parameters included disomy rate (50.0% of laboratories), X and Y bearing sperm ratio (41.3%), diploidy (45.6%), nulisomy (29.8%) and monosomy rates (21.7%). The number of sperm analised was reported by 84.8% of laboratories (mean 1914.8, range 200-104887). In 82.6% of the cases five chromosomes were examined (13,18,21,X,Y), 2.1% screened a different set of five (12,18,21,X,Y), 8.7% analysed seven chromosomes (13,15,16,17,18,21,22,X,Y), and 6.5% examined only three chromosomes (18,X,Y).

The test result was clear in 41 (89.1%) reports, with about half (56.1%) yielding an abnormal outcome. Reference values were given in 58.7% of the reports, but the control population was only well-defined in one laboratory (normozoospermic fertile men). In 10.9% of reports, it was stated that the reference values were obtained from publications, whereas no information about the control population was given for the rest. The test was considered normal when the percentage of abnormalities was lower than the control group (89.1%) or when the difference in abnormality rates reached statistical significance (10.9%).

When disomy was assessed, gonosomes showed higher abnormality rates (17.6%) followed by chromosomes 13 (11.8%), 18 (8.8%) and 21 (5.9%). A high percentage of reports (33.3%) indicated an increased diploidy rate.

Limitations, reasons for caution: While we were able to carry out a full assessment of the way clinical data was reported, we were only able to make inferences about certain aspects of the laboratory method (eg, number of sperm analysed, chromosomes tested, etc). We were unable to examine the accuracy of the FISH protocol.

Wider implications of the findings: Variability in FISH methodology and reporting is challenging for physicians who must interpret results to counsel patients. The creation of a Best Practice Guideline by a panel of experts and the participation in pilot schemes for sperm FISH should be encouraged, in order to maximize diagnostic accuracy and consistent reporting.

Trial registration number: Not applicable

P-744 Frozen-thawed transfer increases birth weight of female babies only, but not male babies from our analyses of 2578 neonates in a solo practice

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Study question: What causes the neonates born after frozen-thawed embryo transfers (FET) heavier than fresh embryo transfers (fresh ET). Is there any difference between female and male babies?

Summary answer: The factors to increase birth weight after FET on each gender were different.Female babies by the process of FET, but male babies by other factors.

What is known already: The previous studies have shown birth weight of babies born after FET is heavier than fresh ET. However, there are no reports to investigate what caused babies born after FET are heavier than fresh ET. Birth weight also is correlated with gestational age and maternal age. Therefore, in the present study, the influence of FET was investigated between male and female neonates to determine if there is any causative factor to increase birth weight in each gender with reference to multivariate analyses adjusted for those parameters.

Study design, size, duration: A total of 2,578 full term neonates (37–41 weeks of gestation) born after FET (n=1959) and fresh ET (n=619) from 2008 to 2016 were investigated. All analyses were carried out both in male (n=1276) and female (n=1302) newborns.

Participants/materials, setting, methods: Birth weight (g), gestational age (weeks) and maternal age (years) were compared between singletons born after FET and fresh ET using student's t-test. Multiple regression analyses were performed to reveal the variables relevant to birth weight among gestational age, maternal age, type of IVF (IVF or ICSI) and type of ET (FET or fresh ET).

Main results and the role of chance: Birth weight, gestational age and maternal age of male babies born after fresh ET vs FET were 3089.0 ±386.2 vs 3153.6 ±390.5 ($p<0.05$), 38.8 ±1.1 vs 39.0 ±1.3 ($p<0.01$), 35.4±4.0 vs 35.7 ±3.6 (ns), respectively. In addition, birth weight (g), gestational age (weeks) and maternal age (years) of female babies born after fresh ET and FET were 2971.2 ±363.4vs 3056.1 ±389.8 ($p<0.01$), 39.1 ±1.2vs 39.1 ±1.3 (ns), 35.1±3.7vs 35.8 ±4.1 ($p<0.01$), respectively. Multiple linear regression identified only gestational age ($p<0.01, \beta=0.42$, 95%CI=116.91-148.57) as a parameter associated with male birth weight. Gestational age ($p<0.01$, $\beta=0.41$, 95%CI=105.76-135.53), maternal age ($p<0.05$, $\beta=0.07$, 95%CI=1.45-11.02) and type of ET ($p<0.01$, $\beta=0.08$, 95%CI=29.91-119.08) were independently associated with female birth weight. The present study showed that cryopreservation was not associated with male birth weight.

Limitations, reasons for caution: Several factors such as BMI, smoking and birth order were not included as potential confounders in the present study.

Wider implications of the findings: It was revealed that the relevance of cryopreservation and maternal age to neonatal birth weight is unequivocal in females compared with males. Though the mechanisms in which those parameters influence baby development are still unclear, it is suggested that female embryos are more susceptible to those factors than males.

Trial registration number: not applicable

P-745 Fibrin (Fibrinogen) degradation products (FDPs) in late pregnancy and estradiol levels: a case-control study

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Study question: What are risk factors for elevated FDP, a clinical indicator of the risk of venous thromboembolism and hemorrhage, in late pregnancy among women undergoing ART?

Summary answer: Fresh embryo transfer(ET) and >10 oocytes retrieved were risk factors for elevated FDP levels in late pregnancy among women undergoing ART.

What is known already: Normal pregnancy is linked to changes in hemostasis and fibrinolysis, characterized mainly by a hypercoagulable state. This can help prepare for the hemostatic challenge at delivery and maintain normal placental function, but can also predispose pregnant women to thromboembolism and other vascular complications. ART procedures can further exacerbate the process by increasing the risk of venous thromboembolism and antepartum and postpartum hemorrhage.

Study design, size, duration: This retrospective case-control study included infertile women who underwent ART procedures and gave birth at International Peace Maternity and Child Health Hospital from January through May in 2016.156 pregnant women with elevated FDP levels and 58 pregnant women with normal FDP levels in the third trimester were included. Only infertile women who underwent ART procedures and gave birth at the same hospital were analyzed to avoid bias.

Participants/materials, setting, methods: 5 μg/mL was chosen as the threshold value of serum FDP during late pregnancy. Pregnant women who had a serum FDP level higher and lower than 5 μg/mL in the third trimester were enlisted in the case(n=156)and control group(n=58). Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to estimate the relationships between elevated FDP levels and ART procedures and were adjusted for confounding factors in multivariate logistic regression analyses.

Main results and the role of chance: Serum FDP levels are much higher in late pregnancy among women undergoing ART compared with spontaneous conception(9.78 ± 11.72μg/mL versus 3.09 ± 1.96μg/mL, p<0.001). Among women undergoing ART, fresh embryo transfer (aOR=3.33, 95%CI, 1.57-7.03) and >10 oocytes retrieved (aOR=2.09, 95%CI, 1.10-3.99) were associated with elevated FDP levels in late pregnancy after adjusting for confounders. Serum estradiol(E2) levels in late pregnancy and on the day of hCG trigger were higher in the high-FDP group than in the low-FDP group. A positive correlation was found between serum E2 levels on the day of hCG trigger and FDP levels in late pregnancy for both fresh (r=0.67, p<0.001) and frozen embryo transfer (r=0.53, p<0.001).No correlation was found between serum E2 levels before embryo transfer and FDP levels in late pregnancy for frozen embryo transfer (r=-0.13, p=0.17).

Limitations, reasons for caution: We were unable to design this study as a prospective cohort study to confirm the influence of estrogen on coagulation and fibrinolysis in late pregnancy since we could not make all participants who underwent ART procedures participate in a follow-up with the obstetrician at our hospital during their pregnancy.

Wider implications of the findings: Fresh ET and higher E2 on hCG trigger day can probably increase the risk of venous thromboembolism in late pregnancy and antepartum and postpartum hemorrhage. Deferring fresh ET to frozen ET may be a better option for patients with a high level of E2 on hCG trigger day.

Trial registration number: not applicable

P-746 Perinatal outcomes in 1134 singletons conceived after frozen embryo transfer (FET); true natural cycle (tNC-FET), modified natural cycle (mNC-FET) and artificial cycle (AC-FET), from 2006-2014.

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Study question: Are perinatal outcomes different for singletons born after varying FET stimulation regimens?

Summary answer: Perinatal outcomes were similar after different FET-protocols, however, the tNC-FET group had significantly higher risk of very preterm birth (VPTB) compared with the mNC-FET group.

What is known already: Children conceived after assisted reproductive technology (ART) treatment have increased risk of being born preterm and small for gestational age (SGA) compared with spontaneously conceived (SC) children.

Furthermore, children conceived after FET have increased risk of being born large for gestational age (LGA) compared with children conceived after fresh embryo transfer and SC. Whether this altered risk profile is due to the ART hormone treatment or the embryo freezing/thawing is unclear. Studies suggest that pregnancy and live birth rates are similar after tNC-FET, mNC-FET and AC-FET in ovulating women. Perinatal outcomes for these three FET stimulation regimes are scarcely explored.

Study design, size, duration: A national register-based cohort study including all singletons conceived after FET (n=1,134) with information on the FET stimulation regimes; tNC-FET (n=167), mNC-FET (n=496) and AC-FET (n=471), in Denmark from 2006-2014. Data were extracted from the national ART and medical birth register and were cross-linked based on the maternal and child unique personal identification numbers.

Participants/materials, setting, methods: Perinatal outcomes were compared using regression analyses with tNC-FET as reference group. The multiple regression analyses were adjusted for the following confounders; fertilization method of the frozen embryos (IVF/ICSI), sex, parity (0 or > 1), maternal age (continuous variable), year of childbirth (categorical variable), blastocyst or cleavage stage transfer, single embryo transfer (sET), and for FET treatment group (tNC-FET, mNC-FET or AC-FET).

Main results and the role of chance: Most children were conceived after either mNC-FET (43.7%) or AC-FET (41.5%) treatment, while only 14.7% of the children were conceived after tNC-FET treatment. Crude analyses on background characteristics showed that sET was less used in the tNC-FET treatment group compared with both the mNC-FET and the AC-FET treatment groups. In addition, more tNC-FET children were conceived after ICSI-treatment and more tNC-FET children were born of nullipara women compared with children conceived after mNC-FET.

In the adjusted multiple regression analyses children conceived after tNC-FET had a significant higher risk of VPTB (before week 32) (aOR 3.30 (95% CI 1.06; 10.30)) compared with children conceived after mNC-FET. No altered risks were found when investigating preterm birth (before week 37), mean birth weight, SGA, LGA, stillbirth after week 28, perinatal or neonatal death**.**

Limitations, reasons for caution: Due to the retrospective design residual confounding may occur. Adjusting for BMI, smoking, cryopreservation technique and cause of infertility were not possible.

The group of tNC-FET is small.

Wider implications of the findings: Hormonal treatment (mNC-FET or AC-FET) was not associated with poor perinatal outcomes. This is important knowledges, as the effect of hormone substitution, for women with regular menstrual cycle, during ART treatment is frequently debated. mNC-FET and AC-FET are often logistically preferred, although tNC-FET may be clinically advantageous.

Trial registration number: Not applicable

P-747 Involvement of serum and glucocorticoid-regulated kinase 1 (SGK1) in term and preterm labor

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Study question: Does the glucocorticoid-regulated kinase 1 (SGK1) play a role in term and preterm labor/birth?

Summary answer: SGK1, by mediating glucocorticoid-induced epithelial sodium channel (ENaC) upregulation and its downstream inflammatory signaling, may play a role in term and preterm labor.

What is known already: We have previously shown in humans and mouse models that ENaC and its regulated inflammatory signaling play essential roles in term and preterm labor. The surge in cortisol levels, a major glucocorticoid in humans, is observed in women as approaching towards the end of pregnancy. SGK1 is phosphorylated/activated by glucocorticoids and promotes ENaC expression in many systems including fetal lung tissues.

Study design, size, duration: Women with spontaneous preterm labor (n=14) and term labor (n=15) were recruited and placental tissues were collected for SGK1 expression. A previously published human transcriptome database from maternal–fetus interface tissues/blood collected at onset of term and preterm labor was used for analysis of SGK1 expression and its correlation with ENaC-regulated signaling. In addition, knockdown of SGK1 was performed in human endometrial epithelial cells in vitro and the regulatory role of SGK1 in ENaC was examined.

Participants/materials, setting, methods: The expression of SGK1, phosphorylated SGK1 (pSGK1), ENaC subunits and inflammatory factors was assessed by immunoblotting or real-time PCR. Comparisons were done between term and preterm women. Correlation tests were performed between different genes. siRNA based knockdown technology was used in vitro.

Main results and the role of chance: Uterine SGK1 is activated at labor in mice. Placental SGK1 expression is upregulated in women with spontaneous preterm labor as compared to those with term labor. Analysis of the human transcriptome database reveals significant and positive correlation of SGK1 with ENaCalpha as well as labor-associated pro-inflammatory factors in labored birth groups (both term and preterm), but not the non-labored group. In vitro in human endometrial cells, treatment with cortisol

enhances ENaCalpha and COX-2 expression, which is attenuated by SGK1 knockdown.
Limitations, reasons for caution: The involvement of SGK1 in animal models of preterm labor in vivo awaits further investigation. Possible role(s) of SGK1 on fetus during preterm labor awaits more detailed study.
Wider implications of the findings: These results have showed that SGK1 may play an important role in labor, suggesting it as a potential target for diagnosis and treatment of preterm labor/birth.
Trial registration number: Not applicable.

P-748 Exposure to gonadotropin-releasing hormone agonist during early pregnancy is associated with increased risk of ectopic pregnancies

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Study question: What is the effect on pregnancy and neonatal outcomes of exposure in early pregnancy to a gonadotropin-releasing hormone (GnRH) agonist?
Summary answer: Exposure to a GnRH agonist during early pregnancy may increase the risk of ectopic pregnancies, but does not affect fetal development.
What is known already: Pituitary downregulation with the GnRH agonist is widely used in *in vitro* fertilization (IVF). A potential drawback is that the patient undergoing IVF may conceive naturally during the mid-luteal phase of downregulation with the GnRH agonist. There is concern that exposure to a GnRH agonist in early pregnancy may adversely affect pregnancy outcomes and neonatal outcomes. However, few individual case reports have been published, and with conflicting results.
Study design, size, duration: This retrospective study with a large cohort analyzed data collected from June 2010 to January 2018. The outcomes of women who conceived naturally prior to IVF treatment (NC) and during downregulation with the GnRH agonist were evaluated relative to women who conceived by IVF, intrauterine insemination (IUI), or artificial insemination by donor (AID).
Participants/materials, setting, methods: The final analysis included 116, 140, 325, and 183 women in the NC, IVF, IUI, and AID groups, respectively. Pregnancy outcomes and neonatal outcomes were compared among the four groups.
Main results and the role of chance: Nine of 129 women in the NC group were lost to follow-up, and pregnancies were ongoing in four women at the end of the study period, leaving 116 for this analysis. The live birth rate of the NC group (57.8%) was lower than that of the IUI (70.9%) and AID (74.3%) groups, but similar to the IVF group (71.2%). However, after adjusting for maternal age, body mass index, type of infertility, and history of previous caesarean section, the live birth rates of the NC and AID groups were comparable. In both the unadjusted and adjusted analyses, the rate of ectopic pregnancies of the NC group (17.5%) was significantly higher than that of the IVF (1.4%), IUI (3.7%), or AID (0.6%) groups. In both the unadjusted and adjusted analyses, all the groups were statistically similar with regard to rates of biochemical pregnancy, pregnancy loss, and birth defects, and gender ratio of newborn, gestational age at delivery, mode of delivery, and birth weight.
Limitations, reasons for caution: This retrospective cohort study is vulnerable to all the associated inherent biases. In addition, the development of the newborns was judged by gross external anatomy and parents' perceptions, and not validated by physical or neurological examination.
Wider implications of the findings: These data provide valuable guidance for both physicians and patients when GnRH agonist is used during the luteal phase. Patients should be informed about the small chance of natural conception and the increased risk of ectopic pregnancies. Physicians should be cautious when applying GnRH agonist for luteal support in IVF.
Trial registration number: not applicable

P-749 Obstertric and perinatal outcome of babies born after Elective Fertility Preservation (EFP).

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Study question: Is there a compromise in the obstetric and perinatal outcome of the children born after EFP trough oocytes vitrification?
Summary answer: Obstetric and perinatal outcome following EFP is comparable to the results observed in autologous fresh ICSI cycles.
What is known already: There is evidence about the obstetric and perinatal results obtained after IVF cycles conducted with vitrified oocytes in the overall population. However, although there is increasing demand in EFP there is a lack of information about the results achieved by women who electively had their oocytes vitrified for fertility preservation (FP) and returned to attempt pregnancy.
Study design, size, duration: Retrospective multicentric study, January 2008-September 2018.
Participants/materials, setting, methods: Private university-affiliated IVF center. Obstetric outcome was obtained from 170 pregnant EFP women who delivered 185 children. Control group included 12262 women who achieved pregnancy after fresh autologous ICSI cycles during the same period and delivered 14914 babies. Chi-square and Student's t-test were used when appropriate. Adjusted odds ratios and their 95% confidence intervals were estimated with each method to evaluate the relative odds for vitrified oocytes compared to the reference group of fresh oocytes.
Main results and the role of chance: Mean age was older for EFP patients (38.5 ± 3.8 vs. 34.8 ± 3.5) $p<0.005$. Delivery route was similar (50.3% vs. 50.7% vaginal delivery) (NS). EFP women and controls delivered during week 39.1 ± 2.9 and 38.5 ± 3.8 respectively ($P<0.005$). There were no differences in terms of preterm births (12.1% vs 16.4%), very preterm births (7.1% vs. 4.3%), perinatal mortality (0.5% vs.0.3%), birth defects (0.5% vs. 0.6%), admission to NICU (5.4% vs 3.3%) and sex of the baby (47.6% vs 49.8% for female neonates) (NS). Birth weight was higher in EFP group (3046.7 ± 722.9 g vs. 2865.9 ± 663.7 g) ($p<0.05$). Low birth weight (LBW:<2500 g) was higher in controls (14.8% vs. 26.3%) $p<0.05$) and very low birth weight (VLBW:1500 g) was comparable (5.4% vs 3.0%) (NS). The impact of EFP on the following variables was rule out as shown by the OR (95%CI): LBW=0.854 (0.337-1.091);VLBW: 1.849 (0.337-1.091); Preterm birth: 0.703 (0.449-1.01); very preterm birth: 1.701 (0.963-3.008); perinatal mortality: 1.795 (0.246-13.089); Birth defects: 0.773 (0.107-5.573); Route for delivery; 1.017 (0.756-1.368) and sex of the baby:1.124 (0.837-1.510);(NS).
Limitations, reasons for caution: Although this study compiles a large series, sample size is still small in EFP group.
Wider implications of the findings: This study suggests that there is no increased risk of adverse obstetric and perinatal outcome in children conceived from vitrified oocytes in elective fertility preservation, thus confirming the safety of the approach. Further analysis as the sample size continues to grow will be mandatory in order confirm our findings.
Trial registration number: N/A

P-750 Reassuring results on the chance of a live born child in women with multiple sclerosis receiving assisted reproduction

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Study question: What is the chance of a live birth after assisted reproductive technology (ART) treatment in women with multiple sclerosis (MS)?
Summary answer: Women with MS did not have a decreased chance of a live born child per embryo transfer, compared to women without MS undergoing ART.
What is known already: The results in women with other autoimmune diseases as ulcerative colitis, Crohn's disease and rheumatoid arthritis, have suggested a reduced chance of live birth after ART treatment. Whether ART treatment affects the disease course in MS is controversial, but women with

MS are more likely to refrain from pregnancy and infertility and MS may also coincide. No previous studies have examined the efficacy of ART in women with MS, in terms of live birth. Therefore, we examined the chance of live birth after embryo transfer in women with MS, compared to women without MS.

Study design, size, duration: A nationwide register-based cohort study comprising all embryo transfers in Denmark during 24 years (1994 to 2017), including follow-up on pregnancies until November 2018.

Participants/materials, setting, methods: Women undergoing ART treatment included 2,297 embryo transfers in 826 women with MS (the exposed cohort), and 203,862 embryo transfers in 68,105 women without MS (the unexposed cohort). We used multilevel logistic regression analysis to compute the crude and the adjusted risk estimates with 95% confidence intervals (CI) adjusting for a number of important confounders. In sub-analysis we examined a potential impact of the use of corticosteroids prior to embryo transfer.

Main results and the role of chance: There were 508 (22.1%) live births in the exposed cohort and 49,185 (24.1%) live births in the unexposed cohort. The adjusted odds ratio (aOR) for a live born child in women with MS, relative to women without MS undergoing ART, was close to unity (aOR = 0.92 (95% CI 0.82-1.03)). Use of corticosteroids within 3 month prior to embryo transfer did not have an impact on the chance of a live birth. This is the first study to examine the impact of maternal MS on the chance of a live born child after ART treatment. On the whole, the study design prevents selection- and information bias, and we obtain relative risk estimates with good statistical precision. Our results are novel, and it is reassuring that the chance of livebirth after ART in women with MS, are equal to women without this chronic disease.

Limitations, reasons for caution: Although this study includes all relevant patients, procedures and diagnoses from the national Danish registries, we did not have access to information on disease severity, and use of MS-modifying medication in relation to ART treatment.

Wider implications of the findings: This is the first study to examine the impact of MS on efficacy of ART, and our results must be confirmed in other settings. The chance of a live birth is similar between women with MS and without MS. These are important information for women with MS and their clinicians.

Trial registration number: The study is notified at the Danish Data Protection Agency under the current joint notification of the Region of Southern Denmark (ref.no. 2012-58-0018).

P-751 A randomized controlled trial comparing the number of mature oocytes following automated-3D versus manual-2D ultrasound follicle assessment, in high-risk for OHSS patients triggered with GnRH-agonist

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Study question: Is the number of mature oocytes(MII) different following automated-3D versus manual-2D ultrasound follicle assessment, in high-risk for OHSS patients undergoing ICSI, triggered with GnRH agonist?

Summary answer: A significant, but clinically irrelevant, increase in MII oocytes was observed following automated-3D versus manual-2D follicle assessment in high-risk for OHSS patients triggered with GnRH-agonist.

What is known already: Accurate assessment of size and number of follicles during ovarian stimulation is important for determining the day of triggering final oocyte maturation and oocyte retrieval, in order to retrieve an optimal number of mature oocytes. Previous studies have shown that timing final oocyte maturation using 3-dimensional SonoAVC (automated-3D) and conventional 2-dimensional (manual-2D) ultrasound assessment is associated with similar numbers of mature oocytes in normal responders. However, no data are available on the comparative efficiency of manual-2D and automated-3D ultrasound for determining the day of triggering final oocyte maturation with GnRH-agonist in high responders at risk of OHSS.

Study design, size, duration: Random patient allocation to automated-3D (SonoAVC;GE Medical Systems) or manual-2D ultrasound assessment was performed at initiation of stimulation by a nurse using a computer-generated randomization list in a single center between 8/2018 and 1/2019. Primary outcome was the number of MII oocytes retrieved. A sample size of 26 patients in each group was required to yield 80% power to detect a difference of 4 MII oocytes, assuming a mean number of COCs retrieved=18 and SD=5.

Participants/materials, setting, methods: This study included women ≤40 years with AMH>3 ng/ml at high risk for OHSS treated with GnRH-antagonists and recombinant-FSH. When three follicles≥17mm were present based on manual-2D or automated-3D ultrasound assessment, 0.2mg triptorelin was administered to trigger final oocyte maturation. Oocytes were fertilised with ICSI and all blastocysts were cryopreserved. Clinicians deciding when to trigger, embryologists and patients were blinded to the method of follicular assessment. Values are expressed as median (interquartile range).

Main results and the role of chance: Age, BMI, AMH and baseline FSH, LH, oestradiol and progesterone levels were similar in the two groups.Compared to manual-2D, automated-3D assessment was associated with a similar duration of stimulation [11 (95%CI: 11.0-12.0) vs 12 (95%CI: 11.0-12.0, respectively] and total dose of recFSH [1787 (1500-1988) vs 1800 (1736-2007), respectively]. Serum hormone levels in the 2D and 3D group on the day of trigger were similar for oestradiol [4567 (3269-4928) vs 3718 (3014-5512)], progesterone [1.1 (0.8-1.6) vs 1.3 (0.94-1.62)] and LH [1.3 (1.2-2.7) vs 1.7 (0.83-2.7)] respectively.

However, compared to manual-2D , automated-3D assessment was associated with significantly more follicles ≥11mm on the day of trigger [22 (21.0-24.0) vs 26.5 (20.9-31.5), p=0.037, respectively], more cumulus-oocyte complexes (COCs) retrieved [18 (7) vs 18.5 (5), p=0.041, respectively), more mature oocytes [14.0 (10.0-16.0) vs 15.0 (14.0-17.0), p=0.016, respectively] and more 2PN oocytes [9.5 (7.4-12.1) vs 12.0 (11.0-14.5), p=0.010, respectively].

