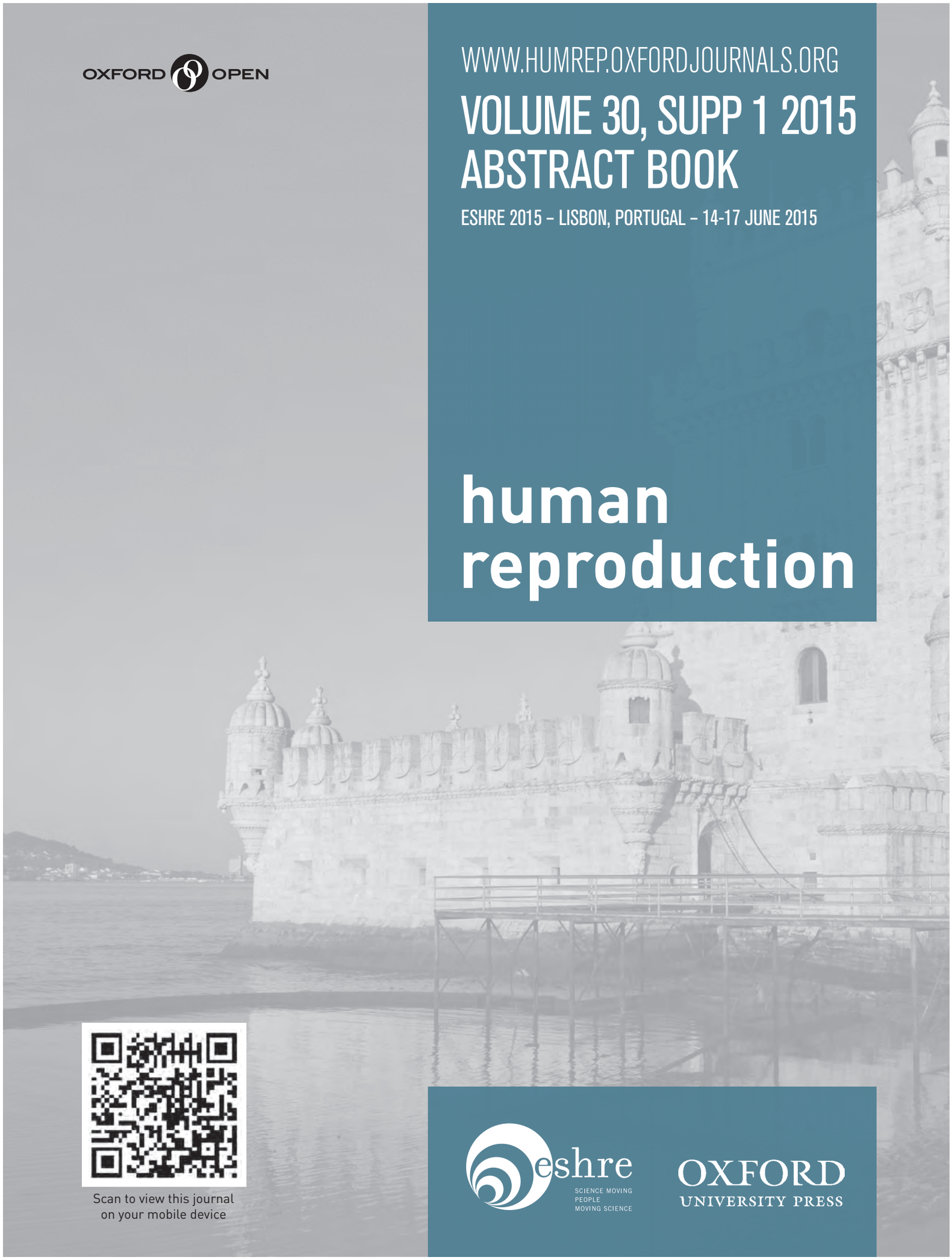

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ESHRE 2015 – LISBON, PORTUGAL – 14-17 JUNE 2015

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Abstracts of the 31st Annual Meeting of the European Society of Human Reproduction and Embryology

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14 to 17 June 2015

Abstracts

31st Annual Meeting of the
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The cover of *Human Reproduction* shows histone acetylation in two human germinal vesicle (GV) stage oocytes. The upper panels show an early-stage GV oocyte with a non-surrounding nucleolus stained for (A) chromatin (DAPI; blue) and (B) histone acetylation (anti-H4K12ac; red). Note the regions of intense chromatin staining in some areas, whereas others show no acetylation (C; overlay). The lower panels, of a more developed oocyte with a surrounding nucleus stained for chromatin (D) and histone acetylation (E), show more condensed chromatin than in the early-stage oocyte (above), although the oocyte still has some acetylated chromatin as shown in E and overlay (F). For more details see van den Berg *et al.*, pp. 1181–1190.