Limitations, reasons for caution: Although significantly more COCs and mature oocytes were retrieved and more 2pn oocytes were available after fertilization in the automated-3D vs the manual-2D group, the detected differences appear to be clinically irrelevant. This is a small albeit powered RCT, necessitating studies with a larger patient population.

Wider implications of the findings: The decision to trigger using automated 3D follicle measurement was associated with significantly more mature oocytes compared with conventional 2D ultrasound, in high-risk for OHSS patients triggered with GnRH-agonist. However, these differences were small and potentially clinically irrelevant. The choice of method depends on equipment availability in each clinic.

Trial registration number: NCT03610009

P-752 Modeling of Low Level and Common Embryotoxic Volatile Organic Compounds (VOCs)–Their Relationship to Concentrations Within the Culture Media, Human Embryo, and Mechanisms of Cytotoxicity

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Study question: To better understand mechanisms of embryotoxicity, can airborne concentrations of VOCs be modeled as concentrations within the supporting culture media and embryo?

Summary answer: Henry's Law and octanol-water partitioning coefficients define the relationship between concentrations of specific VOCs in the gaseous and aqueous phase, and within the embryo.

What is known already: VOCs, organic compounds with a high vapor pressure and thus, a high level of volatility, are a common airborne con-

stituent of outside source air, recirculated laboratory air, HVAC components, tissue cultureware, and IVF procedures. VOCs are ubiquitous and unique in their polarity, molecular weight and biochemical structure. VOCs are embryotoxic and play a critical role in preimplantation toxicology and epigenetic processes. Research has clearly demonstrated that a large range of VOCs exist within the IVF laboratory and exert a negative impact on zygote to blastocyst conversion rates, implantation rates, and clinical pregnancy rates in IVF laboratories.

Study design, size, duration: Henry's Law was used to model VOC mass transfer from the air to the water/culture phase. This model uses the air-water partitioning coefficient and the definition that the ratio between the liquid concentration and the air phase concentration is equivalent to a dimensionless constant. The octanol-water partitioning coefficient was used to correlate the mass transfer from the water/culture phase to the embryo using the ratio between the octanol concentration and the water concentration.

Participants/materials, setting, methods: The 34 most common VOCs in IVF laboratories were studied. Henry's Law and octanol-water calculations determined the relationship between concentrations of each VOC in the culture media, embryo, and time to thermodynamic equilibrium. The VOCs in the air phase were assumed to be dilute and at three constant concentrations (10ppb, 100ppb, 1ppm). The concentration in the embryo was compared to the median lethal dose (LD50), the dose at which 50% of living cells survive.

Main results and the role of chance: Extensive testing demonstrated that total VOC (TVOC) levels range between 100ppb and 1ppm in IVF laboratories. The concentration of each VOC within the embryo at 10ppb in air was compared to LD50 values. Many of the concentrations within the embryos were higher than documented LD50 values indicating these VOCs to be cytotoxic. Research has demonstrated an average concentration of 11.3 $\mu g/m^3$ of formaldehyde as measured in numerous IVF laboratories. The concomitant formaldehyde concentration within the embryo was 19.36 times higher than the LD50 value. Low level airborne VOCs increased in concentration as they partitioned from the air to the cell culture medium, and ultimately, into the embryo. Research has demonstrated that VOCs can exert an embryotoxic effect by disrupting the cellular membrane, increasing intracellular calcium, impairing mitochondrial function, decreasing spindle formation/chromosome alignment, interfering with DNA/RNA replication, and increasing DNA oxidative damage and fragmentation. This model correlates the measured concentration of various airborne VOCs to the final concentration within the embryo. The presence of low level TVOCs is concomitant with reduced cellular metrics and clinical outcomes. This information, in conjunction with LD50 values, can be used to understand, and remove VOCs as a cytotoxic variable in the cell culture process.

Limitations, reasons for caution: The specific density of the human embryo is not clearly known and an approximation for standard cell density was used. Variations in cell density will impact the calculated concentration of each VOC in the culture media and embryo.

Wider implications of the findings: These findings are applicable to all cultured cellular processes utilizing living cells. A deeper understanding of the cytotoxic mechanisms of VOCs in relationship to the living cell culture will provide a paradigm shift in the definition of the optimal *in vitro* culture environment for the living cell.

Trial registration number: Not Applicable

P-753 The influence of advanced paternal age on offsprings' intelligence following assisted reproductive technology

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Study question: Is advanced paternal age a cognitive risk for the offspring born after assited reproductive technology(ART)?

Summary answer: Our study suggests advanced paternal age may result in higher offspring Verbal intelligence quotient(IQ) and Full IQ scores in ART population

What is known already: Recent investigations suggest that advanced paternal age has many deleterious effects on offsprings' developmental outcomes ,especially cognitive, emotional and neuropsychiatric outcome,but there is debate about the relationship between fathers' age and children's intelligence.Few studies have examined the impact of paternal age on offsprings' intellectual development,especially children born after assisted reproductive technology.

Study design, size, duration: The study was divided into four groups based on the age of the fathers:≥40 years;35∼39 years;30∼34 years;25∼29 years(control), The follow-up assessments were taken place between 2009.09 and 2017.07 at ART Offspring Health Monitoring Center 4-6 years old children who born in 2003-2012 was recurited in our Reproductive Center to have a intelligence test-Chinese Version of the Wechsler Intelligence Scale for Children (C-WISC)

Participants/materials, setting, methods: Participants who underwent IVF/ICSI and achieved live births included.Exclusion criterias for parents:

1)sperm and oocyte donation donation,PGD/PGS;

2)mutiple pregnancy,one member of a couple smoked or abused alcohol before pregnancy;

3) serious diseases;

4) data missed in database.

After exclusion,a total of 1934 parents agreed to participate and 1914 singletons completed the intelligence tests(C-WISC).

Adjusted linear regression or logistic regression analyses were used to evluate cognition after control for certain confounders.

Main results and the role of chance: After controlling for multiple possible confounding factors, including the other parent's age,parental education, pregnancy complications,sex ,birth weight and birth complications,singletons' Verbal IQ and Full IQ whose fathers' age ≥40 ,35∼39,30∼34 years are higher than paternal ages 25∼29 years.There are no difference among the four groups in Performance IQ.Our study suggests advanced paternal age may result in higher offspring Verbal IQ and Full IQ scores in IVF/ICSI population.This result is similar to a previous study which suggests advancing paternal age is associated with higher GI in offspring.Older father age may not only bring some deleterious devlopment outcome,but it also has some advantageous developmental outcomes.

Limitations, reasons for caution: This study also has some limitations,the parents' income is not a factor in our confounding factors,because of it is a private information for Chinese pepole.Family economic status always show correlation with childrens' education levels.Therefore ,we considered area as a confounding factor,to some extent ,it represents economic level.

Wider implications of the findings: Most investigations suggest that advanced paternal age has deleterious effect on offsprings' intelligence.Our result show disagreement with most researches.It could be due to different population and participant numbers in these studies.

Trial registration number: Z15H040001

P-754 Conversion of in vitro fertilization cycles to intrauterine inseminations in patients with a poor ovarian response: risk of multiple pregnancies.

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Study question: What is the risk of multiple pregnancies following conversion of in vitro fertilization cycles to intrauterine insemination when a poor ovarian response (POR) is diagnosed during controlled ovarian stimulation (COS)?

Summary answer: In in vitro fertilization (IVF) cycles converted to intrauterine inseminations (IUI) for poor response, the risk of multiple pregnancy (MP) is acceptable (12%).

What is known already: There are no studies that have estimated the risks of MP in IVF cycles converted to IUI for POR. Conversion to IUI can be offered to women with one to four mature follicles. Two factors usually lead physicians to favor IVF over IUI: 1) the hypothetic lower success rates with IUI compared to IVF, 2) the hypothetic risk of MP with IUI in cases with more than two mature follicles. We aimed to assess the risks of MP following conversion to IUI in

patients with POR and to evaluate the MP rates according to the number of mature follicles.

Study design, size, duration: We undertook a retrospective study in our teaching hospital from January 2007 to December 2017. Overall,205 IVF cycles that were converted to IUI in 128 patients were analyzed.

Participants/materials, setting, methods: The inclusion criteria were: (1) age ≥18 and <43 years; (2) IVF cycles, with and without Intracytoplasmic sperm injection, following COS using an agonist (long and short) or an antagonist protocol, with only ≤ 4 mature follicles (≥14 mm) on ovulation trigger day; (3) Serum estradiol level <1000 pg/mL on ovulation trigger day.

Main results and the role of chance: Mean age was 34.1 ± 4.6 years, mean antral follicle count was 11 ± 5.3 and meanAMH was 1.8 ± 2.9 ng/L. The main causes of infertility were unexplained (41%) (84/205) and premature ovarian insufficiency (35%) (72/105). The mean daily dose of gonadotropins was 321.1 ± 89.5 IU. On the day of ovulation triggering, the mean number of follicles ≥14 mm was 2.5 ± 1.3, and the mean level of serum estradiol was 465.9 ± 247.1 pg/mL. Of all the cycles converted to IUI, 53 (26%) had one mature follicle on trigger day, 56 (27%) had 2, 56 (27%) had 3, and 40 (20%) had 4. The live birth rate was 7.3% (15/205) and the miscarriage rate was 40% (10/25). There were 3 twin pregnancies, but no higher order pregnancies; the MP rate was 12% (3/25). One twin pregnancy occurred in a cycle with 1 mature follicle on trigger day, one in a cycle with 2 mature follicles and one in a cycle with 4 mature follicles. All twin pregnancies were bichorial-biamniotic. Finally, there was no significant difference in the MP rate between patients with 1-2 mature follicles and patients with 3-4 mature follicles (15.4% vs 8.3%, p=0.99, respectively).

Limitations, reasons for caution: The main limitation is the retrospective design. Moreover, despite including a considerable number of cycles, the number of live births was not high enough to allow us to compare outcomes between different subgroups and draw more significant conclusions, mostly because of the poor overall prognosis of patients with POR.

Wider implications of the findings: In IVF cycles converted to IUI,the risk of MP is acceptable, even with 3 or 4 follicles ≥ 14 mm on trigger day. Conversion to IUI can be offered to patients when there are ≤4 mature follicles on trigger day with a serum estradiol level < 1000 pg/ml.

Trial registration number: Not applicable

P-755 Factors affecting the quality of successful implantation of thawed cryopreserved embryos: the Welsh experience

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Study question: What are the factors that affect the success rates of thawed frozen embryo implantation?

Summary answer: The greatest implantation success was seen in ages 30-34, 2-embryo transfer (p≤0.05), day-5 thaw, 18-21 days of HRT stimulation, male infertility (p≤0.05), ICSI and vitrification.

What is known already: Although frozen embryo transfer (FET) appears promising, the success rates are not idyllic with only 20-25% of embryos transferred resulting in a birth. Some predictors of success include maternal age, infertility duration, tubal infertility, damage during the freeze-thaw process, embryo quality and quantity, and maternal body mass index (BMI).

Study design, size, duration: A retrospective analysis of patients who underwent FET (after in-vitro fertilisation [IVF] or intra-cytoplasmic sperm injection [ICSI]) at a University Hospital, between January 2007 and December 2017, was carried out.

Participants/materials, setting, methods: Records from 1325 patients were reviewed for factors affecting implantation. Primary outcomes analysed were positive pregnancy tests (PPT) and clinical pregnancy rate (CPR) per embryo transfer cycle and embryo transferred. The variables scrutinised were maternal age and BMI, cycle number, number of embryos transferred, embryo stage at thaw, endometrial thickness, HRT duration, HRT versus natural cycle, infertility type and cause, IVF, ICSI and slow freezing or vitrification. Logistic regression was performed using SPSS Statistics.

Main results and the role of chance: 181 (13.6%) patients were excluded due to failure to thaw (95), incomplete data (53) and other reasons (33). The CPR per embryo transferred and per embryo transfer cycle, were 14.38% and 21.69%, respectively. The biochemical pregnancy rate was 8.0%. The PPT rate and CPR for a 2-embryo transfer (odds ratio [OR] 1.850 and 1.818, respectively) and the CPR for male factor infertility (OR 1.87) were statistically significant (p≤0.05).

The maternal age ranges with the highest (24.69%) and lowest (17.65%) proportion of CPR were 30-34, and greater than 40, respectively. The OR for age was 1.004 for CPR and 1.009 for PPT. One cycle was inferior to multiple cycles (ORs 0.802 for CP and 0.891 for PPT). Thawing on day 5 produced the highest CPR (OR 1.75). HRT cycles yielded a higher PPT rate and CPR than natural cycles (38.50% vs 30.43% [OR 0.957], and 23.34% vs. 19.57% [OR 0.863], respectively). 18-21 days of HRT stimulation produced the highest PPT rate (45.7%) and CPR (27.3%). The optimum endometrial thickness for implantation was 8.1-13mm (ORs 0.979 for PPT and 1.004 for CPR). ICSI was superior to IVF (ORs 1.38 for CPR and 1.05 for PPT).

Limitations, reasons for caution: This study was retrospective, and, thus, the relationships found could be associative: confounding bias may have affected the results, such as lifestyle factors. Also, there could have been a degree of selection bias as incomplete patient records were required to be removed from the analysis.

Wider implications of the findings: Both PPT and CPR reflected other studies. Young age, 2-embryo transfer, and day-5 thaw increased pregnancy rates. Alleviating the debate surrounding HRT, stimulation for 18-21 days produced the greatest success. Contrary to previous results, male infertility had higher implantation rates, possibly due to increased use of, and improved, ICSI technique.

Trial registration number: Not applicable since this was a retrospective audit.

P-756 Obstetric and neonatal outcome of assisted reproductive technology (ART) in patients with polycystic ovaries (PCO): in vitro maturation (IVM) of oocytes versus controlled ovarian stimulation

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Study question: Does in vitro maturation (IVM) of immature oocytes in women with polycystic ovaries (PCO) have an impact on obstetric and neonatal outcome compared to controlled ovarian stimulation (COS)?

Summary answer: Obstetric and neonatal outcomes after IVM appear to be similar to those after COS.

What is known already: Women with polycystic ovary syndrome (PCOS) have an increased risk of adverse pregnancy outcomes and congenital malformations in their offspring. For patients with PCOS who require in-vitro fertilization (IVF), IVM of germinal vesicle (GV)-stage oocytes retrieved from antral follicles has been adopted as a mild approach assisted reproductive technology (ART), with improved pregnancy rates over the last two decades. Although reports of obstetrical and neonatal outcome after IVM have been reassuring, the limited sample size in previous studies precludes firm conclusions and warrants further study.

Study design, size, duration: This is a retrospective observational study analysing obstetric and neonatal data from 1574 pregnancies in patients with PCO who conceived following a cycle of IVM or conventional ovarian stimulation (COS) between January 2010 and December 2016 in a tertiary reproductive centre. In total, 857 pregnancies with a gestational age beyond 20 weeks were included. A phenotypic approach was used for the diagnosis of PCOS. Patients requiring pre-implantation genetic testing (PGT) or testicular biopsy were excluded.

Participants/materials, setting, methods: Outcomes of 209 singleton pregnancies beyond 20 weeks after IVM were compared with those of 648 singleton pregnancies after COS. Pregnancies in the IVM group were obtained after minimal ovarian stimulation and no hCG trigger before oocyte retrieval. Outcomes included obstetric complications and neonatal health parameters, in particular birthweight, prematurity, small/large-for-gestational age, perinatal death and major/minor malformation rates. Data were analysed by multiple linear and logistic regression, adjusted for relevant treatment variables and maternal characteristics.

Main results and the role of chance: The IVM and the COS groups differed significantly ($p<0.001$) for maternal characteristics, including circulating AMH levels and PCOM/PCOS phenotype distribution, with an overweight of PCOS phenotype A in the IVM group and a predominance of PCOM in the COS group.

With regard to obstetric complications in singleton pregnancies, in the unadjusted analysis, mothers to infants in the IVM group had more often hypertensive disorders of pregnancy (HDP) (33/193 (17.1%) vs. 44/568 (7.7%)) than mothers to infants in the COS group. Singletons born after IVM showed a tendency toward higher standard deviation score (SDS) (0.54 ± 0.98 vs. 0.39 ± 1.02, $p=0.09$) and a significantly higher preterm (32–36.9 weeks; 24/191(13.3%) vs. 37/562 (6.8%)) and early preterm birth rate (<32 weeks; 5/191 (2.8%) and 15/562 (2.7%)), compared with peers born after COS ($p=0.02$). The total malformation rate was comparable between IVM (3.5%) and COS (3.9%), $p=0.85$. Multivariate linear regression analysis accounting for relevant confounders demonstrated that parity was the only independent predictive factor ($p<0.001$) for birthweight SDS. Multivariate logistic regression analysis showed that BMI and parity were significantly correlated with the incidence of HDP. The risk of HDP was also increased in patients with PCOS phenotype A following IVM.

Limitations, reasons for caution: The study is limited by its retrospective nature and loss to follow up of a subset of children with no information regarding congenital malformations. Furthermore, data from consecutive pregnancies in the same patient were not excluded from the analysis.

Wider implications of the findings: This study provides further evidence that IVM of oocytes derived from small antral follicles does not adversely affect the neonatal health of the offspring of patients with PCOS compared to COS. The observed increased risk of HDP in patients with PCOS phenotype A following IVM treatment requires further scrutiny.

Trial registration number: The study was approved by the institutional review board of Universitair Ziekenhuis Brussel on March 21st 2018 (B.U.N. 143201835600).

P-757 A prospective RCT sibling study to test the efficacy and safety of the calcium ionophore GM508 in two different indication groups – preliminary results.

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Study question: Does application of artificial oocyte activation (AOA) by calcium ionophore impact on neonatal outcome in IVF patients?

Summary answer: Preliminary results show no evidence for any negative impact on neonatal health due to AOA in this two-armed sibling study.

What is known already: AOA is widely applied in IVF, mainly to overcome failed or impaired fertilization. However, besides globozoospermia, there is no proven indication for AOA. Hence, large prospective studies are needed to prove efficiency and validated indications. As AOA does not result in physiological Ca(2+) oscillations, the question of neonatal health after AOA-therapy is of utmost importance. To date, there are only few studies reporting neonatal outcome after AOA. Additionally, different chemicals inducing Ca(2+) waves by different mechanisms were applied in these studies.

Study design, size, duration: A prospective 2-arm randomized sibling study was conducted in 2016. Inclusion criteria were female age < 44 y; $\geq$ 2MII oocytes and an indication for AOA (male factor infertility or previous IVF-cycles with reduced fertilization/blastocyst rate). Embryo culture was performed with one half of the oocytes undergoing AOA and the other half serving as non-treatment group. Preliminary results of neonatal outcome were evaluated by analyzing data of 43 returned questionnaires

Participants/materials, setting, methods: In all fresh or frozen transfers, the morphologically best-rated blastocyst(s) were chosen for transfer, blinded for AOA treatment. Thereby 18 patients received a transfer with AOA blastocyst(s), 11 with non-AOA blastocyst(s) and 14 two blastocysts one from each group. IVF outcome and clinical outcome were analyzed. Pregnancy course and neonatal health were evaluated by questionnaire encompassing 20 questions including patient's medical history, lifestyle factors, pregnancy course, social environment as well as delivery and obstetric outcome.

Main results and the role of chance: Mean number of transferred blastocysts/transfer in the AOA group was 1.4, in the non-AOA group 1.6 and in the mixed group 2.0. Mean female and male age at oocyte pick-up was: 35.1 and 41.0 years; 35.9 and 41.4 years; 36.7 and 38.6 years, respectively. In the AOA group 25 children were born (11 singletons and 7 twins) with two preterm deliveries in the twin group (28 and 32 gestational week). One twin pair was monozygotic. All children were healthy and experienced normal development during the first months. Syndactyly was reported in one girl as single minor malformation. In the non-AOA group 12 healthy children were born on term (10 singletons and 1 twin pair). In the mixed transfer group 16 healthy children were born (15 singletons and on triplet after double blastocyst transfer). One singleton and the triplet were born preterm. One boy was reported with syndactyly in this group. All children experienced normal development during the first months.

Limitations, reasons for caution: AOA was applied with GM508 Cultactive according to the manufacture's protocol, thus results might be not applicable for other AOA-protocols. Further limitations are the preliminary dataset of this ongoing study.

Wider implications of the findings: Our results encourage that the application of AOA by this protocol does not negatively impact on the obstetric outcome. Thereby, application might be enlarged in patients with risk for reduced fertilization rates due to severe male infertility or other factors.

Trial registration number: DRKS00010456 registered in 2016. This study has received ethical approval by the Ethical Committee of Vorarlberg (Austria)

P-758 Germline transmission of donor mitochondrial DNA in rhesus macaques produced via mitochondrial replacement therapy

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Study question: To define health and fertility of adult trans-mitochondrial monkeys produced by mitochondrial replacement therapy (MRT).

Summary answer: Both male and female MRT macaques are fertile and donor mitochondrial DNA (mtDNA) was passed on from the female MRT monkey to offspring.

What is known already: MRT is a form of germline gene therapy developed to prevent transmission of mtDNA mutations from mothers to children. In a previous study, mature oocytes were collected from female rhesus macaques of diverse genetic backgrounds. The cytoplasm and mtDNA complement of one oocyte was removed and replaced with mtDNA from a different background. MRT oocytes were fertilized and implanted into surrogate females. Trans-mitochondrial monkey offspring have now reached sexual maturity and are being studied.

Study design, size, duration: Four male MRT monkeys were produced in our lab in 2009, followed by an MRT female in 2012. Fertility in adult males was analyzed through sperm collection and analysis as well as natural breeding. Fertility and germline transmission of donor mtDNA were studied in the female MRT monkey and mature oocytes and live offspring were produced by natural breeding.

Participants/materials, setting, methods: This study was performed at the Oregon National Primate Research Center with rhesus macaques used as animal models. All studies were approved on an IACUC protocol. Analysis

started when monkeys were about 5-6 years old, the time of sexual maturity. All births were the result of natural breeding through the time-mated breeding program. MtDNA was analyzed with the amplification- refractory mutation system quantitative PCR (ARMS qPCR) and MiSeq.

Main results and the role of chance: Sperm from MRT monkeys was analyzed for volume, count, motility and morphology and were not distinguishable from controls. MRT males were paired naturally with females and resulted in healthy offspring.

57 mature oocytes were collected from the MRT female and 56 showed entirely donor mtDNA while one showed 1.6% of maternal mtDNA. The female monkey was paired with a male from the time-mated breeding program and also produced a healthy infant. All analyzed peripheral tissues from the infant showed exclusively donor mtDNA haplotype demonstrating successful germline transmission.**Limitations, reasons for caution:**

The number of animals used in this study is small including a sole female.

Wider implications of the findings: MRT clinical trials are ongoing in some countries. Our results on adult MRT monkeys and their offspring provide critical safety assurance. Results show that MRT does not affect fertility in males or females and that donor mitochondria will be passed on from mother to offspring.

Trial registration number: not applicable

P-759 Human and communication errors are the main cause of non-conformances in an IVF laboratory

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Study question: How frequent non-conformances are in the IVF laboratory and why do they occur?

Summary answer: Non-conformances in the IVF laboratory are not frequent and associated mainly to human and communication errors.

What is known already: Notwithstanding the complex procedures normally performed in an ART laboratory, and the emphasis given to quality control measures, reports and analysis of non-conformances in the IVF setting are scant. Likewise it has been noticed in other fields of medicine and human activities such as aviation, the few reports in ART indicate that the majority of the errors are of human and communication origin.

Study design, size, duration: We reviewed all non-conformances reported in our database between January 2012 and December 2018. The 29,357 procedures performed in this period included egg retrievals, fresh and frozen embryo transfers, vitrification, pre-implantation genetic testing and andrology practices. Non-conformances were divided according to the literature into four impact grades (minimal, significant, moderate and major) and eight categories (communication, documents/records, external, facilities, equipment/supplies, human, patient problem/complaint and quality control (QC)/statistical).

Participants/materials, setting, methods: Retrospective study of non-conformances registered in our IVF laboratory database.

Main results and the role of chance: A total of 64 non-conformances were documented during the study period (99.78 % with no non-conformances in all categories). When we analyzed them according to their impact, 50.00 % (32/64) were minimal, 18.75 % (12/64) moderate, 31.25 % (20/64) significant, with no major errors reported. We observed that 43.75 % (28/64) of the non-conformances were due to human errors, 29.69 % (19/64) to communication problems, 15.63 % (10/64) to failures in equipment or supplies, 7.81 % (5/64) to patient problems or complaints and 3.13 % (2/64) external. No facilities, documents/records or QC/statistical non-conformances were reported. The embryology laboratory was the site of the 53.13 % (34/64) of the errors, while 31.25% (20/64) of them occur in the cryopreservation/storage unit, 12.50 % (8/64) in the andrology sector, 1.56 % (1/64) in the PGT laboratory and 1.56 % (1/64) were external. If we consider only moderate and significant errors, non-conformances rates were 1 per 917 procedures (0.11 %) and 1 per 399 cycles (egg retrievals and frozen embryo transfers) (0.25 %).

Limitations, reasons for caution: It would perhaps be unwise to directly extrapolate or compare our results to other laboratories due to different settings, culture systems and classifications in use. With the improvement of our QC programme, the non-conformances documentation has been more precise over the years.

Wider implications of the findings: Our results provide more information about the origin of non-conformances. The coincidence of our data with other published reports suggests that our results could be useful for other embryologists and clinicians to anticipate and minimize risks.

Trial registration number: Not applicable.

P-760 Defining which serum Progesterone cut off levels on day of human chorionic gonadotropin (hCG) trigger can affect implantation in IVF/ICSI: evaluating the quality of service.

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Study question: What is the level of serum Progesterone on the day of hCG trigger that can affect the clinical pregnancy rate (CPR) in IVF/ICSI treatments?

Summary answer: Serum Progesterone levels ≥5nm/L on day of hCG trigger reduces CPR. Frozen embryo transfers (FET) in a subsequent cycle gives better outcomes.

What is known already: Progesterone is a key hormone for IVF to succeed, it changes the endometrium to become receptive, facilitating embryo implantation. A spike in Progesterone levels on the day of hCG trigger in a controlled ovarian hyper stimulated cycle is known as premature luteinisation (PL). Although the pathogenesis is unclear, its detrimental effect on pregnancy has been documented in many papers. However, it is still unclear which cut-off Progesterone level is negatively affecting implantation. It has been suggested that each Unit should audit its practice, generating a reliable cut off level for freezing which could be applied to its patient's population.

Study design, size, duration: Retrospective quantitative data collection was done between September 2017 to April 2018. All patients undergoing IVF or ICSI cycles at Wales Fertility Institute (WFI) had their serum Progesterone levels measured, on the day of the hCG trigger. If the Progesterone levels were >7nm/L, patients were offered embryo freezing and subsequent transfer in a frozen embryo transfer cycle. All other patients with Progesterone levels ≤7nm/L had a fresh embryo transfer.

Participants/materials, setting, methods: A total of 527 patients had their serum Progesterone levels measured on the day of hCG trigger. 133 patients were excluded due to missing data and needing embryo freezing for other reasons such as ovarian hyper stimulation syndrome. Data from 394 patients was therefore used for service evaluation at WFI. Progesterone levels were split into ranges 1-4 nm/L, 5 -7nm/L and >7nm/L. Data was analysed using Chi-squared tests and logistic regression adjusted for confounders.

Main results and the role of chance: Initial analysis showed 37% CPR in the Progesterone 1- 4nm/L and 19% in the 5-7nm/L. This showed that there is a significant drop in the CPR when the Progesterone levels are greater than 4nm/L. CPR was 40% in the >7nm/L group post FET, suggesting that it is beneficial to freeze embryos when Progesterone levels are >7nm/L. There may also be a benefit from freezing embryos with Progesterone levels ≥5nm/L. However, there was weak evidence of an association with CPR due to the small size of the 5-7nm/L group ($p=0.2$). Logistic regression was used to identify women at a higher risk of having high Progesterone levels on the day of the hCG trigger. Three potential groups were identified: women with >15 follicles on the day of the hCG trigger (*OR (odds ratio)= 2.2, 95% CI (confidence interval): 1.0-5.0, p=0.064*), > 11 follicles > 15mm in size on the day of the hCG trigger (*OR=3.6, 95% CI: 1.6-8.2, p=0.022*) and women who had serum oestradiol (E2) levels >10,000pg/mL on the day of the hCG trigger (*OR=8.5; 95% CI: 2.6-27.9, p<0.001*). These are seemingly associated with high serum Progesterone levels on the day of the hCG trigger, the latter two being statistically significant*(p<0.05)*.

Limitations, reasons for caution: The main limitation was a relatively small sample size and some missing data on some variables. Furthermore, the potential risk factors identified are proxy variables for each other which means they are likely to be co-linear. To overcome this, three separate logistic regression models were used in the analysis.

Wider implications of the findings: From this retrospective service evaluation there seems to be a definite benefit to electively freezing embryos when

hCG trigger day Progesterone levels are ≥5nm/L. There are three main patient subgroups seemingly at higher risk. It could be suggested to limit the serum Progesterone assessment only to these groups.
Trial registration number: not applicable

P-761 Is the application of elective single embryo transfer (e-SET) in freeze-all cycles a good strategy?

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Study question: Is the application of elective Single Embryo Transfers (eSET) a good strategy, compared to elective double embryo transfer (eDET), in freeze-all cycles?
Summary answer: Two sequential eSET compared to eDET in IVF cycles using freeze-all strategy result in similar clinical pregnancy rates with the advantage of no multiple gestation.
What is known already: The success of IVF treatment is reliant on embryo, endometrium and interaction between them. Conventionally, embryo transfer is placed in the same cycle of oocyte collection, what is called fresh embryo transfer. However, studies have indicated that fresh transfers can result in endometrium impairment due to supraphysiologic hormone levels, and that better implantation outcomes could be obtained when vitrified embryos are transferred in a later hormone replacement cycle. The freeze-all strategy, where the entire cohort of embryos are vitrified, has been increasing in the last years, but it is still uncertain in which population and situation it is effective.
Study design, size, duration: Retrospective cohort study reviewed 4654 cycles performed between 2011 and 2018 in a private center. A total of 409 cycles were selected for this study as fullfilled the inclusion criteria: ICSI cycles using own oocytes and ejaculated or epididymal sperm, no embryo biopsy for genetic diagnosis, freeze-all embryos (no fresh embryo transfer) and existence of one or two frozen-thawed embryos transferred (ET) in a later cycle with at least one surplus embryo available.
Participants/materials, setting, methods: Patients underwent IVF cycle as routine and were split into two groups: eSET (n=165) and eDET (n=244). For the eSET, 35 women who failed in the 1st eSET had a 2nd SET and the cumulative clinical pregnancy rate (CPR) was calculated by the formula: [CPR at 1steSET + CPR at 2ndSET ∗ (1 - CPR at 1steSET)]. Demographic characteristics and clinical pregnancy rates (CPR) after a transfer of two embryos in each group were compared.
Main results and the role of chance: The choice about the number of embryos to be transferred was performed as a shared decision-making process between patients and doctors, after an explanation of advantages and disadvantages of each situation as routine. Groups were homogeneous to age (eSET: 35.6±4.0 versus eDET: 35.0±4.1; p=0.163), basal FSH measurement (eSET: 6.3±5.9 versus eDET: 6.3±3.2; p=0.907), dose of gonadotropin administered (eSET: 1785.3±402.3 versus eDET: 1866.3±488.0; p=0.088), number of oocytes recovered (eSET: 18.6±11.9 versus eDET: 17.1±9.5; p=0.154), number of embryos vitrified (eSET: 9.2±4.9 versus eDET: 9.1±4.6; p=0.695) and survival rate after thawing (eSET: 92.3% versus eDET: 94.8%; p=0.090). After the 1st frozen-thawed ET, the CPR was statistically similar between groups (eSET: 32.7% versus eDET: 37.3%; p=0.342). When we evaluated the cumulative-CPR in the eSET group after a 2nd ET for patients who did not become pregnant in the 1st ET, the eSET-SET group presented the cumulative CPR (44.2%) slightly higher than eDET (37.3%; p=0.848) despite no statistical significance. Additionally, while the eSET group had no multiple gestation, the eDET group had 29.7% of multiples (p<0.001).
Limitations, reasons for caution: The retrospective characteristic of this study is a limitation. Other points to be considered are that freeze-all strategy was performed mainly when patients presented ovarian hyperstimulation syndrome risk, high levels of estrogen or thin endometrium. Also, not all patients who received an eSET and failed had a second SET.
Wider implications of the findings: Studies have indicated that CPR can be higher in frozen-thawed than in fresh ET, which provide a basis for freeze-all strategy. We demonstrated that freeze-all strategy associated to eSET can be a good option compared to eDET, reducing drastically the multiple pregnancy rate and does not negatively affect the CPR.
Trial registration number: N/A

P-762 ICSI outcome in treated HIV-infected women: a retrospective cohort study

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Study question: Does HIV infection in women affect the embryological and clinical outcome in Intra Cytoplasmic-Sperm-Injection (ICSI) cycles?
Summary answer: The ICSI outcome in treated HIV-infected women was similar to the outcome in matched HIV-uninfected women.
What is known already: As a consequence of improved overall health, quality of life and life expectancy, many HIV-infected patients of reproductive age desire children. Although anti-retroviral treatment (ART) is recommended for all individuals, regardless of $CD4^+$ T-cell count, little is known about possible differences in ICSI outcomes between ART treated HIV-infected women and uninfected women. Large prospective trials are lacking.
Study design, size, duration: In the present study, embryological and clinical outcomes were evaluated in ART treated HIV-infected women attending ICSI cycles (HIV+ group) between May 2012-September 2017. A similar number of HIV-uninfected ICSI cycles (CTRL group) were matched for female age, oocyte retrieval date, stimulation protocol, ethnicity and rank of cycle. Frozen embryo transfer, preimplantation genetic testing or oocyte donation cycles were excluded from the analysis. Women with ongoing hepatitis B or C infections were also omitted.
Participants/materials, setting, methods: A number of 96 ICSI cycles were analysed: 48 HIV+ cycles and 48 CTRL cycles. Fertilization rate (per mature oocytes) and embryo utilization rate (number of embryos frozen and transferred per fertilized oocyte) on day 3 and/or 5 were evaluated and compared between the two groups. The clinical pregnancy rate (per embryo transfer) was also reported. The data were analyzed using the chi-square test. The level of significance was set at $p<0.05$ for all comparisons.
Main results and the role of chance: The mean total amount of gonadotrophin units used per ICSI cycle was 2132.7 ± 898.6 IU in the HIV+ group , comparable with the control group (2426.4 ± 1107.6, $p>0.05$). A number of 787 oocytes were retrieved, of which 390 from the HIV+ group and 397 from the CTRL group.

No statistically significant difference in maturation (309/397 vs 296/397 p= 0.1202) and fertilization rate (225/309 vs 212/296; p=0.7431) was observed between the HIV+ and CTRL groups, respectively. In addition, utilization rate on day 3 (83/225 vs 94/212; p=0.1128) or day 5 (47/225 vs 45/212; p=0.9311) showed similar results in both groups.

In total, 15 cycles had no embryo transfer (ET) due to medical reasons (n=8), insufficient embryo quality for ET (n=3) and abnormal fertilization (n=4). From the 81 cycles with ET (39 from HIV+ group and 42 from the CTRL group), 28 resulted in positive hCG (11 and 17 cycles, respectively). In total, 24 cycles ended in clinical pregnancy with fetal heart beat (10 cycles in HIV+ group and 14 cycles in CTRL group), with no statistically significant difference (p=0.4487) between the two respective groups. Eventually, 20 babies were live born: 8 from HIV+ group and 12 from CTRL group.
Limitations, reasons for caution: To confirm this data, patient numbers need to be enlarged, if possible by conducting a multi-center study. This larger study should also investigate the effect of the antiretroviral treatment (type of medication and length of administration) on embryo quality and clinical outcome.
Wider implications of the findings: The present results reassure that treated HIV infection in women has no deleterious effect on embryological and clinical outcome following ICSI.
Trial registration number: not applicable

P-763 The number of oocytes retrieved is not associated with adverse obstetric and perinatal outcomes in IVF/ICSI cycles with antagonist protocol and fresh embryo transfer

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Study question: Is there an association between the number of oocytes retrieved and adverse obstetric and perinatal outcomes in IVF/ICSI cycles with fresh embryo transfer?

Summary answer: The number of retrieved oocytes has no impact on the perinatal and obstetric outcomes after a fresh embryo transfer in GnRH antagonist protocols.

What is known already: There are increasing concerns regarding the adverse effects of controlled ovarian stimulation (COS) upon the uterine environment, that would lead to poorer clinical and also in obstetric and perinatal outcomes. However, as more oocytes are retrieved higher is the cumulative live birth per cycle. However, few data with controversial results are available evaluating if there is an association between the number retrieved oocytes and obstetric/perinatal outcomes after a fresh embryo transfer. Moreover, the studies evaluating this correlation are based on large national registries with no adjustments for some possible confounders such as stimulation protocol, triggering and luteal phase support.

Study design, size, duration: This is a cohort study evaluating the offspring of IVF/ICSI cycles performed in a private IVF center between 2011 and 2015. Data were collected from 1035 newborns after fresh cleavage (n= 197) or blastocyst (n= 838) embryo transfers in cycles performed with GnRH antagonist protocol, hCG triggering, and vaginal progesterone for luteal phase support.

Participants/materials, setting, methods: Outcomes measure were: low birthweight (LBW), small and large-for-gestational-age (SGA, LGA), preterm birth, congenital anomalies, preeclampsia, gestational diabetes and perinatal mortality. Logistic regression analysis was performed to evaluate the association of the number of the oocytes retrieved (continuous variable) with these outcomes. Adjusted odds ratio (aOR) was performed for all outcomes after adjusting for confounding factors, i.e., female age, BMI, the cause of infertility, embryo developmental stage, fertilization method, and the number of embryos transferred.

Main results and the role of chance: There were no significant association in singleton between the number of retrieved oocytes and LBW (aOR 1.03, 95% confidence interval (CI) 0.98-1.09), macrosomia (aOR 0.97, 95% CI 0.87-1.08), SGA (aOR 1,00, 95% CI 0.94-1.06), LGA (aOR 1.01, 95% CI 0.94-1.07), preterm (aOR 0.97, 95% CI 0.92-1.03), congenital anomalies (aOR 0.97, 95% CI 0.98, 95% CI 0.80-1,18), preeclampsia (aOR 0.60, 95% CI 0.34-1.06), gestational diabetes (aOR 1.03, 95% CI 0.93-1.13), placenta accreta (aOR 0.60, 95% CI 0.22-1.61), and perinatal mortality (aOR 0.67, 95% CI 0.37-1.23). Even when evaluating twin newborns, there was no association between the number of oocytes retrieved and all the perinatal and obstetric outcomes evaluated.

Limitations, reasons for caution: As this is a cohort study, although the outcomes were adjusted for confounding factors, some unknown confounders may affect the outcomes.

Wider implications of the findings: This study allowed to evaluate the correlation between the number of retrieved oocytes and obstetric/perinatal, adjusting the outcomes for maternal and embryological confounders. Moreover, the patients evaluated performed the same stimulation protocol, triggering and luteal phase support, thus eliminating some additional confounders and improving the reproducibility of the findings.

Trial registration number: Not applicable.

P-764 Obstetric and perinatal outcomes of cleavage and blastocyst embryos in antagonist protocol after fresh and frozen-thawed embryo transfer

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Study question: Are there differences in obstetric/perinatal outcomes among antagonist protocol cycles when comparing cleavage and blastocyst embryo transfer in a fresh and frozen-thawed embryo transfer (FET)?

Summary answer: Fresh blastocyst transfer presents a higher chance of newborns with lower birthweight and gestational age when comparing to vitrified blastocyst after hormonal replacement endometrial priming.

What is known already: Observational studies and meta-analysis have associated adverse obstetric/perinatal outcomes when comparing blastocyst to cleavage embryos transfers. Moreover, FET cycles have been associated with better obstetric and perinatal outcomes when compared to fresh cycles. However, most of these data are based on large databases and meta-analysis studies, being impossible to exclude potential bias such as the type of stimulation protocol, i.e. long-agonist or antagonist, type of endometrial priming for FET, and also the embryo developmental stage. Thus, there is a need for evaluating the obstetric and perinatal outcomes in specific scenarios that are commonly used in current assisted reproduction practice.

Study design, size, duration: This is a cohort study evaluating the offspring of IVF/ICSI cycles performed in a private IVF center between 2011 and 2015. Data were collected from 1934 newborns after fresh cleavage (n= 197) or blastocyst (n= 838) embryo transfers, and after FET cycles in cleavage (n= 48) and blastocyst (n= 851) stage. All the IVF/ICSI cycles performed in GnRH antagonist protocol and the FET cycles were performed after hormonal replacement.

Participants/materials, setting, methods: Data from 1151 singletons and 783 twins were evaluated. Outcomes measure were: birthweight, low birthweight (LBW), small-for-gestational-age (SGA), large-for-gestational-age (LGA), gestational age, preterm birth, congenital anomalies, preeclampsia, gestational diabetes, and perinatal mortality. Multiple linear and logistic regression were performed, and the adjusted odds ratio (aOR) was obtained after adjusting for maternal characteristics and treatment variables. To adequately size the effect of gestational age and newborn gender on birthweight, we standardized birthweights using z-scores.

Main results and the role of chance: Singletons born after vitrified-warmed blastocyst transfer presented a significantly higher z-score ($P<0,0001$) and gestational age ($P<0,0177$) in comparison with a fresh blastocyst transfer. Although statistically significant, it is questionable whether these differences present a clinical significance as the mean difference (MD) in birthweight was 157.1g and in gestational age was 2.31 days. When evaluating SGA, LGA, preterm birth, congenital anomalies, preeclampsia, gestational diabetes, and perinatal mortality, the multivariable regression analysis adjusting for maternal confounders (aOR) did not present statistical differences among cleavage vs. blastocyst embryo transfer in fresh neither in FET cycles and also when comparing fresh to FET blastocyst transfers. These findings also were observed in twin pregnancies.

Limitations, reasons for caution: This was an observational study of a single center, making the generalization of its results questionable. Moreover, the sample of newborns after cleavage stage embryo transfer was smaller than the blastocyst stage. Therefore the results from the comparison among cleavage and blastocyst embryo transfers should be interpreted cautiously.

Wider implications of the findings: The findings of this study allow clinicians to select the stage of embryonic development and whether the embryo transfer will be performed in a fresh or frozen cycle embryo based on clinical criteria, without the need for concern with obstetric and perinatal outcomes.

Trial registration number: Not applicable.

P-765 Type 1 Diabetes in Children born after Assisted Reproductive Technology: a register based national cohort study

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Study question: Is there an increased risk of type 1 diabetes in singletons born after ART as compared with singletons born after spontaneous conception?

Summary answer: ART children did not have increased risk of type 1 diabetes, but an association between type 1 diabetes and frozen embryo transfer (FET) was found.

What is known already: Recent studies have raised concerns to whether fertility treatments may have an impact on long-term morbidity in ART children. Elevated blood pressure and altered glucose metabolism have been found both in animal studies and in a few studies in ART children. Type 1 diabetes is one of the most frequent chronic pediatric diseases, showing a markedly increased incidence in childhood during the past decades. Two Danish studies have analyzed the risk of type 1 diabetes in children born to women with fertility problems or fertility treatment. No association between fertility status or fertility treatment and type 1 diabetes was found.

Study design, size, duration: This was a retrospective, register based national cohort study that included all singletons born in Sweden between 1985 and 2015, in total 3,138,540 children with follow-up for onset of type 1 diabetes until 2017.

Participants/materials, setting, methods: The study was population based and all live-born singletons born after ART (n=47,938) or spontaneous conception (n=3,090,602) were included. Several national health registries were cross-linked with data from Statistics Sweden (SCB). The unique personal identification numbers enable linkage of registries and of child and parents.

Main results and the role of chance: In total, 202 children born after ART and 17,916 children born after spontaneous conception developed type 1 diabetes, corresponding to 43.4and 35.5 per 100,000 person-years at risk (hazard ratio [HR] 1.23, 95% confidence interval [CI] 1.07 to 1.42). After adjustment for calendar year of birth the HR for type 1 diabetes was 1.13, (95% CI 0.98 to 1.30), and after full adjustment for sex, year of birth, smoking, parental ethnicity and educational level, and diabetes in the parents, the HR was 1.08, (95% CI 0.94 to 1.24). In a subgroup analysis, an association was found between FET and type 1 diabetes in the child (adjusted HR 1.52, 95% CI 1.08 to 2.14). When comparing ICSI vs IVF no difference was found (adjusted HR 1.08, 95% CI 0.77 to 1.52).

Limitations, reasons for caution: Limitations of the study are the retrospective design, missing data and residual confounding caused by unknown confounders

Wider implications of the findings: With the high number of ART children born globally and the fast introduction of new ART techniques, elucidation of important health problems is of utmost importance. The higher rate of diabetes among children born after FET is a concern in view of the present trend of increasing embryo freezing/thawing.

Trial registration number: ISRCTN 11780826

P-766 External quality controls using the Internet for andrology and embryology laboratories

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Study question: Can the Internet be a reliable support to verify, compare and confirm laboratories performances of analyses in andrology and embryology?

Summary answer: Online Internet Quality Control schemes allow establishing inter- and intra-laboratory quality controls of important semen, oocyte, and embryo parameters.

What is known already: Although very few papers have discussed this subject, very large coefficients of variation between laboratories have been reported: with CVs for concentrations >30%, for total motility >70%, for morphology >40%. The main reasons are: 1) the difficulty to achieve homogeneous dispatching of sperm samples and 2) the non-availability of oocytes and embryos for quality control purposes, and 3) the differences in assessment methods from one laboratory to the other.

Study design, size, duration: Inter-laboratory study of 30 professionals involved in andrology and embryology, in Europe, and North- and South-America. Online access to images and videos was granted during six weeks.

Participants/materials, setting, methods: Images of gametes (sperm, oocyte, embryos), videos (sperm motility), are presented as quality control schemes on a web-based application. Thirty professionals trained in andrology and/or embryology were invited to participate in the schemes. The online produced and saved data were analyzed at the end of the test period. Coefficients of variation (CV) for andrology schemes (semen parameters) and for embryology (degree of agreement on morphological characteristics) were calculated.

Main results and the role of chance: For the andrology schemes, the CVs were for concentration 12.8%, total motility 8.7%, morphology according to strict criteria 73.5% and vitality 4.2%. For the embryology schemes, the CVs were for oocytes 19.2%, cleavage stage embryos 15.6% and blastocysts 20.4%.

Limitations, reasons for caution: As a limited number of experienced professionals participated in this study, the CVs obtained on a larger less experienced population are likely to be higher. Furthermore, better inter-observer agreements could have been achieved through training.

Wider implications of the findings: Compared to external quality controls based on a distribution of biological samples, the online system allows assessing the laboratory performances in a more precise way. Lower CVs can be achieved as all users assess the same images. Higher CVs for sperm, oocyte and embryo characteristics stress the need regular controls.

Trial registration number: None

P-767 Risk of morbidity in pregnancies and deliveries after In Vitro Fertilization: a French comparative observational cohort of 43,084 IVF versus non-IVF deliveries 2013-2016

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Study question: Is there an excess of maternal morbidity (MM) in ongoing pregnancies and deliveries after In Vitro Fertilization (IVF) techniques when compared to spontaneous conceptions (SC)?
Summary answer: In this 4-years cohort study, univariate and multivariate analysis identified increased risks of obstetrical complications after IVF and fresh transfer pregnancies, compared to non-IVF population.
What is known already: Large observational studies identified that Assisted Reproductive Techniques (ART) pregnancies are associated with a significant risk of complications as compared with pregnancies after SC. These complications include preterm delivery, gestational diabetes, pre-eclampsia and placental anomalies, leading to increased fetal loss, intrauterine growth restriction, and low birthweight for neonates, all events being a significant concern. Although initially it was believed that these complications were more prevalent after ART due to the high incidence of multiple pregnancies, a high risk was also demonstrated in ART singleton pregnancies (Pinborg, 2013, Ombelet, 2016, Wang, 2016, Qin, 2016, Luke, 2017, Vermey, 2018).
Study design, size, duration: This is an observational, exposed-unexposed national cohort study comparing deliveries and births following IVF, standard or using Intra Cytoplasmic injection (ICSI), and fresh transfers, to non-IVF controls. The study included all 2,832,578 national deliveries registered between 2013 and 2016 in France, among which 1.5% (43,084) from IVF. Births were considered when occurring beyond 22 weeks of gestation (WG), and a term birth was defined when it occurred at 37 WG and beyond.
Participants/materials, setting, methods: Pregnancies and deliveries were analyzed by extracting the Information Systems Medicalization Program (PMSI) French database. The main identified MM indicators for 43084 IVF and 2 789 494 non-IVF pregnancies were: venous and arterial thrombosis (VT, AT), gestational diabetes mellitus (GDM), pre-eclampsia (PE), placenta praevia (PP), placenta abruption (PA) hemorrhage at delivery (HD). The risks of MM in IVF were estimated in multivariate analysis after adjustment for maternal age, smoking and obesity, primiparity and multiple deliveries.
Main results and the role of chance: The mean maternal age was 33.2 and 29.9 years in the IVF and control groups ($p < 0.0001$). The rate of multiple deliveries was 1.68%, of which 13% if IVF conception. Diabetes and hypertensive disorders during pregnancy were more common in the IVF *vs* non-IVF group: 1.01% *vs* 0.9% ($p = 0.01$) and 1.04% *vs* 0.9% ($p < 0.001$). Tobacco dependence and obesity were less common in the IVF *vs* non-IVF group (2.2% *vs* 4.5%, and 3.9% *vs* 4.3%, $p < 0.001$). The frequency of premature deliveries was higher in IVF *vs* non-IVF: 19.3% *vs* 6.9% ($p < 0.0001$), persistent for single births (9.0% *vs* 5.7%, $p < 0.001$). The risk of MM (GDM, PE, PP, PA) was higher in IVF *vs* non-IVF (20.9% *vs* 14.3%, $p < 0.0001$), even if single pregnancies (19.6% *vs* 14.1%, $p < 0.0001$) except arterial thrombosis. The risk of MM increased significantly with age for all events except for PE. In multivariate analysis, IVF is a significant risk factor for all MM events except arterial thrombosis. The adjusted risk of the occurrence of at least one concern after IVF is 1.20 [1.16-1.22] at all and 1.23 [1.20-1.27] in single deliveries. This risk is stable over the four years.
Limitations, reasons for caution: While the strength of this study lies in the number and completeness of subjects studied, its limitations are its retrospective and register-based nature, not allowing to refine the risk evaluation, according to 1.obstetrical events only notified if hospitalization, 2. Undetailed IVF data, such as technique or embryonic stage at transfer.
Wider implications of the findings: These large national cohort data provide evidence for increased adjusted risk of maternal morbidity after IVF, including single pregnancies, and essential tool for informing without worrying couples candidate for IVF, and analyzing neonatal health of IVF-children. Future developments should allow to refine the knowledge of more or less at-risk subgroups.
Trial registration number: not applicabe

P-768 Birth size in children born after cryopreservation of embryo in assisted reproduction compared to fresh embryo transfer and spontaneous conception

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Study question: Is the previously observed high birthweight after cryopreservation of embryo (cryo-ART) associated with a corresponding increase in other measures of fetal growth?
Summary answer: Children born after cryo-ART had a higher weight, length and head circumference at birth compared to spontaneously conceived children, but a similar ponderal index.
What is known already: In pregnancies following cryo-ART, the risk of preterm birth and small for gestational age is lower than in ART pregnancies with fresh embryo transfer (fresh-ART). However, cryo-ART fetuses carry a higher risk of being born large for gestational age, as measured by birthweight. It is not known whether this is a result of increased longitudinal, but symmetrical, growth, as reflected by other measures of birth size.
Study design, size, duration: Nation-wide registry-based cohort study of 3838 singletons born after cryo-ART, 18 144 singletons born after fresh-ART and 1 390 259 singletons born after spontaneous conception from 1988 to 2016 in Norway.
Participants/materials, setting, methods: Data were collected from the Medical Birth Registry of Norway and the Norwegian National Education Database. Main outcome measures were: gestational age, birthweight, birthweight for gestational age, birth length, head circumference at birth, ponderal index (birthweight relative to birth length), placental weight and birtweight:placental weight ratio. We estimated mean differences between children born after spontaneous conception, fresh-ART and cryo-ART in linear regression models, reporting 95% confidence intervals (CIs) on differences between ART methods and SC.
Main results and the role of chance: Children born after cryo-ART had a higher weight (Δ=70 g, 95% CI 50 to 90), length (Δ=0.29 cm, 95% CI 0.19 to 0.38) and head circumference (Δ=0.23 cm, 95% CI 0.16 to 0.30) at birth compared to spontaneously conceived children, but a similar ponderal index (Δ=-0.002 95% CI -0.01 to 0.01). The larger size at birth after cryo-ART was more pronounced for boys than girls. Children born after fresh-ART had a lower weight (Δ=-94 g, 95% CI -103 to -84), length (Δ=-0.37 cm, 95% CI -0.42 to -0.33), head circumference (Δ=-0.26 cm, 95% CI -0.29 to -0.23) and ponderal index (Δ=-0.023 95% CI -0.027 to -0.019) at birth than spontaneously conceived children. Placental weight was larger in both cryo-ART and fresh-ART compared to spontaneously conceived pregnancies, also relative to birthweight. Adjustments were made for parity (0/1/2/3+), age of mother and child year of birth. In a sub-sample with available information, further adjustments for maternal pre-pregnancy body mass index, height, smoking and education did not alter conclusions.
Limitations, reasons for caution: We were unable to adjust for cause and duration of infertility, number of oocytes retrieved, good quality embryos available, total dose of gonadotropins, information on type of culture medium and method used for cryopreservation (i.e. slow-freeze or vitrification).
Wider implications of the findings: Our study suggests that the high birthweight seen in cryo-ART is associated with an overall increased, but symmetrical growth. However, the implications of these findings for the future health of children born after cryo-ART are unclear and warrant further investigation.
Trial registration number: None

P-769 assisted reproductive technology (ART) and neurodevelopmental outcome at 4 years of preterm infants

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Study question: Is assisted conception associated with neurodevelopmental impairment at 4 years age in preterm infants born before 34 weeks of gestational age?
Summary answer: At 4, fine motor skills are the most fragile domain of neurodevelopment in ART preterm and affect less than 10% of infants
What is known already: Some risk factors of neurodevelopmental impairment in ex preterm are well-known, such as gestational age, birthweight, severe neurologic complications of prematurity but also male sex and low socio-economic conditions. Assisted conception appears to increase the rate of preterm births, though few studies have analyzed late outcomes for these preterm infants.
Study design, size, duration: All infants born alive between 24+0 and 33+6 weeks of gestational age between 2003 and 2014 enrolled in the Loire Infant Follow-up Team (LIFT) prospective longitudinal cohort and assessed for neurodevelopmental outcome at 4 years are included. The neurodevelopmental outcome at 4 years of corrected age was determined by a physical examination and ASQ parental questionnaire.
Participants/materials, setting, methods: All infants born alive before 33+6 weeks of GA between 2003 and 2013 and included in LIFT are included. ART preterm was extracted and described for neonatal characteristics and 4 years ASQ outcome. ASQ is widely validated tools including 30 items exploring five domains of neurodevelopment: communication abilities, gross motor skills, fine motor skills, problem solving abilities and personal-social skills. ART and neurodevelopment association is explored matching 1:1 with preterm from LIFT cohort.
Main results and the role of chance: Among 378 preterm born after ART included who have reaching age of 4 years: 16 were non included, 1 died, 8 were lost of follow-up, 10 moved, 23 refused secondarily follow-up, 9 were out of follow-up for sequelae. 311 ASQ have been posted, 253 (81%) have been completed by parents. Neurodevelopmental difficulty at ASQ was revealed for 7.5%, 4.8%, 9.9%, 5.2% and 1.2% respectively for communication abilities, gross motor skills, fine motor skills, problem solving abilities and personal-social skills. According to ART, difficulty were highest for artificial insemination in communication abilities (12.5%), whereas it was highest for ovulation induction in gross motor skills (9.3%), fine motor skills (16.4%) and in problem solving abilities (9.1%). No neurodevelopmental difficulties were diagnosed for oocyte donation preterm excepted for fine motor skills (3.4%)and personal-social skills (3.4%).
Limitations, reasons for caution: Several limitations have to be take into account. Firstly, lost of follow-up preterm is a particular social high risk subgroup of neurodevelopment disabiliites. Secondly, although ASQ is a well-validated questionnaire; this tool may lack of sensibility. Considering these limits, long term disabilities of preterm in this cohort may be underestimated
Wider implications of the findings: At 4, neurodevelopmental disabilities remain unfrequent in ART preterm. Further studies are still necessary to assess later behavioral outcome of this specific preterm infants.
Trial registration number: NA

P-770 The role of Cabergoline and post-collection Gonadotropin Releasing Hormone – antagonist administration for Ovarian Hyperstimulation Syndrome prevention after agonist trigger.

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Study question: What is the role of Cabergoline 0.5mg orally daily for 7-days and post-collection GnRH-antagonist 250mcg SQ daily for 7-days administration for OHSS prevention after GnRH-agonist trigger?
Summary answer: The addition of luteal phase GnRH-antagonists may reduce the risk of OHSS and manage discomfort beyond Cabergoline alone, which is better than agonist trigger alone.
What is known already: Induction of ovulation with GnRH-agonists is one of the most efficient methods to prevent OHSS (Mourad *et al.* 2017), Cabergoline has been proven in several studies to reduce the risk of OHSS (Tsunoda *et al.* 2003; Manno *et al.* 2005; Álvarez *et al.* 2007; Carizza *et al.* 2008). It has been shown that restarting GnRH-antagonists inhibit the expression of VEGF and thus may reduce the rate of vascular permeability.
Study design, size, duration: This is a retrospective cohort study of 480 patients who underwent IVF treatment at the University Health Centre from 2011 to 2018, with agonist trigger and freeze all embryos.
Participants/materials, setting, methods: IVF/ICSI patients at high risk of developing OHSS were treated with GnRH-agonists triggering. 208 patients received no other treatment (group-1). 167 patients were treated with Cabergoline 0.5mg for 7 days (group-2), and 105 patients (group-3), were treated with a combination of Cabergoline 0.5mg for 7 days and a GnRH-antagonist 250mcg for 7 days. Statistical analysis was performed with ANOVA with post hocks or Chi-Square testing. Normal distribution was confirmed. Data is mean±SD or percentage.
Main results and the role of chance: The three groups did not differ for female age, male age, basal serum FSH, and PCOS diagnosis (P>0.05). AFC was higher among group-3 than group-1 or 2 (p=0.0001). No severe OHSS occurred. For mild or moderate OHSS group-1 had more than group-2 (38% vs. 29%, p=0.048) or group-3 (38% vs. 18%, p=0.006) and group-2 had more than group-3 (29% vs. 18%, p=0.046) (total p=0.001). Group-3 had lower levels of haemoglobin (12.2±1.9 vs.13.8±1.0 vs. 13.3±1.2g/dl, p=0.0001), higher serum albumin (31.6±5.4 vs. 27.0±3.4 vs. 30.1±5.4 g/L, p=0.0001) than group-1 or 2, respectively. In group-3, 18% complaints of bloating/discomfort vs. 33% and 21% in group-1 and 2 (p= 0.005).Group-3 had higher serum sodium than did group 1 or 2 (135.3±2.0 vs. 132.9±1.4 vs. 133.5±1.9mEq/L, p=0.0001) and lower serum potassium (3.6±0.5 vs. 4.8±0.3 vs. 4.1±0.8mEq/L, p=0.0001). (Please note when group 1 is less than group 2 or groups 3 respectively post hock testing was significant (p<0.05).
Limitations, reasons for caution: As a retrospective cohort study, it may exhibit selection biases. The groups had significant differences in the AFC, which may influence OHSS development. However, the group with the highest mean AFC was group 3, which exhibited the lowest rates of OHSS and symptomatology.
Wider implications of the findings: The addition of Cabergoline to patients who received GnRH agonist trigger; minimizes hematologic abnormalities, decreases patient discomfort and prevents the development of mild and moderate OHSS. Adding the GnRH-antagonist to Cabergoline leads to further decreases in peritoneal fluid accumulation, hematologic abnormalities and most importantly increase patient comfort.
Trial registration number: not applicable

P-771 Higher risk of preeclampsia in singleton pregnancies, from donor versus autologous oocytes (AO) with similar endometrial preparation, in a healthy, young cohort: a prospective study

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Study question: Is the risk of hypertensive disorders increased in healthy women ≤37 years with singletons from donor oocytes [fresh-transfer] versus AO [elective frozen embryo transfer (eFET)]?
Summary answer: Amongst the hypertensive disorders, there was a higher risk of preeclampsia, and an increased risk of iatrogenic preterm delivery (PTD) in donor oocyte (DO) pregnancies.
What is known already: Recent meta-analyses show an increased risk of hypertensive disorders and PTD in DO versus AO pregnancies. Most of the studies are retrospective and conducted in elderly women. Main confounding factors are age, parity, and plurality.

Very few studies highlight an increased risk of hypertensive disorders in DO pregnancies in younger women. Proposed etiologies are immunological and altered endometrial hormonal milieu. The latter, in itself could be a potential

confounder for perinatal outcomes when comparing DO with AO pregnancies with fresh embryo transfer. Limited studies, controlling for endometrial preparation, show a higher perinatal morbidity with DO FET compared with AO FET.
Study design, size, duration: A prospective cohort study conducted at a tertiary referral centre from October 2012 -December 2018

A total of 108 singleton pregnancies diagnosed on ultrasound at 10 weeks were studied. The DO group (n = 49) had fresh ET and AO group (n=59) had vitrification followed by eFET. Both groups were similar regarding ICSI, ET policy (2-3 cleavage stage) and programmed endometrial preparation. The IVF programme, antenatal care and deliveries were conducted at the same institution.
Participants/materials, setting, methods: All participants were ≤37 years old with a BMI <30kg/m2. Oocytes donors were <30 years, in good physical and mental health. Pregnancies were followed for hypertensive disorders as a primary outcome, including chronic hypertension, preeclampsia, eclampsia and superimposed preeclampsia. Additionally, PTD (<37weeks) both iatrogenic and spontaneous, was also studied.

Chi-square and t-tests were used to compare proportions, and means respectively. Adjusted odds ratios were calculated using logistic regression (significance was set at $P < .05$, two-tailed).
Main results and the role of chance: Mean age and BMI were comparable in DO and AO groups at 31.96 ± 3.20 versus 30.92 ± 2.64 years (*P*=.07), and 23.31 ± 1. 36 versus 23.69 ± 1.25 kg/m2 (*P*=.14) respectively. All women were non-smokers, with no history of chronic hypertension, and not on aspirin. Overall, there were 103(95.4%) nulliparas. There was no case of eclampsia.

Hypertensive disorders were significantly more in the DO group at 22.45% versus 8.47%, (OR 3.12; 95% CI 1.00-9.73; *P*.04). This difference could not be validated after adjusting for age (AOR 2.47; 95% CI 0.77-8.81; *P*=.13) This implies that the risk of hypertensive disorders in DO pregnancies, in a young cohort compared to an autologous group with comparable mean age, (*P*=.07), may still be age-dependent.

Amongst hypertensive disorders, preeclampsia was significantly higher in the DO group (14.29% and 3.39%, *P*=.04) while gestational hypertension was comparable (*P*=.52).

Although overall PTD rate was similar (*P*=.15), iatrogenic PTD was higher in the DO group at 26.53% versus 8.47% (OR 3.80; 95% CI 1.25 -11.55; *P*=.01) After adjusting for preeclampsia this difference lost statistical significance (AOR 2.41; 95% CI 0.68-10.52; *P*=.13), implicating preeclampsia to be a key risk factor for iatrogenic PTD in women with DO pregnancies.
Limitations, reasons for caution: We have compared pregnancies from DO with fresh ET to AO with eFET, as similar endometrial preparation was needed to study end points. Adequate number of patients with DO FET as a study group were not available. Vitrification may have affected our results. Also, larger studies are needed for confirmation.
Wider implications of the findings: Our results suggest an increased risk of preeclampsia in young, healthy woman with DO pregnancies. Therefore, they need to be counselled and require specialized care for optimum maternal and perinatal outcomes. Lowering the risk of preeclampsia with specific endometrial preparation protocols needs to be explored.
Trial registration number: Not Applicable

P-772 IVF incident reporting system: a customized tool for the application of the Quality management system in IVF laboratories

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Study question: How to manage the approach to the clinical risk management in IVF laboratories in accordance with the Italian State-Regions Conference of March 15th, 2012?
Summary answer: The IVF incident reporting system enhances the safety level of procedures by monitoring non-conformances and/or adverse events that could be prevented with specific corrective measures.
What is known already: Quality measures require IVF laboratories to satisfy stringent criteria to document, track and report items ranging from minimal inconvenience to extreme harm that can impact on the outcome of IVF treatments or on gametes'/embryos'/patients' safety. In Italy, the Board of the Region of Emilia Romagna acknowledged the State-Regions Conference of March 15th/2012 throughout the deliberation n. 972/2013. It defines functional, structural and technological requirements that the Health Service hospitals must have in accordance with Quality Control and Quality Assurance rules of the Italian Law n.40/2004 referred to the donation, supplying, control, storage and distribution of human gametes and embryos.
Study design, size, duration: Based on 2013 regulations, an expert team of embryologists and Quality Referees designed a specific electronic database to record any non-intentional deviation from protocols adopted daily in our IVF Center by embryologists, gynecologists and nurses. Since the introduction in 2015, this tool is currently used as an incident reporting system defining three possible levels of detection: the IVF laboratory, the outpatients' room and the department of Obstetrics and Gynecology.
Participants/materials, setting, methods: Retrospective analysis of an electronic database monitoring and documenting non-conformances and adverse events at a large IVF Center with an average of 1300 IVF cycles per year. Events detected by the IVF laboratory were divided into three categories based upon their content regarding the management of samples prior to, during or after the analytical phase, the management of laboratory parameters or the operators training.
Main results and the role of chance: From January 2015 until December 2017, 330 non-conformances were recorded with an average of 110 events yearly. The 75% (245/330) of non-conformances regarded IVF laboratory responsibilities: the 82% (200/245) concerns the management of samples prior to, during or after the analytical phase demonstrating that this is the most critical part of the IVF treatment, the 17% (42/245) was about the management of laboratory parameters (forgetting to log daily maintenance, parameters of incubators or environmental controls) and 1% (3/245) concerns operators' deviations away from standard working procedures. The 23% (77/330) of non-conformances was assessed to the IVF laboratory-outpatients' room interface (incorrect patient ID assessment and/or documentation, communication problems with the surgical or nursing departments) and the remaining 2%(8/330) was due to inaccuracies of clinical engineering service and others. All non-conformances have been frequently discussed by the IVF team, who shared and introduced more than 10 corrective actions in clinical procedures. The same approach has been used to monitor subsequent outcomes. For example, in 2017, the number of non-conformances related to the partial integrity of clinical folders was quite null compared to 2016 following the introduction of a novel check list to be completed at the end of the IVF cycle.
Limitations, reasons for caution: It is important to keep on sensitizing and encouraging all the operators involved in clinical practice as the usage of the reporting system is founded on a voluntary attitude leading to the risk of underestimating errors.
Wider implications of the findings: A robust system of incident reporting mirrors what happens in clinical activity, impacting on the perception of clinical risk. This tool should be recommended to consciously increase the management of clinical risk and combined with other tools for the perspective and/or retrospective detection of errors.
Trial registration number: Not applicable.

P-773 Frozen-thawed embryo transfer is an independent risk factor for third stage of labor complications in singleton vaginal deliveries

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Study question: Is pregnancy conceived by frozen-thawed embryo transfer (FET) associated with an increased risk for third stage of labor complications in singleton vaginal deliveries?
Summary answer: Conception by FET is an independent risk factor for post-partum hemorrhage, requirement for manual lysis and revision of uterine cavity in singleton vaginal deliveries.

What is known already: IVF pregnancies are associated with a higher prevalence of perinatal complications than spontaneous gestations, even after correction of confounding factors. Little is known about the prevalence of third stage of labor complications in IVF pregnancies. In a previous study from our group, the prevalence of these complications was increased in gestations conceived through IVF and fresh embryo transfer. The endometrial preparation for FET varies from fresh cycles and the outcome of gestations conceived by FET is different from those conceived through fresh embryo transfer.

Study design, size, duration: Cohort study reviewing delivery files of all pregnancies conceived through FET and successfully delivered vaginally in the same tertiary medical center between 2007 and 2017. The study group consisted of 88 FET gestations (cases), and 176 matched parturients that conceived spontaneously (controls).

Participants/materials, setting, methods: The FET cases group were matched according to maternal age, gravidity, parity, and gestational age at delivery in a 2:1 ratio to a control group of women who conceived spontaneously and underwent a singleton vaginal delivery at our center during the same period. Women with multiple gestations, deliveries by cesarean sections, intrauterine fetal demise and missing data regarding the third stage of labor were excluded.

Main results and the role of chance: Baseline characteristics were similar in cases and controls except for a lower prevalence of induction of labor in the control group (36.3% vs. 23.3%, p=0.03). The rate of post-partum hemorrhage was 13.6% in cases and 5.7% in controls (OR=2.62, p=0.018). Manual lysis was required in 17% of the case deliveries and in 2.3% of the controls (OR=8.83, p<0.001). Revision of the uterine cavity was required in 21.6% of cases and 6.8% of controls (OR=3.76, p<0.001). Multivariable analysis adjusted for age, previous cesarean section, induction of labor and the type of anesthesia demonstrated that deliveries after FET were independently associated with an increased risk for the above mentioned third stage of labor complications (estimate OR=4.44, p=0.002).

Limitations, reasons for caution: Retrospective case-control design. FET group size number is limited due to the primary inclusion criteria (FET and successful singleton vaginal delivery in the same center). Nevertheless, our findings are clinically and statistically significant.

Wider implications of the findings: FET is an independent risk factor for third stage of labor complications warranting anticipating active management. Together with similar findings in deliveries following fresh transfers, this data suggests that the implantation of embryos created in-vitro adversely affect placental separation and uterine contraction after delivery, regardless of type of endometrial preparation.

Trial registration number: Retrospective study, IRB approved.

P-774 The effect of maternal pre-pregnancy BMI on intelligence and growth of singletons following IVF/ICSI: follow-up of a large sized, cohort study

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Study question: To evaluate the effect of raised maternal pre-pregnancy body mass index (BMI) on intelligence and growth of singletons following IVF with or without ICSI.

Summary answer: Maternal pre-pregnancy obesity is associated with increased risks for obesity, overweight and intellectual disability at early ages of offspring conceived through IVF/ICSI.

What is known already: Pregnancy in obese women is associated with escalating risks of complications for fetus, such as increased risks of preterm birth, macrosomia and fatal growth restriction. Besides, longer-term consequences of studies suggest that maternal obesity risks offspring's health later in life and increases susceptibility to adult disease. Offspring of obese mother increase the risks of obesity and metabolic syndrome, hypertension, asthma, central nervous system developmental problems, depression, attention-deficit hyperactivity disorder.

Study design, size, duration: This is a cohort study with 1904 singletons aged 3-6 years were followed up from 2009 to 2017, born to infertile couples who received autologous IVF/ICSI cycle from 2002 to 2012.

Participants/materials, setting, methods: Singletons at 3-6 years old who were consent to attend the follow-up study had the metabolic and Intelligent assessment. Age- and sex-specific BMI z-scores by Chinese standard were used to assess growth and development of children. Chinese Version of the Wechsler Intelligence Scale for Children (C-WISC) was performed to evaluate verbal intelligence quotient (VIQ), performance intelligence quotient (PIQ), and full intelligence quotient (FIQ) of children.

Main results and the role of chance: After adjusting for confounders, the obese women were more likely to have obese children (20.0% vs 5.1%, P =0.01) than normal BMI women, and overweight women had increased risks of having overweight children (13.6% vs 8.2%, P =0.02) or obese children (10.1% vs 5.1%, P =0.01) than normal weight women. Maternal BMI had no effect on serum lipids of IVF/ICSI offspring. When not adjusting for parental educational level, offspring of the overweight women scored lower in VIQ (97.72 vs 99.65, P =0.02), PIQ (111.33 vs 112.93, P =0.04) and FIQ (104.82 vs 106.71, P =0.02) than the normal weight women, while offspring of the obese women lower in both PIQ (109.28 vs 112.93, P =0.04) and FIQ (102.69 vs 106.71, P =0.03). However, after adjusting for parental educational level, this result was not consistently observed in four groups. But after adjusting for confounders, offspring of obese women showed increased prevalence of intellectual disability (IQ < 80) in VIQ (16.9% vs 8.5%, P =0.03) and FIQ (10.8% vs 3.9%, P =0.03) compared with normal BMI women.

Limitations, reasons for caution: The enrolled children were 3 to 6 years old whose intelligence levels were still in the rapid development stage whose intelligence levels were still in the rapid development stage. And it still wasn't clarified the mechanism of effect of maternal BMI on children obesity and decreased intelligence.

Wider implications of the findings: A growing number of obese women are appealing to ART for infertility, however maternal obesity has been found to impair ART outcomes and offspring's health. Nevertheless, it's quite important to inform public health policy in terms of advising women to manage weight and diet prior to and during ART.

Trial registration number: not applicable

P-775 The pregnancy outcomes of women with a congenital didelphus uterus after in vitro fertilization-embryo transfer

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Study question: Are the pregnancy outcomes of women with a didelphus uterus worse than those of women with a normal uterus?

Summary answer: The didelphus uterus associated with increased risks of premature delivery and low birth weight (LBW), but with a similar live birth rate as normal uterus.

What is known already: Although normal pregnancies can occur in some patients with a clinically asymptomatic didelphus uterus, this condition has been reported to be in relation with miscarriage, premature delivery, perinatal mortality and other complications in ordinary population. The prevalence of didelphus uterus was estimated to be 0.2-0.5% in infertile women. Then, what about the pregnancy outcomes in infertile women with a didelphus uterus undergoing in vitro fertilization- embryo transfer (IVF-ET)?

Study design, size, duration: A retrospective, single-center case control study was conducted of 50 women with a didelphus uterus and 567 women with a normal uterus, who received IVF-ET treatment from January 2004 to December 2017.

Participants/materials, setting, methods: All included patients obtained singleton pregnancies via IVF-ET. A comparison of the pregnancy outcomes was conducted between the didelphus group and the control group. The main outcome measures included miscarriage, preterm delivery, LBW and so on. In order to avoid selection bias, only the first pregnancy of each patient was considered. Multiple pregnancy and spontaneous/selective reduction were excluded from analysis.

Main results and the role of chance: The didelphus group and the control group were statistically similar regarding the maternal age (p = 0.768), body mass index (p = 0.088), endometrium thickness on transfer day (p = 0.104),

infertility type (p = 0.51), cause of infertility (p = 0.052) and basic follicle-stimulating hormone (p = 0.390).

The presence of a didelphus uterus was associated with increased risks of premature delivery (16.0% vs. 3.9%, OR 4.72; p < 0.001) and LBW (12.2% vs. 2.6%, OR 5.28; p = 0.001); Additionally, significantly lower term delivery rate (66.0% vs. 78.7%, OR 0.53; p = 0.039) and live birth weight (3026.0 ± 573.0 vs. 3388.0 ± 451.0 g; p < 0.001) were also observed in the didelphus group. However, the live birth rate (82.0% vs. 81.3%; p = 0.904) was statistically similar in 2 groups. Furthermore, although the rates of miscarriage (18.0% vs. 14.3%; p = 0.476) and cesarean section (85.4% vs. 73.9%, OR 2.06; p = 0.106) were higher, and the gestational weeks at delivery (38.2 ± 2.1 vs. 38.9 ± 2.3 weeks; P = 0.064) and ectopic pregnancy rate (0 vs. 3.2%; p = 0.201) were lower in the didelphus group, the difference were not significant.

Limitations, reasons for caution: All pregnancy outcomes were tracked by telephone calls or faxes in our reproductive center. Thus, some obstetric complications were not detailed recorded. Additionally, the control group in this retrospective study was not screened randomly, which may cause selection and confounding biases.

Wider implications of the findings: Our findings can be used to counsel the pregnancies in women complicated by a didelphus anomaly and help guide appropriate antenatal treatment and surveillance.

Trial registration number: None.

P-776 The Assisted Reproductive Technology (ART) Capability Assessment: experiences from 24 pilot assessments with fertility clinics from 14 countries

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Study question: To test our assessment in the field in order to evaluate whether it can detect and quantify capability variations between fertility clinics

Summary answer: The results of our pilot assessments suggest that there is a considerable amount of capability improvements to be made across many fertility clinics

What is known already: ART treatment is not without risk and unsuccessful treatment approaches can cause considerable psychological stress to patients. Treatment access, effectiveness and safety vary greatly between countries. Several factors are known to affect ART treatment outcomes, in particular the quality of the embryo and the woman's age. However, outcome variations between clinics are also due to different stimulation protocols, culture methodology and embryo transfer techniques used as well as the quality management and control systems in place

Study design, size, duration: The ART Capability Assessment is based on relevant international guidelines, scientific publications and input from leading experts in the field. It is conducted by a team of experienced consultants over the course of two days and involves interviews, observations as well as document analysis. Between May 2016 and November 2017, we piloted our assessment with fertility clinics from Europe (N=10), Asia (N=7), Australia (N=2), the Middle East (N=2), Latin America (N=2) and North America (N=1)

Participants/materials, setting, methods: Interviews were conducted with senior clinic staff, including clinicians, biologists, nurses and technicians. Processes, operations and management areas were also assessed by means of observations. The assessment comprised five major categories (clinical processes, clinical operations, laboratory processes, laboratory operations and management), 43 subcategories and more than 400 assessment criteria. The criteria were rated on a four point-scale (0%, 33%, 66% and 100%), allowing category scores and an overall assessment score to be calculated

Main results and the role of chance: The mean overall assessment score for all clinics was 72% (median 75%), ranging from 39% to 89%. The clinics achieved the highest mean score for clinical processes (77%) and the lowest for management (66%). Several areas were identified where many clinics performed very well, for example, endocrinology (when conducted in-house) (89%), laboratory cleaning (87%) and medication storage & handling (85%). On the other hand, there were also several areas where the performance of many clinics was significantly below best practise standards, particularly consumable selection and use (53%) and cryopreservation & storage (53%). In addition, one clinic each from Europe and Asia underwent re-assessment more than one year after the initial assessment. The clinics had been provided with the results of the initial assessment in the form of a detailed report, containing suggestions for improvement. According to the results of the re-assessment, mean overall assessment scores improved from 58% to 61% and from 75% to 80%, respectively. The improvements were attributable to various small incremental changes throughout multiple areas as opposed to any single major change. This suggests that the assessment results were meaningful, allowing the clinics to implement changes which lead to capability improvements

Limitations, reasons for caution: Our sample of fertility clinics was a so-called convenience sample that included clinics from various countries willing to participate in a pilot. Thus, the results cannot be considered representative. Moreover, we did not assess whether the capability improvements observed in our re-assessments were associated with improvements in clinical outcomes

Wider implications of the findings: There is a need for more standardised processes, operations and management across fertility clinics in order to increase the chances of a successful treatment, to reduce treatment-related risks and to decrease the psychological burden on patients. The ART Capability Assessment provides guidance as to how to accomplish this

Trial registration number: Not applicable

P-777 Pregancy outcomes of patients with a congenital unicornuate uterus: an analysis of 406 women following in vitro fertilization embryo transfer

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Study question: What are the pregnancy outcomes in women with a unicornuate uterus after in vitro fertilizaiton-embryo transfer (IVF-ET)?

Summary answer: The presence of a unicornuate uterus was associated with some adverse outcomes in singleton pregnancies, but the live birth rate was satisfactory.

What is known already: Unicornuate uterus is a rare form of uterine malformation with an estimated prevalence of 8.0–13.3% in infertile patients. Although normal pregnancies can occur in some patients clinically asymptomatic, most literature regarded this kind of anomaly to be associated with increased risks of maternal and neonatal complications as well as infertility. However, most studies were based on small sample or case studies and thus these analyses were not comprehensive enough.

Study design, size, duration: Four hundred and six women with a unicornuate uterus who obtained clinical pregnancies via IVF-ET from January 2012 to December 2017 were retrospectively analyzed. According to the number of gestations, the pregnancies were divided into singleton pregnancies (n = 279) and multiple pregnancies (n=127). Among the multiple pregnancies, there were 121 cases of twin pregancy (including 5 monochorionic twins), 5 cases of triplet pregnancy (including 4 dichorionic triplet pregnancies) and 1 case of quadruplet pregnancy.

Participants/materials, setting, methods: This study was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). Pregnancy outcomes including the rates preterm delivery, cesarean section, live birth and perinatal mortality, birth weight were analyzed in singleton and multiple pregnancy groups.

Main results and the role of chance: The total miscarriage rate of patients with a unicornuate uterus was 16.5% (67/406): the early pregnancy loss rate was 15.3% (62/406), and the late miscarriage rate was 1.2% (5/406). The rates of preterm delivery and term delivery were 21.9% (89/406) and 60.0% (244/406), respectively. The ectopic pregnancy rate was 1.0% (3/406) (2.4% (3/279) in singleton pregnancies and 0.8% (1/127) in multiple pregnancies. In addition, there were 2 cases of induced abortion due to congenital chromosomal abnormalities.

The number of babies born was 386, including 361 cases of live births and the live birth rate was 88.9% (361/406) (77.7% in singleton (213/274) and 85% in multiple (12/14) pregancies). The overall perinatal mortality was 6.2% (25/406), including 16 cases of still birth and 9 cases of neonatal death. There was a high cesarean section rate with 71.2% (275/386). Furthermore, the average gestational age at delivery was 37.2 ± 3.4 weeks of gestation.

Among the multiple pregnancies, there were 37 cases experienced spontaneous reduction and 26 cases received selective reduction. What is more, the 26 women with twin pregnancy who underwent selective reduction all resulted in live births.

Limitations, reasons for caution: This was a descriptive analysis and the results were not compared with women with a normal uterus. Additionally, all pregnancy outcomes were obtained via telephone calls or faxes in our center and thus some detailed information were not recorded.

Wider implications of the findings: The results can be used to guide appropriate antenatal treatment and surveillance for women who are pregnant or plan for a future pregnancy but complicated with a unicornuate uterus.

Trial registration number: None

P-778 Lower total gonadotropin dose per oocyte retrieved and top-quality embryos in the fresh cycle affect live birth rate in frozen-thawed embryo transfer

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Study question: Which factors of the fresh cycle affect live birth rate (LBR) after frozen-thawed embryo transfer (FET)?

Summary answer: Lower total gonadotropin dose per oocyte (dose/oocyte) and presence of ≥1 top-quality embryo are associated with higher LBR in FET.

What is known already: Factors that are known to affect LBR include female age, number of oocytes retrieved, ovarian response and number of top-quality embryos created. It is known that high gonadotropin doses are associated with poor endometrial function in the fresh cycle but it is unclear if FET cycles are affected. Previous studies have evaluated the effect of characteristics of the fresh cycle on LBR after fresh transfer or cumulatively. Only a few analyses have focused on FET and little is known about fresh cycle characteristics that might affect LBR in FET.

Study design, size, duration: Retrospective cohort study of data from a national infertility database. A total of 9465 FET cycles with single embryo transfer performed in 2000 – 2017 were included in this study. Only cycles up to the first live birth (LB) after FET (gestational age >24 weeks) of 5905 women were analysed. Fresh cycle characteristics were matched to corresponding FET treatments.

Participants/materials, setting, methods: The study consisted of 1525 (16.1%) natural cycles, 4091 (43.2%) natural cycles with luteal support and 3849 (40.7%) hormonally substituted cycles. Blastocyst transfer was performed in 1064 (11.8%) cases. We analyzed the effect of characteristics of the fresh cycle, such as number of oocytes retrieved and number of top-quality embryos created on LBR using general estimating equations. Dose/oocyte was used as measure of ovarian response.

Main results and the role of chance: Overall, LBR after FET was 20.2% (1915/9465). It was highest in patients with low dose/oocyte in the fresh cycle (<200 IU/oocyte: 21.5%, 1434/6675) and lowest in those with 300-400 IU/oocyte (13.5%, 85/630, P<0.0001). In patients with no top-quality embryos created in the fresh cycle, LBR was only 17.1% (208/1219) but increased to 21.3% in those with >3 top-quality embryos (469/2200, P=0.002).

The final model showed that the chance of LB after FET was highest after stimulations with dose/oocyte <200 IU/oocyte and decreased in cases with 200-300 (odds ratio [OR] 0.79, 95% confidence interval [CI] 0.67-0.94) and 300-400 IU/oocyte (OR 0.58, 95% CI 0.45-0.74). Presence of ≥1 top-quality embryo in the cohort also increased the odds of LB in FET, compared with no top-quality embryos created (OR 1.39, 95% CI 1.36-1.66). Blastocyst transfer did not significantly affect results. Age >36 years decreased the odds of LB (OR 0.63, 95% CI 0.56-0.71). Compared with spontaneous cycles with luteal support, both purely spontaneous cycles (OR 0.80, 95% CI 0.66-0.98) and hormonally substituted treatment cycles (OR 0.65, 95% CI 0.57-0.75) were associated with lower chance of LB after FET. The number of oocytes collected was excluded from the final model.

Limitations, reasons for caution: It was not possible to evaluate the effect of different embryo freezing methods on results (vitrification vs. slow freeze). As there still is no consensus on embryo quality grading after thawing, the quality of the transferred embryo was not analysed.

Wider implications of the findings: Dose/oocyte reflects ovarian function and oocyte quality. Stimulation should be optimized so that dose/oocyte remains as low as possible because of lower LBR in FET with >200 IU/oocyte. Day of freezing does not affect LBR but even a single created top-quality embryo is associated with higher LBR in FET.

Trial registration number: Not applicable

P-779 Impact of oocyte donation on pregnancy complications at very advanced maternal age

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Study question: We aimed to compare the complication rates between oocyte donation and spontaneously conceived singleton pregnancies at very advanced maternal age.

Summary answer: Oocyte donation pregnancies in the late 5th decade of life were associated with significantly higher rates of gestational hypertensive complications compared with spontaneous pregnancies.

What is known already: Deliveries in the fifth and sixth decades have risen in accordance with a shift in family planning trends and the increased use of assisted reproductive technologies. Oocyte donation has become a common treatment option among women at the edge of the age of fertility. Oocyte donation pregnancies have been linked with higher rates of obstetrical complications, including gestational diabetes, hypertensive disorders, placental abnormalities, preterm labor and cesarean deliveries. Still, it is unclear whether the reason for this is attributed to the advanced age of the recipients, to the IVF itself, or to the use of non-autologous gametes.

Study design, size, duration: A retrospective cohort study, consisting of all women aged 45 to <47 years old at time of delivery who gave birth to singletons in our hospital between March 1, 2011 and May 1, 2018. The final cohort included 150 women in the oocyte donation group and 76 women in the spontaneous conception group.

Participants/materials, setting, methods: All women aged 45 to <47 years old at time of delivery who gave birth to singletons at 24 weeks' gestation or later. The oocyte donation group included pregnancies achieved after IVF with donated oocytes. The spontaneous conception group included pregnancies achieved following coitus or intrauterine insemination. Exclusion criteria were multiple gestation pregnancies, pregnancies achieved by IVF with autologous oocytes or patients that underwent iatrogenic fetal reduction.

Main results and the role of chance: Women in the oocyte donation group were mostly nulliparous compared with those that conceived spontaneously (62.0% vs. 7.9%, p<0.001 respectively). The rates of gestational hypertension (14.7% vs. 5.3%, P=0.036, respectively) and preeclampsia (14.7% vs. 1.3% P=0.002, respectively) were significantly higher in the oocyte donation group compared with the spontaneous conception group, as well as the rate of cesarean delivery (83.3% vs. 35.5%, P<0.001, respectively). In the multivariate analysis, conducted to test possible association between the mode of conception and gestational hypertensive complications, oocyte donation pregnancies had almost four times the risk of gestational hypertensive complications compared with spontaneous conception group after controlling for age, parity, marital status and use of LMWH during pregnancy (OR 3.92; 95%CI 1.28-11.98; P=0.017).

Limitations, reasons for caution: First, it is possible that our oocyte donation population included women who are healthier compared with others at the same age. Second, the study groups differed in parity rates which is a risk factor for preeclampsia. We tried to overcome this confounder by including it in the multivariate analysis.
Wider implications of the findings: Oocyte donation pregnancies at very advanced maternal age were associated with a high risk of hypertensive complications. This may suggest an immunological imbalance as the culprit of hypertensive disorders in this population. The use of spontaneous pregnancies as a control group may help to isolate the effect of oocyte donation.
Trial registration number: SMC 1411-14

P-780 Number of children per couple after one or several IVF/ICSI treatments – a cohort analysis of 5385 couples with singleton live births

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Study question: How many singleton children do IVF/ICSI patients have and which factors affect the number of children born?
Summary answer: On the average, there were 1.14 children per couple, and this number was affected most strongly by age, total gonadotropin dose/oocyte retrieved (dose/oocyte) and BMI.
What is known already: The endpoint of most infertility analyses is the first pregnancy or live birth after IVF/ICSI. However, many infertile couples wish for more than one child. Factors that are known to affect outcome from treatment include female age, number of oocytes retrieved, total gonadotropin dose used, number of top-quality embryos created and tubal factor infertility. Dose/oocyte, a measure of estimating ovarian response, is inversely related to cumulative live birth rate, with higher values indicating lower chances of live birth. However, it is not known how this and other factors relate to the number of children born after one or several IVF/ICSI.
Study design, size, duration: Retrospective cohort study of data from a national infertility database. We analysed data of women with single embryo transfer in the first cycle who had ≥1 live birth of a singleton after 1-18 stimulations during 2000-2017. We examined the effect of background and treatment characteristics of the first stimulation cycle on the number of children born. Patients had 1-5 singletons but the main analysis was restricted to those with 1-3 deliveries (>90% of all).
Participants/materials, setting, methods: We analysed data of 5385 women who had a total of 9007 singleton deliveries after fresh or frozen-thawed embryo transfer. Dose/oocyte was used for evaluating ovarian response to stimulation. Other factors analysed included age, BMI, total gonadotropin dose, number of oocytes retrieved and number of top-quality embryos. Data were studied by ordinal logistic generalized linear models. Inclusion into final model was based on Akaike information criterion (AIC), in which lower values indicate a better model.
Main results and the role of chance: In all, 4685 (87.0%) patients had one child, 665 (12.3%) had two and 35 (0.6%) had three children after IVF/ICSI treatment. Women who had three children had similar numbers of oocytes retrieved (12.5±4.6 vs. 11.8±5.6, P=0.70), dose/oocyte (176.3±128.3 vs. 192.0±127.2, P=0.74) but had more stimulations (3.9±1.8 vs. 2.2±1.8, P<0.0001), compared with patients having one child.

Due to collinearity, the number of oocytes retrieved and dose/oocyte were included into separate analyses. The model featuring dose/oocyte had lower AIC and was chosen as the final model. Age >36 years (odds ratio [OR] 0.40, 95% confidence interval [CI] 0.30-0.54, vs. age ≤36 years), dose/oocyte >300 IU/oocyte (OR 0.62, 95% CI 0.46-0.84, vs. ≤300 IU/oocyte) and BMI 30-34.9 kg/m^2 (OR 0.56, 95% CI 0.37-0.85, vs. BMI 20-24 kg/m^2) were associated with lower odds for having several children. The chance of having several children increased with >3 stimulations (OR 5.08, 95% CI 3.96-6.51), compared to one. The odds of having >1 child also increased with the number of top-quality embryos (OR 1.09, 95% CI 1.05-1.14). In the final model, dose/oocyte, age and BMI were more important factors than the number of ovarian stimulations performed and the number of top-quality embryos.
Limitations, reasons for caution: It was not possible to determine whether each couple attained the desired number of children through IVF/ICSI treatment as reasons for discontinuation of treatment were not available. Characteristics of cryopreserved cycles and of subsequent stimulations were also not evaluated.
Wider implications of the findings: Modifiable factors for having more children are dose/oocyte and BMI. Both of these factors are more important for having >1 child from IVF/ICSI than multiple treatments. Dose/oocyte is more reflective of the overall quality of ovarian stimulation than the number of oocytes retrieved and should be kept low.
Trial registration number: Not applicable

P-781 Ovarian stimulation response and extended embryo culture, but not assisted zona hatching, correlate to monozygotic multiple pregnancies

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Study question: Are monozygotic twin pregnancies after assisted reproduction correlated to assisted zona hatching (AZH), extended embryo culture (EEC) or number of oocytes retrieved?
Summary answer: Monozygotic twin pregnancies (MTP) after assisted reproduction are correlated to ovarian stimulation response and EEC, but not to AZH.
What is known already: The increased incidence of MTP after assisted reproductive techniques (ART) has for long been a matter of debate, yet its causes are still controversial. Many factors, ranging from prolonged embryo exposure to the culture media, to the use of AZH or to intrinsic factors of the oocytes following ovarian stimulation, have been presented as potential causes. While the use of AZH has been reported to increase the chance of twins, blastocyst transfer is generally accepted to play a major role. In addition, higher levels of estradiol have been documented in early multiple pregnancies but this correlation during implantation is unknown.
Study design, size, duration: Retrospective analysis of 1596 clinical pregnancies after ovarian stimulation and conventional in vitro fertilization or intracytoplasmic sperm injection, of which 4% corresponded to MTP. Pregnancies were grouped according to the number of embryos transferred (ET) and the number of fetal heart rates (FHR) detected. For each of the groups, data referring to the use of AZH, the day of transfer and the number of oocytes retrieved was collected.
Participants/materials, setting, methods: Participants undergone ART treatment at Next Clinic Praxisklinik Frauenstrasse in Ulm, Germany. Assisted zona hatching was performed on day 3 of culture using the OCTAX pulse laser. Intrauterine embryonic transfer of 1 or 2 embryos took place either early (day 2-3) or after extended embryonic culture (day 5-6). Fetal heart rate was monitored by Doppler ultrasound starting at 4 weeks after intrauterine embryonic transfer. Statistical multivariate analysis was performed by Chi-Square Test.
Main results and the role of chance: Our results indicate a 10-fold increased incidence of MTP after ART, compared to the normal population.

Analysis of 61 MTP versus 1535 non-MTP controls showed a correlation of MTP to EEC (93% MTP had EEC versus 69% of controls). MTP were also positively correlated to a higher number of oocytes retrieved, corresponding to a higher estradiol environment during oocyte intra-follicular development (>3,000pg/ml). MTP presented a statistically significant ($p<0.01$) increased incidence of cases with >15 oocytes retrieved (43%), compared to controls (16%). AZH was slightly more frequent in the MTP group (28%) than in the controls (22%). When the pregnancies were further subdivided into single ET leading to single pregnancies (control A, n=233) or MTP (group A, n=10) and 2 ET leading to 2 FHR (control B, n=1302) or >2FHR (group B, n=51), the correlation of MTP to ovarian stimulation response was reconfirmed ($p<0.01$), with higher response present in 20% of group A (control A, 9%) and 47% of group B (control B, 17%). EEC was more frequent in MTP, although statistical power was lacking for one group (group A, 60%; control A, 49%, $p>0.1$; group B 100%; control B, 72%, $p<0.01$). Interestingly, no correlation was found between AZH and MTP.

Limitations, reasons for caution: This is a multivariate analysis study that limits the ability to determine the consequence of a single variable (AZH, EEC or the effect of ovarian stimulation) on the outcome (MTP). The term monozygotic also needs to be taken with caution as genetic analysis of the twins was not performed.

Wider implications of the findings: Single embryo transfer is advisable to patients with more than 15 oocytes retrieved to avoid the risk of MTP. AZH performed on day 3 does not show a correlation to MTP, in contrast to previous reports. EEC benefits should be balanced with MTP possibility in patients with health risks.

Trial registration number: not applicable

P-782 THIRD PARTY REPRODUCTION: THE ITALIAN SOCIETY OF HUMAN REPRODUCTION (SIRU) CONSENSUS MEETING

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Study question: What is the recommended assessment and management of reproductive treatments with donated gametes, based on the best available evidence and clinical expertise?

Summary answer: A consensus meeting was organized among all different professional figures involved in ART procedures, patients' associations and lawyers to promote evidence-based care on donor-assisted reproduction.

What is known already: In Italy donor-assisted reproduction is presently permitted by law. An increase in number of treatments (from 2800 to 6247,+123%), patients (from 2462 to 5450,+121%), and deliveries (from 601 to 1457,+142%) was observed from 2015 to 2016. However, since Annex III of EC Directive 17/2006 was only partially accepted, a dramatic lack of donors is currently observed. In 2016, 84.4% of sperm donation and 94% of oocyte donation treatments were performed with gametes imported from international banks. The Italian regulatory limitations negatively impact on the feasibility of treatments so that couples' needs are not being met and gaps still persist.

Study design, size, duration: The Italian Society of Human reproduction (SIRU) has organized a meeting in order to define the best scientific evidence for Italian healthcare professionals. The consensus conference was held in Lampedusa from 11 to 13 October 2018 involving 28 experts, equally distributed between public and private centers (55% vs 45%) and gender (59% men vs 41% women). Conflicts of interest were managed in order to guarantee transparency and disclosed by all the participants.

Participants/materials, setting, methods: SIRU organized a consensus conference based on the methodology derived from ESHRE Manual for development of recommendations for good practice. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework was applied for evaluation of 301 bibliographic references.

Main results and the role of chance: We have developed the first consensus meeting on third party reproduction based on a pre- and post-process rigorous methodology. The final document provided nine consensus statements, one clinical consensus recommendation and six clinical practice points aroused from discussion during the meeting. High quality GRADE statements referred to risks of treatments, number of embryos to transfer, recipients' clinical evaluation, risks of pregnancy in advanced maternal age and endometrial preparation before transfer. Moderate quality GRADE statement referred to outcomes of reproductive treatments with fresh or frozen oocytes. Low quality GRADE statements referred to genetic and psychological counselling for donors and recipients and reimbursement for donation. A clinical recommendation was proposed on the use of low dose aspirin during pregnancy in advanced maternal age. The clinical practice points suggested the minimum genetic tests required for donors and the number of oocytes useful for each patient. Finally, the expert working group discouraged the use of preimplantation genetic screening for donor-assisted reproduction, the routinely use of endometrial scratching, the use of donor sperm after repeated implantation failures in couples with normozoospermic men and the indication to reproductive treatments when the prognosis is very poor especially in advanced maternal age.

Limitations, reasons for caution: The consensus statements were developed in relation to the Italian practice. A different panel of international experts would likely have yielded subtly different statements. Embryo donation was not considered because it remains presently forbidden in Italy.

Wider implications of the findings: For the first time in Italy, a scientific society representative of all figures involved in donor-assisted procedures, has convened to evaluate the evidence on management of treatments using donor gametes. The impact of the consensus statements in reducing multiple pregnancies and increasing effectiveness of treatments should be evaluated prospectively.

Trial registration number: not applicable

P-783 Long term management of OHSS syndrome in a large patient population

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Study question: Does a different management of IVF cycle in a patient at risk of OHSS influence complication and pregnancy rate?

Summary answer: An accurate evaluation of individual risk factors for OHSS is the most important factor to prevent complications and improve IVF outcomes in hyper-responders.

What is known already: OHSS is the main complication of ovarian stimulation with a mortality of 3 out of 100.000 cases. The main risk factors are: young age (<30), BMI <19kg/m^2, antral follicles count higher than 24, AMH >3.4ng/ml, polycystic ovarian syndrome, number of follicles at the time of induction > 20, estradiol plasmatic levels >3500 pg/ml, personal history of OHSS.

Even though it is impossible to completely eliminate the risk of OHSS the strategies for OHSS prevention are: primary prevention, such as election of proper gonadotropin dose and antagonist protocol and secondary prevention such as GnRH agonist triggering and freeze all strategy.

Study design, size, duration: Retrospective cohort study. Among 14103 patients undergoing an IVF treatment 1318 were included because considered at risk for OHSS. The patients were divided into 3 groups based on the number of retrieved oocytes and estradiol levels at trigger day: Group A including 377 patients that underwent oocytes freeze all; Group B including 62 patients that underwent embryos freeze all; Group C including 879 hyper responders patients who underwent fresh embryo transfer.

Participants/materials, setting, methods: Participants treated at the IVF and Infertility Center of Bologna University from 1991 to 2018 had comparable age, FSH and AMH level. 1471 cycles of ovarian stimulation were performed using low dose of gonadotropin associated with GnRH antagonist protocol. Monitoring was performed with seriated estradiol blood tests and pelvic ultrasounds. hCG trigger 36 hours before pick-up was used. According to the profile risk of the patients, fresh embryo transfer or oocytes/embryos freeze all was performed.

Main results and the role of chance: AFC was significantly higher in group A compared to the other groups ($p<0,001$). Both days of stimulation and total

dose of gonadotropin were similar among the groups. Patients of group C had lower estradiol levels (pg/ml) on trigger day (2504±869 vs. B 2890±1036, vs. A 3919±1312 p<0,001). Furthermore, group C patients had lower number of follicles at the ultrasound (11±3.4 vs. B 12.6±6.6 vs. A 16.2±5.1 p<0,001) and lower number of oocytes retrieved (9.5±5.8 vs. B 14.9±6.1 vs. A 19.4±9.6 p<0,001). Based on these characteristics Group C had a relatively lower risk to developing OHSS, thus fresh embryo transfer was performed. The mean hospitalization rate for OHSS was 14.5%, but significantly higher in group B patients (38,1%), mainly due to mild and moderate cases. Whereas the rate of hospitalization for severe OHSS was significantly higher in group C compared to group A (15.5% vs. 5.6% p<0,001). The complication rate was low (6.8%) among all the groups and referable to clinical ascites (70%). Finally, pregnancy rate obtained from fresh or frozen embryo transfer was comparable in the three groups (A 39.6% vs. B 40% vs. C 41.8% p=ns).

Limitations, reasons for caution: The main limitation of the study is a possible bias of patient selection particularly in the fresh ET group. In fact, due to the long duration of the study the clinical criteria for choosing fresh ET could have changed as supported by reduction of OHSS rate over the years.

Wider implications of the findings: The main limitation of the study is a possible bias of patient selection particularly in the fresh ET group. In fact due to the long duration of the study the clinical criteria for choosing fresh ET could have changed as supported by reduction of OHSS rate over the years.

Trial registration number: /

P-784 Outcome in singletons born after vitrification and slow-freeze – a CoNARTaS study of 11,773 singletons born after frozen embryo transfer in Sweden and Denmark

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Study question: Is transfer of vitrified blastocysts associated with increased obstetric risks compared with slow-freeze cleavage stage embryos in frozen embryo transfer (FET)?

Summary answer: Transfer of vitrified blastocysts does not seem to alter the well-known risks described in singletons born after slow-freeze cleavage-stage transfer, as compared to fresh transfer.

What is known already: Transfer of frozen-thawed embryos have a central role in modern fertility treatment, limiting the risk of ovarian hyperstimulation syndrome and multiple pregnancies. However, several studies report higher risk of being born macrosomic and large for gestational age after FET. In recent years, the introduction of new techniques has increased treatment success. The slow-freeze technique combined with cleavage stage (CT) transfer has been replaced by vitrification and blastocyst transfer (BT). Only a few studies compare obstetric outcomes after vitrification and slow-freeze, most indicating similar perinatal outcome in the two groups. However, studies indicate higher risk of preterm birth after blastocyst transfer.

Study design, size, duration: This registry-based cohort study includes singletons born after fresh and frozen-thawed transfers following the introduction of vitrification in Sweden and Denmark, in 2002 and 2009, respectively. The study includes 3650 children born after vitrified BT, 8123 children born after slow-freeze CT and 4469 children born after fresh BT during 2002-2014. Obstetric outcome in singletons, born after vitrified BT, was compared with singletons born after slow-freeze CT and singletons born after fresh BT.

Participants/materials, setting, methods: Nordic collaboration study from the CoNARTaS (Committee of Nordic ART and Safety) group. Based on national registries in Denmark, Finland, Norway and Sweden, the ConARTaS cohort includes all children born after ART-treatment in public and private clinics. Obstetric outcomes were assessed with logistic multivariate regression analysis, adjusting for country, year of birth of child, maternal age, parity, BMI, smoking, parental educational level, fertilization method (IVF/ICSI), single embryo transfer, number of gestational sacs and child´s sex.

Main results and the role of chance: The adjusted odds ratio, aOR [95% CI], for preterm birth (PTB <37 weeks) was significantly higher in the vitrification BT group compared with the slow-freeze CT group 1.33 [1.09–1.62]. Similar risks for low birthweight (<2500 g) aOR 0.91 [0.70–1.19], for small for gestational age (SGA) aOR 0.85 [0.63–1.13], for macrosomia (>4500 g) aOR 0.93 [0.77–1.15] and for large for gestational age (LGA) aOR 1.10 [0.91–1.32] were observed. Moreover, similar risks for Apgar score <4 at 5 min aOR 1.28 [0.70–2.34] and malformation aOR 1.16 [0.91–1.47] were observed. Regarding maternal outcomes no differences were seen in the risk of hypertensive disorders in pregnancy (HDP) aOR 0.97 [0.81–1.17] and placenta previa aOR 1.48 [0.98–2.24]. When comparing vitrified BT with fresh BT, we found a higher risk for macrosomia (>4500g) aOR 1.77 [1.35–2.31] and LGA aOR 1.47 [1.18–1.84]. Further, the risk of HDP was higher in singletons born after vitrified BT aOR 1.47 [1.19–1.81]. However, the risk of SGA was lower aOR 0.58 [0.44–0.78] as was the risk of placenta previa, aOR 0.35 [0.25–0.48].

Limitations, reasons for caution: Since vitrification was introduced simultaneously as blastocyst transfer in Sweden and Denmark, it was not possible to explore the effect of vitrification or blastocyst transfer *per se* in this study. The number of singletons born after slow-freeze combined with blastocyst transfer was too small for sensitivity analysis.

Wider implications of the findings: Replacing slow-freeze CT with vitrified BT does not seem to alter obstetric risks in singletons conceived after FET, although this comparison includes two variables. The increase in risk of PTB is probably related to blastocysts. Increased risks of macrosomia and HDP remain increased compared with singletons born after fresh BT.

Trial registration number: ISRCTN11780826

P-785 Use of a subcutaneous catheter (I-port Advance) during ovarian stimulation in oocyte donors before IVF does not affect the clinical outcomes compared to conventional injections.

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Study question: Does the placing of a subcutaneous catheter in donors' skin during the ovarian stimulation for hormones administration could impact negatively the in vitro outcomes?

Summary answer: The placing of a subcutaneous catheter in the donors' skin at any time of the ovarian stimulation does not impact negatively the in vitro outcomes.

What is known already: It is estimated that about 10% of the adults are afraid of needles. The use of port (I-port Advance, Medtronic) a subcutaneous catheter has resulted in a decrease of the number of needle sticks used, from 12.5 to 3.3 injections, during the ovarian stimulation without negatively impacting the stimulation length, gonadotrophic consumption, on number of retrieved eggs in oocyte donors. A small percentage of users have reported skin irritation or unease with the usage of the device, so they returned to regular injections. There is not information if the use of this catheter could put at risk the IVF outcomes.

Study design, size, duration: A prospective randomized study was performed form August 2017 to December 2018: 120 cycles of ovarian stimulation of 34 different donors were included to participate on this study. The subcutaneous catheter was used for a maximum time of 4 days before replacing it for a new port and the donated oocytes were used for In vitro fertilization.

Participants/materials, setting, methods: 120 Recipient´s husbands with normal semen parameters (WHO 2010), were included into this study. 3 groups were formed as following: Group A (40 oocyte donations) which used the I-port for only 8 days of the ovarian stimulation and the remaining part of the stimulation with regular injections. Group B (40 oocyte donations) used

the subcutaneous catheter during all ovarian stimulation and Group C (40 oocyte donations), which used subcutaneous catheters during all the ovarian stimulation.

Main results and the role of chance: 36 hours after the HCG trigger shot the egg collection of the donors was performed. According to our statistical analysis comparing the 3 groups (group A vs Group B vs Group C), we did not find any significant differences in the mean number of oocytes retrieved was (14.4± 4.3, 16.3±4.2 and 15.2±3.3) percentage of matured eggs (87%, 86% and 85%), fertilization rate (68 ,71 and 70%), number of good quality embryos at day 2 of the development (47%, 52% and 48%), blastocyst formation (50%, 46%, 49%), mean of embryos transferred (2.0, 2.1 and 2.0) and pregnancy rate (64%, 63%, 65%) $p>0.05$. For the first time we analyzed the impact of the clinical results when a subcutaneous catheter is used during all the ovarian stimulation and also when its use was canceled at the middle of the ovarian stimulation showing both similar results compared to the conventional ovarian stimulations using syringes.

Limitations, reasons for caution: We did not analyze the comfort and the psychological stress generated with the use of the syringes compared to the use of the subcutaneous catheter.

Wider implications of the findings: The use of a subcutaneous catheter during 4 days of ovarian stimulation showed similar clinical outcomes compared to the conventional use of syringes. The decrease from an average of 32 injections to only 4 injections (I-port advance) could improve the comfort during the in vitro fertilization cycles.

Trial registration number: Not applicable

POSTER VIEWING
STEM CELLS

P-786 Pathogenic mtDNA mutations are abundant in oocytes but eliminated during fetal development

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Study question: Does purifying selection against deleterious mitochondrial DNA (mtDNA) mutations occur during oocyte development?

Summary answer: Purifying selection against mtDNA mutations does not function in female germline in ovary but operates during early fetal development depending on heteroplasmy levels of mutations.

What is known already: Inherited mtDNA mutations are common and associated with multiple diseases in humans. However, only few recurring mutations account for the majority of known familial cases. This phenomenon suggests the existence of intergenerational purifying selection. While the exact timing or developmental stage at which such negative selection operates is unknown, it has been suggested that most deleterious mtDNA mutations are eliminated in the female germline, during oocyte development.

Study design, size, duration: We performed whole mtDNA genome sequencing in mature oocytes and specific developmental stages in offspring of heterozygous *PolG*$^{mut/wt}$ females generated by paring with wild type *PolG*$^{wt/wt}$ males. The effect of specific protein-coding gene mutations on fetal development was examined in chimeric animals.

Participants/materials, setting, methods: Whole mtDNA sequencing (Miseq) was performed to examine the spectrum of mtDNA mutations in single skin fibroblasts of *PolG*$^{mut/wt}$ mothers, MII oocytes, *PolG*$^{wt/wt}$ blastocysts, *PolG*$^{wt/wt}$ viable fetuses at E9.5, *PolG*$^{wt/wt}$ live pups, and MII oocytes of *PolG*$^{wt/wt}$ pups. Embryonic stem cells (ESCs) carrying specific mtDNA mutations were generated and injected into host ICR embryos. Chimeric blastocysts were transferred to recipients to generate chimeric embryos and fetuses.

Main results and the role of chance: *PolG*$^{mut/wt}$ mother's somatic cells showed a mean 10.0±1.7 mtDNA mutations per skin fibroblast while *PolG*$^{wt/wt}$ live pups inherited a significantly lower 4.6±0.7 mutations. Remarkably, MII oocytes, *PolG*$^{wt/wt}$ preimplantation embryos and ESCs, but not viable fetuses of *PolG*$^{mut/wt}$ females, carried abundant deleterious mtDNA mutations. The yield of MII oocytes in *PolG*$^{mut/wt}$ mice was comparable to control *PolG*$^{wt/wt}$ mice, while the number of viable E9.5 fetuses in *PolG*$^{mut/wt}$ females was reduced. Moreover, the majority (95%) of mutations transmitted to the live pups were at low heteroplasmy (≤10%) levels, while most of the mutations with high and moderate heteroplasmy levels presenting in mothers were transmitted to their oocytes and to their fertilized embryos including blastocysts. High heteroplasmy of nonsynonymous mutations for *mt-Nd4l*, *mt-Nd5*, *mt-CoxI*, *mt-CoxII*, *mt-CoxIII* and *mt-Atp6* genes were abundant in mothers and MII oocytes, but not in the live pups. Conversely, high heteroplasmy mutations in other protein-coding and RNA-coding genes were transmitted to the live pups. Chimeric mice carrying *mt-CoxIII* gene mutations showed a high degree of fetal lethality before E9.5. These results demonstrate that the purifying selection against deleterious mtDNA mutations operates during early fetal development and acts on the specific genes resulting in embryonic and fetal demise.

Limitations, reasons for caution: Limitation may be due to the small number of ESC lines with specific mtDNA mutations and chimeric mice generated. More research in RNA-coding gene mutations and their impacts on embryonic and fetal development is needed.

Wider implications of the findings: Our data indicate that deleterious mtDNA mutations causing mitochondrial dysfunction are abundant in mammalian oocytes and may be responsible for unexplained pregnancy loss in humans.

Trial registration number: not applicable

P-787 New role of neogenin in the mammalian testis at the early phase of spermatogenesis

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Study question: There is a lot of neogenin expression in the testicular tissue of mammals but remain unknown as the main role of Neogenin for spermatogenesis. What is the major role of neogenin in spermatogenesis?

Summary answer: Neogenin is specifically localized in the spermatogonial germ cells and it regulates with the early phase of spermatogenesis for differentiation.

What is known already: Neogenin has reported a gene level expression in the mammalian testis.

Study design, size, duration: This control study for the identification of the neogenin function during spermatogenesis in the seminiferous tubule of the mouse testis. And we performed the loss of the functional study with a male mouse that has been postnatal for 12days using the Crisper/CAS9 with the Neogenin knockdown gRNA.

Participants/materials, setting, methods: We performed immunohistochemical study to identify the neogenin expression of testis and expression analysis with western blot. Therefore, we designed that neogenin knockdown genetical edition with the Crisper/CAS9. Then we underwent spermatogenesis to analyze the seminiferous tubule of the Neogenin knockdown.

Main results and the role of chance: Neogenin abundant expression between the spermatogonia, primary and secondary spermatocyte differentiating phase. After advanced aging, testis (over 40weeks) decreased, compared to the one that has been postnatal for 24days based on Western blot data. And then other germ line development for related proteins like Oct4, SOX2 and Nanog shows the similar pattern of Neogenin expression. Especially SOX2 shows an abundant expression at the spermatocyte phase with neogenin. Nanog is highly expressive after the secondary spermatocyte phase compared with Oct4 and SOX2. Oct4 shows well matching with a pattern of neogenin expression. Therefore, Neogenin knockdown mouse

animals, we found a significant decrease in the early differentiation phase from type B spermatogonia compared with control testis. These study results suggest that neogenin may play an important role in initial spermatogonia differentiation.

Limitations, reasons for caution: Animal controlled experiment for spermatogenesis. We need confirm in the human testis whether the expression of neogenin and its defects were related spermatogonia arrest relationship.

Wider implications of the findings: We found the specific role of neogenin by loss functional study with Crisper/CAS9. This platform can apply for specific factor functional study with postnatal testis. And gene editing technology can apply defect gene replacement by normal gene for overcome for spermatogenesis arrest.

Trial registration number: none

P-788 DNA demethylation accelerates the induced differentiation of human primordial germ cells from pluripotent stem cells

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Study question: Can DNA demethylation accelerate the induction of human primordial germ cells (PGCs) from pluripotent stem cells (iPSCs) *in vitro*?

Summary answer: DNA methyltransferase inhibitor 5-aza-2-deoxycytidine (DAC) promoted the induced differentiation of human PGCs from iPSCs *in vitro*. The induced PGCs from iPSCs showed hypomethylated status.

What is known already: There are two waves of global demethylation in human PGCs from the migrating stage to the gonadal stage *in vivo*. The global erasure of DNA methylation creates a super-hypomethylated germline genome. It has also been confirmed that human iPSCs can differentiate into PGCs. But we did not know the methylation status of differentiated PGCs from iPSCs *in vitro*, and the relationship of DNA methylation and the induced differentiation of PGCs from iPSCs *in vitro*.

Study design, size, duration: VASA-GFP iPSCs were used for induction differentiation experiments. The induction efficiency of PGCs from VASA-GFP iPSCs were compared between two groups, which one is induction medium adding the DNA methyltransferase inhibitor (DNMTi) 5-aza-2-deoxycytidine (DAC) and other is no adding DAC. During inducted differentiation, the methylation status was analysed at day 4 and day 8.

Participants/materials, setting, methods: The DNMTi DAC is added after pre-induction for demethylation at day 5. The final concentration was 0.05 uM. These cells were cultured three days with DAC and PGC medium for downstream experiments. We analyzed the DNA demethylation dynamics in human PGCs at day 4 and day 8 by bisulfite sequencing in three imprinted genes *H19*, *HEG1* and *SNRPN*, and the efficiency of induced differentiation to ask whether the DNA demethylation accelerated the differentiation of human PGCs from iPSCs *in vitro*.

Main results and the role of chance: Our results showed the human PGCs derived from iPSCs at day 8 displayed much lower genome-wide 5mC level, indicating hypomethylation status, and also showed the percentage of VASA-GFP positive cells after 4 day induction using DAC and without DAC group was 17.42 ± 1.85%, 16.63 ± 1.73%, respectively; the percentage of CD38-positive cells after 4 day induction using DAC and without DAC group was 10.28 ± 1.85%, 9.21 ± 1.21%, respectively; after 8 day induction, the percentage VASA-GFP positive cells was 54.38 ± 3.75%, and 46.35 ± 2.27%, respectively; the percentage of CD38- positive cells was 36.21 ± 1.41%, 43.36 ± 1.89%, respectively, indicating DNA demethylation accelerated the induction of human PGCs from iPSCs *in vitro*.

Limitations, reasons for caution: In this study, the global epigenetic reprogramming process during induction differentiation of PGCs from iPSCs *in vitro* remains to be determined.

Wider implications of the findings: This provides a new and high-efficiency way for PGC induction differentiation from iPSCs *in vitro*.

Trial registration number: not applicable

Study funding/competing interest(s)

This study was supported by research grants from Natural Science Foundation of Guangdong Province (2016A030313010, 2018A030313506), The Basic Research Project of Science and Innovation Commission of Shenzhen (JCYJ20160429185525629) and Natural Science Foundation of Shenzhen University General Hospital (0000040558). The authors declare.

P-789 Human placenta-derived mesenchymal stem cells restore the ovarian function in ovariectomized rat model via activated PI3K/Akt pathway

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Study question: To assess outcomes of ovarian function including therapeutic mechanisms by transplanted human placenta-derived mesenchymal stem cells (PD-MSCs) in a rat model with ovary dysfunction.

Summary answer: The findings offer insights into further understating of stem cell-based therapeutic mechanisms for reproductive system and provide new avenues to develop therapies in degenerative diseases.

What is known already: Premature ovarian failure (POF) is lack of follicle and infertility in women younger than 40 years of age. Because hormone replacement therapy (HRT) has limitations such as high risk of developing breast cancer are known, the development of new stem cell-based therapies for ovarian dysfunction is required. We reported that follicular development is promoted by mesenchymal stem cells (MSCs) transplanted into an animal models with ovarian dysfunctions. Recently, PD-MSCs, which have their activities for self-renewal and immunomodulation were higher than those of bone marrow- and adipo-derived MSCs, have emerged as new stem cell resources compared with others.

Study design, size, duration: The rats were randomly divided into three groups (NTx: non-transplantation; DTx: directly transplantation; TTx: tail vein transplantation). After all rats were anesthetized by Avertin, one ovary of rat was removed by excision. One week after the ovariectomy, PD-MSCs were transplanted directly into the ovary tissues (DTx) or by the tail vein transplantation (TTx), respectively. And then, the therapeutic mechanisms of PD-MSCs were analyzed at 1, 2, 3, and 5 weeks after transplantation, respectively.

Participants/materials, setting, methods: Blood and ovary tissue were analyzed after post-transplantation. The hormone levels in the plasma was measured by using ELISA. To analyze the engrafted human PD-MSCs in the ovary tissues, the expression of human specific Alu sequence was analyzed using qRT-PCR. The expression for folliculogenesis-related genes and PI3K/Akt signal pathway in the ovary tissues were analyzed using qRT-PCR and Western blot, respectively. The number of ovarian follicles in the ovarian tissues was evaluated by histological analysis.

Main results and the role of chance: The human Alu sequence gene expression was significantly increased in both DTx and TTx groups compared with NTx groups ($*p<0.05$). The levels of E2 and AMH in blood were increased both DTx and TTx groups compared with NTx groups ($*p<0.05$). The number of follicles in ovary tissues was increased in both DTx and TTx groups compared with NTx groups($*p<0.05$). In qRT-PCR analysis, the mRNA expression levels of Nanos3, Nobox, and Lhx8 were significantly increased in both DTx and TTx groups after PD-MSCs transplantation compared with NTx groups ($*p<0.05$). Their proteins expressions in both DTx and TTx groups were also significantly increased compared with NTx groups ($*p<0.05$). Interestingly, the mRNA expression of Lhx8 was increased in both DTx and TTx groups compared with NTx groups ($*p<0.05$). The number of ovarian follicles in the ovary tissues after PD-MSCs Tx was more significantly increased than NTx group ($*p<0.05$). Especially, the number of follicles in TTx group were significantly increased compared to DTx group at long-term periods after Tx. ($*p<0.05$). Furthermore, PD-MSCs trigger significantly activation of PI3K/AKT signal comparing to NTx groups, otherwise, the expression of caspase-3 and caspase-9 were significantly decreased ($p<0.05$).

Limitations, reasons for caution: We need further studies to analyze more in deep the molecular mechanisms by which secreted signal molecules of PD-MSCs for restore ovarian function in ovarian failure model.
Wider implications of the findings: Pooled results from evaluated studies suggest that engrafted PD-MSCs into ovary tissue of rat model with ovarian dysfunction can restore ovarian function by increasing folliculogenesis via the PI3K/AKT signal pathway. Our findings suggest that PD-MSCs could be used as an alternative therapy for patients with ovarian dysfunction, including infertility.
Trial registration number: NA

P-790 Stem cell therapy induces a shift from an inflammatory environment towards an immune tolerant scenario promoting endometrial tissue regeneration

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Study question: Could bone marrow derived stem cell (BMDSC) transplantation induce an immunomodulatory milieu helping tissue recovery in human and murine models with endometrial pathologies?
Summary answer: Differences in Neutrophil Elastase (NE) expression in treated women and a specific cytokine/chemokine profile in mouse models have evidenced a favourable ambient inducing endometrial regeneration.
What is known already: Asherman Syndrome and Endometrial Atrophy (AS/EA), producing endometrial destruction, are two of the most frequent causes of uterine-type infertility. Cell therapy using $CD133^+$BMDSCs has been described for treating these pathologies. It has been demonstrated that factors secreted by these cells stimulate tissue regeneration, for example in pathological situations where Neutrophil Elastase (NE) is overexpressed, creating a microenvironment based on pro-inflammatory and immunomodulatory processes. Our previous studies detected up- and down-regulated genes like SERPINE1/IL4/JUN and CXCL8/CCDND1 respectively. Interestingly, NE can be inhibited by SERPINE1 and increases the release of CXCL8, a potent chemoattractant, thus potentiating the inflammatory response.
Study design, size, duration: Our study design involves on one hand a retrospective study based on endometrial tissue analysis from 8 patients with endometrial pathologies (AS/EA) before and after autologous $CD133^+$BMDSCs treatment (ClinicalTrials.gov-NCT02144987): gene and protein analysis were performed to elucidate how the regenerative process occurred. On the other hand, uterus from immunocompromised mice with damaged endometrium, where human $CD133^+$BMDSCs were injected intrauterus (n = 5) or through the tail vein (n = 5), were analysed by Multiplex technologies.
Participants/materials, setting, methods: We collected 2 endometrial biopsies from each of these 8 women: before/Control and after the Treatment. Genetic analysis from our previous study was assessed with bioinformatic analyses (*t*-test), and validated by individual PCR. All samples were analysed by immunohistochemistry against NE (M0752, Dako), the differential expression was analysed using Image-ProPlus-6.0 (Mann-Whitney test). These results were also supported from the animal model (intrauterus and tail vein) where Multiplex Immunoassays (ProcartaPlex Mouse-26plex Cytokine&Chemokine, ThermoFisher) were carried out.
Main results and the role of chance: Bioinformatic analysis revealed 5 significant genes: JUN, SERPINE1 and IL-4, up-regulated, while CCND1 and CXCL8, down-regulated; when Treatment condition was compared to Control. These gene pattern correlates with the subsequent protein analysis we performed. Expression of NE protein resulted significantly lower in the Treatment group (p=0.0286) when compared to Control, which correlates with CXCL8 gene downregulation (p=0.036). The down-regulated expression of CXCL8 and NE (reliable markers of inflammation) in the human endometrium, caused by the transplantation of $CD133^+$BMDSCs, results in a diminished inflammatory cascade in AS/EA patients promoting probably endometrial regeneration. All together correlates with the NE inhibitory capacity of SERPINE1 (p=0.026), which in absence of NE turned to be up-regulated. This change from an inflammatory environment toward an anti-inflammatory/tolerant phenotype allows proliferation, differentiation, anti-apoptosis and chemotaxis events. We observed evidences of these pro-regenerative processes, when analysing in detail our animal model by Multiplex immunoassays, we obtained a specific cytokine/chemokine pattern (IL-27, MCP-1, MIP-1β and MIP2 among others) in the affected area after cell therapy. All these molecules seemed to be involved in tissue recovery after damage, pro-angiogenic events, wound healing and BMDSCs secretions, processes in that SERPINE1, IL-4 (p=0.041) and JUN (p=0.037) are also implicated.
Limitations, reasons for caution: The limitation of our study is the low quantity of tissue collected due to human endometrial biopsies were coming from women suffering from AS/EA. And, in our animal model the use of Formaldehyde Fixed-Paraffin tissues to perform the Multiplex assays.
Wider implications of the findings: These results support the regenerative effect of CD133+BMDSCs in endometrial pathologies like AS/EA via immune and inflammatory responses; what would bring us one step forward to understand the specific mechanisms of this cell therapy. The decrease of endometrial NE identified here may diminish the inflammatory response giving a proliferative scenario.
Trial registration number: NCT02144987.

P-791 A novel mtDNA knockout approach to investigate mitochondrial gene mutations in mice

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Study question: Can chimera-competent mouse embryonic stem cell (ESCs) with pathogenic mitochondrial DNA (mtDNA) mutations be generated?
Summary answer: Wildtype ESCs with specific mtDNA variants can be derived from heterozygous PolG mutator females, introduced into host embryos and disease phenotypes studied in chimeric mice.
What is known already: Mammalian mtDNA is critical for cellular energy production. It contains 37 genes encoding 13 subunits of oxidative phosphorylation (OXPHOS) complex, 2 rRNAs, and 22 tRNAs. Specific mtDNA mutations in humans are associated with severe syndromes. However, no animal model of human mtDNA disease exist due to limitations of directly manipulating mtDNA genome.
Study design, size, duration: Heterozygous $PolG^{mut/wt}$ females were bred with wt mice and resulting blastocysts were used for ESC isolation. Wildtype ESC lines were selected and screened for mtDNA mutations by whole genome sequencing. Mutant ESCs were compared with human mtDNA database to predict phenotypes.
Participants/materials, setting, methods: Whole mtDNA sequencing (Illumina Miseq) was used to determine mtDNA mutation profile in each wildtype ESC lines. DNA sequences were analyzed with NextGene software to identify mtDNA variants.
Main results and the role of chance: Multiple mutant ESC lines were generated and three lines (ND6-100, ND2-50, ATP8-62) carrying specific heteroplasmic and homoplasmic mutations were selected for studies in chimeras. Implantation, full-term and live pup development rates for ND6-100, ND2-50 and ATP8-62 high ESC-chimeras were comparable to controls. ND6-100 chimeras displayed significantly reduced complex I and IV activity, ATP8-62 chimeras were deficient for complex V while ND2-50 animals showed reduced complex I activity compared to controls. Glucose levels in all chimeras were significantly elevated compared to controls. The optokinetic response indicated vision impairments. The mean ejection friction of ATP8-62 chimeras was significantly lower than controls and the mean diastolic left ventricle volume was higher. These animals showed evidence of bi-ventricular failure, suggesting reduce pumping function with chamber enlargement.
Limitations, reasons for caution: Some specific mtDNA mutations are embryonic lethal and cannot be studied in adult chimeras. Other mtDNA mutations survive in chimeras but not transmitted to offspring due to lethality.

Wider implications of the findings: Our simple and effective approach provides a long sought mean of generating mtDNA mutant mouse chimeras and examination of in vivo phenotypes.
Trial registration number: 'not applicable'

P-792 Testicular sperm extraction derived cells conditioned medium as an in vitro niche supports germ cells development from human embryonic stem cells

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Study question: Can testicular sperm extraction (TESE) derived cells conditioned medium (TCM) support male and female germ cells development from human embryonic stem cells (hESCs)?
Summary answer: TCM supports germ cell development from hESCs as assessed by gene expression profile.
What is known already: Embryonic stem cells (ESCs) can form post meiotic germ cells in mammals. Different growth factors and conditioned medium were used so far to direct this process. It has been reported previously that conditioned medium from testicular cells supports germ cell development in mice and Buffalo ESCs. Recently it was shown that co-culture with human testicular cells can facilitate germ cell progression from hESCs as shown by microarray analysis and gene expression.
Study design, size, duration: TCM was collected following culturing TESE-derived cells in different basal media including, DMEM+20%FBS and EB medium. TCM collected every 4 days following seeding cells. For germ cell development, embryoid bodies (EBs) from hESCs cultured for 4, 7 and 14 days. EBs were cultured in several induction conditions: (i) spontaneously differentiation (SD) using EB medium (SD-EB), (ii) EB medium/40% conditioned EBs medium (TCM-EB). (iii) EB medium/40%DMEM+20%FBS without conditioning (SD-DMEM), (iiii) EB medium/40% conditioned DMEM+20%FBS (TCM-DMEM).
Participants/materials, setting, methods: Yazd2 hESC line (46, XY) was used for *in vitro* generation of germ cells. Following differentiation in all groups, EBs were fixed and immunofluorescent (IF) staining was performed for evaluation the expression of SSEA-1, C-KIT, DAZL, VASA, and SCP3 markers. Moreover, Q-PCR for pluripotency (*NANOG, OCT4*), primordial germ cells (PGCs; *SOX17, DAZL),* pre-meiotic gonocytes *(VASA, SCP3*), and post-meiotic germ cells (*H1T, PRM1, GDF9* and *ZP3*) genes was performed. Experiments were performed in triplicate.
Main results and the role of chance: Interestingly, both TCM-EB and TCM-DMEM showed positive induction role for male and female germ cell development from hESCs in comparison with SD groups similar to previously reported in mice and Buffalo. Co-localization of C-KIT and DAZL as PGCs markers was revealed at days 4 and 7. SSEA-1 and VASA were expressed in differentiated cells after 4 and 7 days. Moreover, SCP3 was expressed at days 7 and 14. Q-PCR data revealed that lower expression level of *NANOG* and *OCT4* in TCM groups than other groups on days 4, 7 and 14. While, *SOX17* as master regulator of human PGCs which expressed from migrating to gonadal PGCs was increased significantly using TCM, similar to *SCP3* after 7 and 14 days ($P<0.05$). *DAZL* and *VASA* with similar expression patterns following differentiation, significant increase ($P<0.05$) was demonstrated in TCM groups. Moreover, high expression level of *H1T*, *PRM1, GDF9* and *ZP3* was detected 14 days following differentiation as post-meiotic developmental stage which was significantly increased in TCM groups ($P<0.05$) and confirmed potential of TCM groups to form an *in vitro* niche for germ cell development.
Limitations, reasons for caution: The major limitation of this study was obtaining TESE samples according to our including criteria.
Wider implications of the findings: Our results confirm previous reports in mice and Buffalo which indicates that TCM could be used as a suitable niche to mimic germ cells development from hESCs.
Trial registration number: Not applicable.

P-793 Cumulus cells conditioned medium as an in vitro niche for differentiation of human embryonic stem cells to female germ cells

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Study question: Can cumulus cells conditioned medium (CCCM) improve differentiation of human embryonic stem cells (hESCs) to female germ cells?
Summary answer: In comparison with spontaneously differentiation (SD) of embryoid bodies (EBs) formed from hESCs, CCCM improved the differentiation of hESCs to female germ cells.
What is known already: Embryonic stem cells (ESCs) in different mammals can form post meiotic germ cells either spontaneously or using different growth factors as confirmed using gene expression profile assessments. On the other hand, cumulus cells secret growth factors like BMP15 and VEGF which provide a niche to support oogenesis. It was shown previously that CCCM improve female germ cell development from Buffalo ESCs.
Study design, size, duration: EBs from Yazd4 (hESC line;46,XX) were divided into 4 groups: 1) spontaneously differentiation (SD) with 100%EB medium (SD-EB), 2) treated with 40%CCCM [40%EB medium (conditioned) +60%EB; CCCM-EB], 3) SD-DM with 40%DMEM (not conditioned) +60%EB and 4) treated with 40%CCCM [40%DMEM (conditioned)+60%EB; CCCM-DM]. EBs from all 4 groups were cultured for 14 days. Gene expression profile of EBs in 4 groups was evaluated using IF and Q-PCR at the time of days 0, 4, 7 and 14.
Participants/materials, setting, methods: EBs from SD-EB, CCCM-EB, SD-DM and CCCM-DM groups were collected at the time of days 0, 4, 7 and 14. Expression of *NANOG, SOX17, DAZL, DDX4 (VASA), CTDSPL (SCP3), GDF9* and *ZP3* were examined in EBs from each group by Q-PCR. In addition, immunofluorescent (IF) staining was done for pluripotency and germ cells markers, TRA-2-49, SSEA1, VASA, GDF9 and ZP3.
Main results and the role of chance: Q-PCR data revealed that between 4 groups, CCCM-EB had the best impact for female germ cell differentiation from hESCs. However, between groups with similar basal medium, CCCM-EB and CCCM-DM shown to provide a better condition for germ cell development in female. On the other hand, comparison between basal media revealed that EB medium provides a better condition for female germ cells development from hESCs. Using IF, co-localization of TRA-2-49 and SSEA1 in some parts of early differentiated cells were observed indicating primordial germ cell (PGC) formation. SSEA1 and VASA were expressed mainly in days 4 and 7 respectively in all groups. However, GDF9 and ZP3 were not observed in none.
Limitations, reasons for caution: Cumulus cells culture faced some difficulties such as contaminations in first attempts which were solved with more cautious in oocyte pick up procedures. High risk patients including HIV, HBS and HCV positive females were excluded from the study.
Wider implications of the findings: CCCM can improve the female germ cell development from hESCs as discovered by Q-PCR data. The basal medium itself plays a critical role for hESC differentiation.
Trial registration number: No applicable

P-794 Successful development of organoids from fresh and cryopreserved biopsy catheter derived endometrial tissue of subfertile women

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Study question: Can organoids be established from minimal invasive endometrium biopsies of subfertile women, and are the organoid characteristics comparable between fresh and cryopreserved biopsies?

Summary answer: Organoids can be developed from fresh and cryopreserved catheter-derived endometrium from subfertile women and are comparable regarding morphology, formation efficiency, expandability, gene and protein expression.

What is known already: Organoids are three-dimensional *in vitro* structures that self-organize from tissue subunits or stem cells, recapitulating key features of the organ of origin, and are largely expandable while remaining phenotypically-genetically stable. They offer possibilities to mimic patient- and disease-specific pathologies and to develop personalized treatments. Previously, endometrial organoids have been established from fresh biopsy catheter derived tissue (Turco et al. 2017) and fresh laparoscopically obtained tissue (Boretto et al. 2017) (healthy and cancerous). Endometrial organoids have not yet been developed from subfertile women, nor been grown from cryopreserved biopsy catheter derived tissue and directly compared with organoids from matched fresh tissue.

Study design, size, duration: In two nested cohort studies within the SCRaTCH randomized controlled trials (RCT), endometrial tissue was obtained during scratching in the luteal phase of the natural cycle or during an oral contraceptive cycle, using an endometrial biopsy catheter (Pipelle®). Ethical approval was obtained for this pilot study, in which tissue of 6 subfertile patients was subjected to endometrial organoid development after obtaining patients' broad written consent.

Participants/materials, setting, methods: Women with unexplained infertility and a good prognosis for spontaneous conception were eligible for one RCT, while the other RCT included women undergoing in-vitro fertilization (IVF) who have not become pregnant in the first full cycle. Endometrial tissue was cultured for organoid development (as described by Boretto et al. (2017)) immediately after the biopsy and after at least 1 week of cryopreservation. Briefly, tissue was digested, embedded in Matrigel and cultured under 'wingless/integrated' (WNT)-activating conditions.

Main results and the role of chance: Organoids developed from both fresh and cryopreserved catheter derived endometrial tissue in a similar manner: glandular fragments self-organized during the first 24 hours and grew into endometrial organoids within 1 week of culture, which could be consecutively expanded every 10-14 days. The organoids from both conditions showed similar morphology (cystic structures with epithelial cell border), comparable formation efficiency (n=3, mean % (organoids ≥100 µm/seeded cells) ± SD, fresh vs cryopreserved tissue, 1.48% ± 0.74% vs 2.10% ± 3.31%, p=1.000 (day 7), and 5.15% ± 5.57% vs 8.00% ± 13.07%, p=1.000 (day 10)), expandability (both up to at least passage 13) and immunophenotype (expression of ERα, PAEP, PR, E-cadherin, acetylated α-tubulin). Proliferation was not statistically different (Ki67 immunoreactivity in fresh vs cryopreserved tissue derived organoids, mean ± SD (n=3): 15.5% ± 2.5% vs 9.1% ± 2.9%, p=0.2500 (cultured without hormones), 21.5% ± 5.8% vs 18.1 ± 10.5%, p=0.7500 (treated with estradiol) and 10.1% ± 1.2% vs 7.4% ± 1.9%, p=0.2500 (treated with progesterone)). Gene expression analysis by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) showed no statistically significant differences between fresh and cryopreserved tissue derived organoids regarding the expression of several endometrial/epithelial markers (e.g. *FOXA2, ESR1, MUC1, PAEP*).

Limitations, reasons for caution: More patient samples may be needed for more solid conclusions of gene expression profiles, although the current sample size already clearly showed no effect on organoid morphology, expandability and immunophenotyping (protein expression).

Wider implications of the findings: The applicability of cryopreserved tissue to develop endometrial organoids allows organoid-based translation in fertility clinics when organoid research laboratories are not nearby. Furthermore, endometrial organoids will provide a valuable model to study many sorts of 'omics' in endometrial-related reproductive diseases and develop personalized treatments.

Trial registration number: 1. SCRaTCH trial, NTR5342, registered July 31st, 2015; 2. SCRaTCH-OFO trial, NTR6687, registered August 31st, 2017.

P-795 Autologous Mitochondria transfer to Improve Outcomes in Women With IVF Failures Due to Low Oocyte Quality: A Prospective Self-Controlled Study

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Study question: Can Bone marrow mesenchymal stem cell-Derived autologous mitochondria transfer into compromised oocyte change embryonic development kinetics and improve outcomes in Women With multiple IVF failures due to Low oocyte quality?

Summary answer: This study demonstrated that bone marrow mesenchymal stem cell-Derivedautologous mitochondria transfer doesn't alter embryonic development kinetics

What is known already: Qocyte quality plays an important role in fertilization and development to high -quality embryos. All of these development stages require high energy consumption, increasing the synthesis of mitochondrial adenosine triphosphate, which provides the energy necessary for the rupture of the germinal vesicle and the resumption of meiosis, for a normal fertilization, and for reducing the incidence of errors during the second meiotic division. Moreover, mitochondrial dysfunction has been suggested as a major cause of age-induced decline in oocyte quality.

Study design, size, duration: This Prospective Self-Controlled Study plans to conduct in our clinical center from August 2018 to August 2020. By means of a time-lapse system (EmbryoScope; Unisense FertiliTech, Aarhus, Denmark), this study determined the timing of a number of developmental parameters including cleavage timing from a zygote to a 8-cell embryo (t2, t3, t4, t5, t6, t7, t8) and assessed fragmentation at each stage.

Participants/materials, setting, methods: Infertile women <42 years of age, body mass index <30 kg/m2, at least one previous failed IVFdue to low embryo quality were included. Their oocytes were randomly and averagely divided into two groups i.e. Mitochondria transfer groups (MT) and control group. Mitochondria from bone marrow mesenchymal stem cell were isolated by differential centrifugation. In Mitochondria transfer groups (MT), 4000-5000 copies mitochondria DNA were injected into each oocyte during intracytoplasmic sperm injection.

Main results and the role of chance: A total of 18 patients were included and we got169 oocytes in total. till now. Their average age was 32.72 years old and antimullerian hormone leve was 3.28 ng/ml. 72.22% patients was primary infertility and the major cause of infertility was tubar factor. The results showed that the timings of all embryo cleavage stages (from t2to t8) together with fragmentation values showed no significant differences between embryos deriving from oocytes with MT or without MT. In addition , 2PN fertilization rate was similar between two groups (51.1% vs. 50%, P=0.959)

Limitations, reasons for caution: We will further follow up clinical pregnancy outcomes of embryos in two groups. A randomized clinical trial will be necessary to determine the true extent of any clinical benefits.

Wider implications of the findings: This is the first investigation to evaluate the impact of Bone marrow mesenchymal stem cell-derived Autologous mitochondria transfer on embryonic development kinetics. Results confirm that bone marrow mesenchymal stem cell-derived Autologous mitochondria transfer doesn't alter embryonic development kinetics. Autologous mitochondria injection is safe.

Trial registration number: NCT03639506

P-796 COMPARISON OF THE EFFECTS OF ALLOGENIC BONE MARROW AND ADIPOSA-DERIVED STEMCELLS TRANSPLANTATION ON REPRODUCTIVE FUNCTIONS OF MICE WITH CYCLOPHOSPAMIDE-INDUCED OVARIAN FAILURE

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Study question: Is the ability of allogenic ADSCs (adipose-derived stem cells) better than BMSC (bone marrow derived stem cells) on reproductive function of POF (premature ovarian failure) mice model?
Summary answer: POF mice model that received ADSCs had higher effect on reproductive functions than those who received BMSC.
What is known already: Several studies have been conducted on the role of stem cells in repairing ovarian reserve, while the effect of administering stem cells on endometrial receptivity is still unclear. Researchs showed that stem cells from different sources can have the same effect in saving ovarian function. Previous research has shown that BMSC and ADSCs transplants increases follicle growth and maturation, improves microevironment in the follicles. These processes then rebuild normal hormone function and decreasing the apoptosis process. It is not clear yet which one is more optimal between BMSC and ADSCs in improving reproductive function.
Study design, size, duration: Randomized post test control group design. This study was divided; Pre-experimental (making POF model mice) and the experimental. The size of the sample was obtained by replicating the formula for a complete random randomized trial ($n \geq 6$). Dependent variables on this study; serum AMH (anti-mullerian hormone) levels, integrin $\alpha v\beta 3$ expression of endometrial tissue, and pregnancy outcomes (birth characteristics i.e. number, birth weight, birth length). This research had been done in 2 months (October-December 2016)
Participants/materials, setting, methods: 56 female mice (wistar strain *Micetus norvergicus*)on diestrus phase were outbred
1.C(-): intraperitoneal injection of 0.9% NaCl
2.C(+): injection of Cyclophosphamide (Cyc) and NaCl 0.9%
3.BMSC group: Injection of Cyc and BMSC
4.ADSCs group: injection of Cyc and ADSCs
were mated, then for two groups, 7 mice were terminated (day-4) to assess serum AMH and integrin levels and 7 other were performed cesarean sections (day-19) to assess the characteristics of the offspring born. Two mice from each group were labeled
Main results and the role of chance: Pre-experimental found ovarian histopathological findings showed no follicles with intraperitoneal dose of 15 mg/kgBW Cyc for 2 weeks. Experimental stage on 56 mice, found significant differences in the serum AMH levels ($p = 0.001$), with higher values observed for ADSCs and BMSC group compared to the C (+) group. serum AMH levels in the ADSCs group were higher than the BMSC group, but not significant ($p = 0.7$). There was 11 folds increase in serum AMH levels at ADSCs and a 9 folds increase in BMSC group.

There were significant differences in the integrin $\alpha v\beta 3$ expression of endometrial tissue ($p = 0.01$), with higher values observed for ADSCs and BMSC group compared to the C (+) group. There was an increase in integrin $\alpha v\beta 3$ expression of endometrial tissue 2.4 folds upon administration of ADSCs and a 2.2 folds increase upon administration of BMSC.

There were significant differences in the number of offspring ($p = 0.005$), birth weight ($p = 0.002$) and birth length ($p = 0.02$), with higher values observed for ADSCs group compared to the BMSC group.

Tissue sample from labelled mice was examined under fluorescence microscopy and showed higher endometrial and ovarian tissues "homing" in ADSCs compared to BMSC group.
Limitations, reasons for caution: In this study there was no histopathological examination to the extent of damage of endometrium due to cyc inj. It is not known how long the effect of stemcells settles (optimally homed) on the reproductive organs. Special examination is needed to determine the rejection reaction in this study.
Wider implications of the findings: Research shows that ADSCs have several advantages in easily available sources, are not invasive, minimal rejection reaction. Allogenic stem cells can be considered to be an alternative therapy for stem cells without host rejection reactions risk, although further clinical trials are needed in this matter.
Trial registration number: -

P-797 Transcriptome analysis reveals potential role of autophagy in embryo fragmentation

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Study question: Is the fragmentation of In *vitro* fertilized (IVF) embryos derived from assisted reproductive technology (ART) caused by abnormal autophagy?
Summary answer: Alterations in autophagy could lead to molecular changes in multiple processes, and may influence cell growth and development.
What is known already: For IVF embryos derived from ART, fragmentation is a common feature, which could generate embryos with poor quality and reduce the success rate of ART. After fertilization, the maternal mRNA and proteins stored in oocytes are rapidly degraded and new mRNA and proteins encoded by the embryonic genome are synthesized. The autophagy pathway can drive the rapid cellular changes necessary for proper development. Although it has reported that programmed cell death or apoptosis is triggered in arrested fragmented human embryos, the exact molecular mechanism of autophagy in maintaining the intracellular homeostasis in embryogenesis is still unclear.
Study design, size, duration: In the primary study, we *in vitro* activated and inhibited the autophagy pathway in mouse embryonic stem cells (mESCs), respectively. Next, we performed the RNA-sequencing for each group, as well as normal control (2 samples per group), and analyzed their expression profiles.
Participants/materials, setting, methods: We used a mTOR kinase inhibitor (AZD8055) to block the mTOR pathway and activated the autophagy in mESCs. In another group, the autophagy process was down-regulated by inhibiting the lysosomes using chloroform. After extracting the total RNA from cells and depleting the ribosome RNA, libraries were generated and sequenced with paired-end reads. Then the expression profile was analyzed and the comparison was performed for activation group (AZD) and inhibition group (Chl) separately.
Main results and the role of chance: After treatment, we observed a significant increase of mESCs death in the AZD group and defined 1495 differentially expressed genes (DEGs) in the transcriptome analysis. Besides, about 366 genes aberrantly expressed in the Chl *vs* control. The main biological processes regulated by these genes included cell kill (*Tap2*), growth (*Wisp1, Rarg, Pou4f2, Dmbx1, Bmp4, Ntn1, Prox1, et al*), biological adhesion (*Nckap1, Plau, Jam2, Ccdc80, Tgfb2, Tnc, et al*) and metabolic process (*Cbr3, Apoe, Atp2b2, Abca1, Fgf8, Ighbp3, Tll1, Wnt3, et al*). In addition, we found a shared set of down-stream pathways was regulated in both groups, such as MAPK signaling pathway, Rap1 signaling pathway, TGF-beta signaling pathway, ECM-receptor interaction, Cell adhesion molecules and tight junction, which are essential pathways for normal embryo development. These molecular changes caused by altering the process of autophagy may result in abnormal cell growth and development.
Limitations, reasons for caution: We have only conducted preliminary exploration in mESCs and investigated the consequence of abnormal autophagy. The further comparison can be carried out between arrested fragmented human IVF embryos and normal embryos.
Wider implications of the findings: Studying the molecular changes influenced by autophagy could help to understanding its importance in regulating the embryo development. Our results will provide a basis on how autophagy impact the fragmentation of IVF embryos and contribute a potential new insight on optimizing culture conditions to improve the treatment of infertility.
Trial registration number: Not applicable.

P-798 Genome editing by CRISPR-Cas9 reveals that pre-implantation development in the mouse is compromised in Pou5f1-null embryos

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Study question: What is the role of *Pou5f1* in mouse pre-implantation development?

Summary answer: In mouse, *Pou5f1* expression is required for successful development to blastocyst stage, as *Pou5f1*-null embryos, edited by CRISPR-Cas9, exhibited a significantly lower blastocyst formation rate.

What is known already: Early mammalian embryogenesis is controlled by mechanisms that govern the balance between pluripotency and differentiation. The transcription factor Oct4, encoded by *Pou5f1*, is a key component of the pluripotency regulatory network. At blastocyst stage, the outer cells of the embryo differentiate into the trophectoderm, and *Pou5f1* expression becomes downregulated and restricted to cells of the inner cell mass (ICM). CRISPR-Cas9 has been used to examine the role of OCT4 during mouse and human preimplantation development, but the details of its effect on mouse embryonic development remain unclear.

Study design, size, duration: We compared CRISPR-Cas9 component delivery in two developmental stages, as it was previously shown in human that gene editing at an earlier stage, resulted in increased editing efficiency.

The degree of mosaicism (gene-edited vs non-edited cells within the embryo), and the mutational spectrum (different mutations within the embryo) were evaluated by immunofluorescent detection of Oct4 (n=29), PCR Fragment size Analysis (n=44) and Next Generation Sequencing (NGS) (n=24).

Participants/materials, setting, methods: An sgRNA-Cas9 mixture targeting exon 2 of *Pou5f1*, encoding the Oct4 protein, was either microinjected in mouse zygotes (n=21), or was co-injected with mouse sperm during ICSI in Metaphase-II oocytes (MII) (n=19).

The control groups consisted of sham-injected zygotes/ICSI-oocytes (n=10) and non-injected media-control zygotes/ICSI-oocytes (n=10).

Reconstructed embryos were cultured for 5 days in sequential KSOM/Cook Blastocyst medium. Embryonic development was assessed daily, with detailed scoring at blastocyst formation on day 5.

Main results and the role of chance: Genomic analysis of embryos revealed a very high gene editing efficiency at the blastocyst stage, similar for both groups, with 95.2% of embryos being edited in the zygote group and 100% in the MII group.

Within the edited embryos, no mosaicism was detected. More specifically, the edited embryos contained only mutant alleles while no wild type alleles were identified.

In addition, the mutational spectrum was similar for both groups. In the zygote group, 30% of embryos contained 1 mutant allele, compared to 40% in the MII oocyte group. The majority of embryos harbored 2-4 mutant alleles, being 70% for the zygote-group vs 60% for the MII group.

We further observed a stereotypic pattern in the type of indels introduced in independently targeted embryos, including a 28 bp deletion present in 50% of the edited embryos.

The development of *Pou5f1*-targeted embryos was compromised. In both groups, an average of 55,35% of the reconstructed embryos were arrested at the morula stage, while 32,15% formed blastocysts with reduced size blastocoel cavity and 9,5% formed blastocysts with reduced ICM. Blastocyst formation rates in the control and sham-injected groups were 86% and 88%, respectively.

Immunofluorescence analysis of reconstructed embryos confirmed efficient loss of Oct4 expression.

Limitations, reasons for caution: One of the major hurdles of CRISPR-Cas9 genome-editing in mouse embryos is the presence of a wide mutational spectrum, which may complicate phenotypic analysis of the injected embryos.

Furthermore, the efficiency of genome modification depends on the intrinsic properties of the targeted locus, and the effects may vary between targeted regions.

Wider implications of the findings: Early injection of CRISPR-Cas9 components results in absence of mosaicism and a similar mutational spectrum compared to later stage injections.

Our study reveals that CRISPR-Cas9-technology can be efficiently applied in the mouse model, paving the way for further studies on the role of key gene regulators on pre-implantation development.

Trial registration number: Not Applicable

P-799 Erythroblast Differentiation of Human Endometrium derived Induced Pluripotent Stem Cells as a Source of Autologous Transfusion

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Study question: Are human endometrium derived induced pluripotent stem cells (iPSc) compatible as a stable source of erythroblast cells for the development of a novel autologous transfusion strategy?

Summary answer: In an effort to develop autologous transfusion source, iPS cells driven from human endometrial cells stably yielded cells of eryhroid differentiation of upto orthochromatic normoblasts.

What is known already: Due to its regenerative potential and plasticity, endometrial tissue has been described in the literature as a favorable source of tissue regeneration. Human endometrium derived iPSc have been previously described as an efficient cell source for reprogramming into a pluripotent state. Resident adult stem cells in the basalis layer of the endometrium has been proposed as a source for in vitro differentiation into various lineages, owing to its high plasticity. Although erythroid differentiation has been attempted using embryonic stem cells or fibroblast driven iPSc, endometrium driven iPSc from hysterectomy patients are tested as a stable material for autologou transfusion.

Study design, size, duration: Discarded endometrial cells were obtained from 6 donor women receiving hysterectomies due to benign conditions. The endometrial layer was dissected from the myometrium and loose endometrial cells were dissociated enzymatically. By passaging these primary cells, endometrial stromal cells were allowed to dominate. A total of 15 iPSc lines were driven from these donors using the defined Yamanaka factors, followed by a two phase feeder and non-feeder hematopoietic differentiation procedure.

Participants/materials, setting, methods: pMIG-human sox2,oct4,klf4 and c-Myc vectors were used to transduce the endometrial stromal cells. Directed differentiation of established iPS cells were conducted in two phases, first on murine bone marrow stromal feeder cells(OP-9) followed by feeder free conditions with hydrocortisone, stem cell factor, interleukin-3, recombinant erythropoietin and poloxamer188. Expression profiles of KDR,CD235a+,CD34+,CD43+ and CD 71+ were analyzed by flow cytometry and Wright-Giemsa staining was performed for morphological analysis and differential counting.

Main results and the role of chance: The treatment of donor endometrial stromal cells with β estradiol at a concentration of 0.1μM/ml resulted in iPS cell reprogramming efficiency of approximately 166 ± 5% compared with the non-treated control cells set as 100%. As a result of inducing these cells to hematopoietic fate via a 9 day co-culture with murine stromal fibroblasts, yields ranging from 8-13% was observed depending on the donor. Because the potential of these co-cultured cells to further differentiate into erythroblastic fate may not be confined to defined cell surface markers, all of the OP-9 co-cultured cells were transferred to a feeder-free system composed of hydrocortisone, stem cell factor, interleukin-3, recombinant EPO and poloxamer 188 for 17 days, stably yielding over 80% of polychromatic and orthochromatic normoblasts. Flow cytometry expression profiles of KDR, CD235a+, CD34+, CD43+ and CD 71+ were compatible with the pattern of embyonic hematopoiesis and erythropoietic differentiation. A significant yield of 52 ± 4% polychromatic erythroblasts on day 7th and 30 ± 2% orthochromatic erythroblasts were observed on the 14th day of differentiation. The fraction of orthochromatic

erythroblasts further expanded to approximately 40% on day 17, showing clinically useful yields.

Limitations, reasons for caution: Erythropoietic differentiation up to the point of counting the ratio of polychromatic and orthochromatic normoblasts were evaluated. The process of retrieving enucleated reticulocytes was not selected as the end-point in this study and still requires refinement with further extension experiments.

Wider implications of the findings: A complete process of deriving iPSc with discarded human endometrium and consecutively directing differentiation into erythroid lineage was fully described. Significant yield of polychromatic erythroblasts on day7 and orthochromatic erythroblasts were observed on day 14, which may serve as a pilot study for developing a novel autologous source of transfusion.

Trial registration number: not applicable

P-800 Urine-derived stem cells facilitate endogenous spermatogenesis restoration of busulfan-induced non-obstructive azoospermia mice by paracrine exosomes

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Study question: whether transplantation of USCs or USCs exosomes (USC-exos) could promote endogenous spermatogenesis restoration in a busulfan-induced NOA mice model.

Summary answer: USCs could facilitate endogenous spermatogenesis restoration of busulfan-induced NOA mice through paracrine exosomes, but could not protect the mice testicles at early stage of destruction.

What is known already: Non-obstructive azoospermia (NOA) is the most severe issue without effective treatments in male infertility. Urine-derived stem cells (USCs) possess multipotent differentiation capacity and paracrine effects to participate in tissue repair and regeneration.

Study design, size, duration: NOA mice devided into 8 groups: Normal group, groups treated with no injection, PBS or USCs on the 0 days after busulfan administration respectively, groups treated with no injection, PBS, USCs or USC-exos on the 36 days after busulfan treatment separately.Thirty days after USCs and USC-exos transplantation, all mice were sacrified to detect spermatogenic markers.

Participants/materials, setting, methods: USCs were cultured and indentified. High-density USCs were cultured in hollow fiber bioreactor for exosomes collection. USC-exos were isolated from USCs conditional media and identified by transmission electron microscopy, western blot and Flow NanoAnalyzer. USCs and USC-exos were transplanted into interstitial space in testes of NOA mice (40 mg/kg).Immunofluorescence staining and hematoxylin and eosin (H&E) staining, *qRT-PCR* and western blot analysis were used to detect spermatogenic markers.

Main results and the role of chance: Thirty days after USCs and USC-exos transplantation, the spermatogenesis was restored by both USCs and USC-exos in NOA mice of 36 days after busulfan-treated confirmed by immunofluorescence staining and hematoxylin and eosin (H&E) staining. Moreover, spermatogenic genes (Pou5f1, Prm1 and SYCP3) and spermatogenic protein UCHL1 were significantly increased in both USCs 36 and USC-exos36 group compared to PBS treated group demonstrated by using *qRT-PCR* and western blot analysis. However, transplantation of USCs at day 0 after busulfan treatment didn't improve the spermatogenesis of NOA mice.

Limitations, reasons for caution: Despite the encouraging effect of USCs and USCs exosomes on NOA mice, the mechanism remains mysterious.

Wider implications of the findings: Our study provides a novel insight in the treatment of NOA.

Trial registration number: SYSU-IACUC-2018-000258

P-801 Increased primordial germ cell (PGC) specification from human embryonic stem cells (hESC) derived in Activin A

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Study question: Does pluripotency state affect differentiation of hESCs into PGC-like cells and can this be enhanced by supplementing with Activin A(ActA) or using hESCs derived in it?

Summary answer: HESCs derived in ActA, in '4i' state of pluripotency support best the derivation of PGC-like-cells, which is further enhanced by supplementation with ActA during differentiation

What is known already: Stem cell derived gametes can serve as a treatment as well as a model to understand gametogenesis and infertility. Human gamete precursors or PGC-like cells can be obtained from hESCs differentiated as embryoid bodies (EBs) in the presence of Bone Morphogenetic Protein4 (BMP4), Stem-Cell Factor (SCF), Epidermal Growth Factor (EGF), human-Leukemia Inhibitor Factor (hLIF) & Rho-Kinase inhibitor (ROCKi). In mouse, 'naive' ESCs cultured in ActA form a germline competent epiblast-like condition, eventually resulting in derivation of functional oocytes and fertile offspring. In human, it remains unclear if the pluripotency state or exposure to ActA affects PGC derivation.

Study design, size, duration: Female hESC lines, UGENT 11-2 and UGENT-11-4A (derived in ActA), cultured in distinct conditions (primed, Wnt-I (Wnt pathway inhibited) and naïve (4-inhibitors (4i) and RSeT). Another male line UGENT12-3A derived in ActA was cultured in the 'primed' condition without and with continuous supplementation of ActA (UGENT-12-A2) followed by conversion to '4i' state. PGC-like cells were derived all conditions, immunostained using cell surface antibodies and quantified using flow cytometry.

Participants/materials, setting, methods: HESCs in all conditions were force-aggregated (6500 cells/EB) and cultured 4 days in embryoid body differentiation medium (EBDM) containing BMP4, SCF, EGF, hLIF and ROCKi or in EBDM with ActA (20ng/ml) in hypoxic conditions. Day 4 EBs were immunostained for OCT4, SOX17 and PDPN to identify PGC-like cells, imaged as z-stacks and quantified using FIJI, followed by analysis with one-way ANOVA. Additionally, Day4 EBs were also dissociated, immunostained with TNAP and PDPN, quantified using FACS.

Main results and the role of chance: We found that the specific pluripotency state affects the germline induction potential of hESCs. PGC-like cells co-expressing OCT4, SOX17 and PDPN, were observed in EBs from all conditions, with 4i-hESCs showing highest competency. In UGENT 11-2, 4i-hESCs yielded significantly higher number of PGC-like cells per EB than primed-hESCs ($p=0.03$) and RSeT-hESCs ($p=0.002$). In ActA-derived UGENT11-4A, the number of PGC-like cells per EB was highest in 4i-hESCs ($p=0.0003$).

We found that ActA-derived lines responded better to germline derivation. The average PGC-like cells yield from the '4i' EBs of in the conventional UGENT 11-2 was 281 PGC-like cells per EB while the ActA-derived UGENT 11-4A was 1102 PGC-like cells per EB. EBDM+ActA increased this yield significantly ($p<0.0001$).

Continuous culture of primed ActA-derived hESC in ActA did not improve the PGC-like cell yield when converted to the '4i' state . Instead, it compromised their competence to form PGC-like cells in UGENT 12-A2. The '4i' UGENT 12-2A hESCs responded to EBDM with 56% TNAP-PDPN double-positive live single cells in Day 4 EBs and showed an increased yield of TNAP-PDPN double-positive cells to 71.7% with EBDM+ActA.

Limitations, reasons for caution: The trancriptome of the stem cells and PGC-like cells derived in EBDM and EBDM+ ActA are currently being analysed.

This data would add robustness to the study as well as highlight markers in ActA-derived lines which lead to increased yield of PGC-like cells

Wider implications of the findings: The current study aims to identify PGC markers and conditions to standardise the process of deriving gametes from human pluripotent stem cells. Adaptation of culture conditions based on this knowledge may ultimately advance efforts to derive germ cells in vitro for patients currently facing sterility.

Trial registration number: This study is funded by Innovation by Science and Technology in Flanders (IWT, Project Number: 150042). The authors declare they have.

P-802 Differentiating mouse embryonic stem cells into primordial germ cell-like cells and early meiotic stages by exposure to adult interstitial and neonatal testicular cells

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Study question: Does culturing mouse embryonic stem cells with adult interstitial and neonatal testicular cells increase efficiency of differentiation to primordial germ cell-like cells and induce meiosis?

Summary answer: Mouse adult interstitial and neonatal testicular cells were able to support differentiation of mouse embryonic stem cells (mESCs) into male germ cells.

What is known already: Multiple studies have been conducted outlining the differentiation of mESCs into epiblast-like cells (EpiLCs), primordial germ cell-like cells (PGCLCs), and later into spermatid-like cells, through cultured media. The spermatogenesis pathway has been replicated in vitro with the goal of treating and alleviating male infertility. Expressions of Oct3/4, Nanog, DAZL, and VASA at different times of the differentiation process can identify the stage of the cells. Interstitial cells from mouse testis are known to play an important role in the development of the reproductive system and support the completion of the spermiogenic process through secretion of hormones and other factors.

Study design, size, duration: Interstitial cells isolated from adult or neonatal testes of B6D2F1 mice were plated on mesh interphases. mESCs with mouse embryonic fibroblasts were plated below and differentiated in this conditioned medium. Three wells with increasing ratios of adult or neonatal testicular cells to mESCs were used (1:5, 2:5, 3:5). Three wells with no mesh interphase were the control. Each contained approximately $1.2x10^6$ mESCs. Germ cell stage-specific biomarkers were assessed after a few days of culturing.

Participants/materials, setting, methods: Over a 6-month period, mESCs were differentiated into EpiLCs and PGCLs. Interstitial cells were isolated from adult or neonatal mouse testes using differential plating and were plated on mesh interphases. After 7 passages, mESCs were plated and cultured in the mesh interphase system at 37°C and 5% CO_2. Immunofluorescence was used to analyze the efficiency and stages of differentiation by staining with Nanog, Oct4, DAZL, and VASA.

Main results and the role of chance: At day 3 of differentiating mESCs in mesh interphase-conditioned medium containing Activin A, bFGF, and KSR, and co-culture with interstitial cells derived from adult or neonatal testis, some cells were trypsinized and analyzed by immunofluorescence. A decreased positivity of Nanog indicated the successful differentiation of mESCs into EpiLCs. Continued expression of Oct3/4 was detected in the cells at day 3, suggesting the retention of stemness. Expression of DAZL in the cytoplasm at day 5 demonstrated differentiation to PGCLC, indicating progression to the germ cell lineage. On day 8, approximately 30% expressed VASA positivity, indicating entrance to meiosis. Cells cultured in wells with a 1:5 ratio containing adult interstitial cells had greater expression of DAZL and VASA. DAZL and VASA expression was negative in the control group, suggesting the important role of the supporting testicular cells. This indicates that the presence of interstitial cells may further differentiate the EpiLCs into PGCLCs earlier on.

Limitations, reasons for caution: Despite successfully differentiating mESCs into EpiLCs and PGCLCs and early meiotic stages, the efficiency of differentiation through culture with adult and neonatal cells into spermatid-like cells need to be confirmed. Additional studies on further differentiation should be conducted to more completely study spermatogenesis in vitro.

Wider implications of the findings: Coaxing the differentiation of mouse embryonic stem cells into the early male gamete can be accomplished in the presence of factors secreted from the testicles of adult mice. Reproducing spermatogenesis in vitro may provide valuable information on overcoming male infertility due to spermatogenic arrest or germ cell aplasia.

Trial registration number: N/A

P-803 Activation of endogenous Steroidogenic factor 1 (SF-1) expression in 46,XY human pluripotent stem cell-derived gonadal-like cells guides the development of testicular cell fates

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Study question: What is the role of Steroidogenic factor 1 (SF-1) in the development of two testicular somatic cell types, Sertoli cells and Leydig cells, in human?

Summary answer: SF-1 is required for guiding the differentiation of bipotential gonad stage cells into Leydig cell-like steroidogenic and Sertoli cell-like non-steroidogenic cells early in human development.

What is known already: Steroidogenic factor 1 (SF-1), an orphan nuclear receptor encoded by *Nuclear receptor subfamily 5 group A member 1* (*NR5A1*), is essential in the reproductive and adrenal development and in endocrine regulation. In mice, loss of *Nr5a1* (Sf-1) leads to gonadal and adrenal aplasia, and in human mutations in *NR5A1* (SF-1) have been identified in patients suffering from complete or partial gonadal dysgenesis. Sf-1 expression initiates in the bipotent genital ridge and persists in testicular pre-Sertoli and Leydig cells. In mice, Sf-1 is required for fetal Sertoli and Leydig cell development and for differentiation towards steroidogenic cell lineages.

Study design, size, duration: This study was conducted using human induced pluripotent stem cells containing a Tetracycline-inducible dead Cas9 (dCas9), a Trimethoprim (TMP)-regulatable destabilization domain and CRISPR guide RNAs for targeting the *NR5A1* (SF-1) promoter. Cells were differentiated for 10 days. The levels of steroidogenic hormones were measured from culture supernatants in triplicates and induction of cyclic adenosine 3', 5'-cyclic monophosphate (cAMP) from duplicate samples.

Participants/materials, setting, methods: Cells were differentiated in basal medium containing Activin A, CHIR-99021, Dorsomorphin and Y-27632 (1d), BMP-7 and CHIR-99021 (2d), followed by differentiation in basal medium supplied with/without doxycycline (DOX) and TMP for 6 days. At day 8 of differentiation cells were stimulated with follicle stimulating hormone (FSH) and luteinizing hormone (LH) for 1, 8 or 24 hours. Gene expression levels were monitored with quantitative polymerase chain reaction (qPCR) at 7-8 time points.

Main results and the role of chance: Cell differentiation into primitive streak (PS) -like stage and intermediate mesoderm (IM) was demonstrated by detecting an increase in the expression levels of respective markers; BRACHYURY for PS and PAX2, LHX1 and OSR1 for IM. By day 8 of differentiation, bipotential gonadal markers (GATA4, WT1, LHX9 and EMX2) were expressed in the differentiated cells of both non-treated (-DOX, -TMP) and treated (+DOX, +TMP) conditions. Conditional activation of SF-1 by DOX and TMP in cells differentiating towards gonadal fate initiated expression of SF-1 at day 6 of differentiation, and the expression persisted until the completion of culturing. Notably, in cells where SF-1 was activated (+DOX, +TMP) the expression levels of steroidogenic markers (STAR, CYP11A1, CYP17A1 and HSD17B3) were greatly increased relative to the undifferentiated cells. Furthermore, by day 8-10 of differentiation an increase in the expression levels of several testicular markers: anti-Mullerian hormone (AMH), inhibin alpha subunit (INHA) and SOX9 (specific markers of Sertoli cells), and luteinizing hormone receptor (LHR, a Leydig cell marker) could be detected. By contrast, in non-treated cells the expression of SF-1, steroidogenic, and testicular markers remained at basal level.

Limitations, reasons for caution: The cell identity could not be entirely resolved with the methods applied in this study and thereby, the differentiated cells likely represent a heterogeneous population.

Wider implications of the findings: This study demonstrates that SF-1 is required for the development of testicular steroidogenic cells and for the induction of Sertoli cell-specific gene expression in human. This is the first human *in vitro* model, in which endogenous activation of SF-1 defines the testicular steroidogenic and non-steroidogenic cell fates.
Trial registration number: not applicable

P-434 Effect of microgravity on frozen human sperm samples. Can they be sent to space?

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Study question: How can microgravity affect frozen human sperm samples? Can frozen sperm samples be safely sent to space for possible future use under different gravitational conditions?
Summary answer: Frozen sperm samples preserved in cryostraws and stored in a specific nitrogen vapour cryoshipper do not suffer significant alterations after exposure to microgravity (μG).
What is known already: Microgravity affects lipid structures and enzymatic reactions in mammalian cells. The effects of microgravity on the cardiovascular system, muscular-skeletal apparatus, nervous system and male and female reproductive systems among others have been reported. There is relatively little known about the effects of different gravitational environments than that of Earth's gravity (1G) on human gametes/embryos. Some data suggest a significant decrease in the motility of human fresh sperm samples but nothing is known about the possible effects of gravitational differences on frozen human gametes, in which state they would be transported from earth to space.
Study design, size, duration: Prospective study carried out in a University-affiliated ART centre in collaboration with a Polytechnic University and an Aeroclub specialized in aerobatic parabolic flights for scientific research. A CAP10B plane that offers short-duration hypogravity exposure was chosen to obtain microgravity conditions. The plane executed series of 20 parabolic manoeuvres,x which means 8 seconds of microgravity for each parabola. Overall 10 sperm samples obtained from 10 healthy donors were analysed after exposure to microgravity and ground gravity.
Participants/materials, setting, methods: Samples were split in two and frozen using glycerol as cryoprotector, aliquoted in high security straws and stored in liquid nitrogen until the day of the experiment when they were exposed to μG and 1G conditions. After thawing, evaluation of sperm concentration and motility using a computerized semen motility analyzer, vitality(eosin-nigrosin staining), morphology (eosin-tiazin staining), DNA fragmentation (Sperm Chromatin Dispersion) and apoptosis degree by magnetic activated cell sorting using annexin-V microbeads were performed.
Main results and the role of chance: Comparing mean values between control group (1G) and study group (μG) no significant statistical differences were found in any of the parameters analysed ($p \geq 0.05$):

- Sperm concentration (M/ml) 48.15±41.20 vs 48.05±35.37 (0.10 95% CI [-5.56;5.76])
- Sperm motility (M/ml) 16.01±12.98 vs 17.16±13.78 (-1.14 95% CI [-4.33;2.05])
- Progressive a+b sperm motility (%) 23.95±13.17 vs 22.08±11.26 (2.22 95% CI [0.94;1.25])
- Sperm vitality (%) 43.23±10.21 vs 43.94±7.80 (0.97 95% CI [0.87;1.08])
- Morphologically normal spermatozoa (%) 8.54±3.93 vs7.40±2.97 (1.14 95% CI [0.96;1.35])
- DNA sperm fragmentation (%) 12.25±5.43 vs 12.38±5.07 (0.98 95% CI [0.83;1.16])
- Apoptotic spermatozoa (%) 19.55±14.24 vs 13.09±10.34 (1.39 95% CI [0.72;2.70])

100% concordance was observed in vitality diagnosis and DNA sperm fragmentation diagnosis after μG exposure in comparison with the initial diagnosis at 1G. 90% concordance was observed in sperm concentration and sperm progressive motility diagnosis according to the World Health Organization standards, and 80% in morphological assessment according to Kruger Criteria. Minor differences observed were more probably related to heterogeneity of the sperm sample than to the effect of exposure to different gravity conditions.
Limitations, reasons for caution: Aerobatic parabolic flights offer a limited time of microgravity (5-8 seconds) as well as short periods (1-3 seconds) of hypergravity preceding and following μG. Other platforms such as Random positioning machines or the Space Station could be considered. More cases have to be analysed to confirm the results.
Wider implications of the findings: The lack of differences observed in the sperm characteristics between frozen samples exposed to microgravity and those maintained in ground conditions open the possibility of safely transporting male gametes to space and considering the possibility of creating a human sperm bank outside Earth.
Trial registration number: NCT03760783

Human Reproduction, Vol.34, No.Suppl, pp. i512–i543, 2019

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