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INVITED SESSION

SESSION 01: ROBERT G. EDWARDS' MEMORIAL KEYNOTE SESSION

Monday 15 June 2015 **08:30–09:30**

O-001 Human reproduction keynote lecture – preconception stress increases the risk of infertility: results from a couple-based prospective cohort study, the LIFE study

C. D. Lynch[1], R. Sundaram[2], J. M. Maisog[2], A. M. Sweeney[3], G. M. Buck Louis[2]
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[3]*Texas A&M Health Science Center, Department of Epidemiology and Biostatistics, College Station, U.S.A.*

Study question: Are women's stress levels prospectively associated with fecundity and infertility?
Summary answer: Higher levels of stress as measured by salivary alpha-amylase are associated with a longer time-to-pregnancy (TTP) and an increased risk of infertility.
What is known already: Data suggest that stress and reproduction are interrelated; however, the directionality of that association is unclear.
Study design, size, duration: In 2005–2009, we enrolled 501 couples preconceptionally in a prospective cohort study in Michigan and Texas, USA. Couples were followed for up to 12 months and through pregnancy if it occurred. A total of 401 (80%) couples completed the study and 373 (93%) had complete data available for this analysis.
Participants/materials, setting, methods: Enrolled women collected saliva the morning following enrollment and then the morning following their first observed study menses for the measurement of cortisol and alpha-amylase, which are biomarkers of stress. TTP was measured in cycles. Covariate data were captured on both a baseline questionnaire and daily journals.
Main results and the role of chance: Among the 401 (80%) women who completed the protocol, 347 (87%) became pregnant and 54 (13%) did not. After adjustment for female age, race, income, and use of alcohol, caffeine, and cigarettes while trying to conceive, women in the highest tertile of alpha-amylase exhibited a 29% reduction in fecundity (longer TTP) compared with women in the lowest tertile [fecundability odds ratio = 0.71; 95% confidence interval (CI) = (0.51, 1.00); $p < 0.05$]. This reduction in fecundity translated into a more than twofold increased risk of infertility among women these women [Relative Risk = 2.07; 95% CI = (1.04, 4.11)]. In contrast, we found no association between salivary cortisol and fecundability.
Limitations, reasons for caution: Due to fiscal and logistical concerns, we were unable to collect repeated saliva samples and perceived stress questionnaire data throughout the duration of follow-up. Therefore, we were unable to examine whether stress levels increased as women continued to fail to get pregnant.
Wider implications of the findings: This is the first US study to demonstrate a prospective association between salivary stress biomarkers and TTP, and the first in the world to observe an association with infertility.
Study funding/competing interest(s): This study was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (contracts #N01-HD-3-3355, N01-HD-3-3356, N01-HD-3358). There are no conflicts of interest to declare.
Trial registration number: NA.
Keywords: fecundity, infertility, stress

O-002 How a dead duck can be fertile

Moeliker Kees[1]
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For witnessing and publishing 'The first case of homosexual necrophilia in the mallard duck (*Anas platyrhynchos*)' the speaker was awarded the 2003 Ig Nobel Prize in the field of Biology. Thanks to this much coveted award he became known as ´The Duck Guy´ and people from all over the globe send him their own observations of and/or publications on non-reproductive sexual behavior in animals. Here he presents highlights from his ever growing 'Necrophilia Files', including some severe cases that were overlooked for decades.
To address the topic of human reproduction too, the speaker will also share his insights in possibly the most dramatic example of current habitat destruction and loss of biodiversity – the disappearance of the once-ubiquitous crab louse (*Pthirus pubis*) due to excessive removal of pubic hair among sexually active people.
Keywords: ducks, animal behaviour, non-reproductive sexual behaviour, necrophilia, pubic louse

SELECTED ORAL COMMUNICATIONS

SESSION 02: NEW PERSPECTIVES ON EMBRYO SCORING

Monday 15 June 2015 **10:00–11:30**

O-003 Embryo quality scoring: a correlation between trophectoderm development and aneuploidy rate in developing blastocysts

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Study question: Blastocysts cultured for IVF are graded based on stage of hatching and organization of the inner cell mass and trophectoderm. Common practice is to implant embryos based on quality score in cases where preimplantation genetic screening (PGS) is not used. Is blastocyst embryo quality score correlated with chromosomal ploidy?
Summary answer: PGS is critical to improving success rates in implantation and live birth during IVF. In cases where PGS is not used prior to implantation, embryo quality score can be used to determine the likelihood of embryo aneuploidy based on strong correlations between embryo aneuploidy and *in vitro* trophectoderm development.
What is known already: It is common to select embryos for transfer based on morphology during IVF. A retrospective study assessing ploidy and general blastocyst morphology suggests that blastocysts graded "excellent" have a higher euploid rate than embryos graded "good," "average," and "poor," but the value of this information is limited to the general definitions of morphology used. A better understanding of how ploidy effects blastocyst morphology and organization may help improve efficacy in non-PGS related IVF transfers.
Study design, size, duration: Embryo quality scores have been obtained for 528 blastocysts from 112 patients all using the same IVF center. Trophectoderm biopsies from 528 blastocysts underwent PGS for chromosomal abnormalities using array CGH or Next Generation Sequencing and the results were compared to embryo quality score to determine any existent correlations.
Participants/materials, setting, methods: Blastocyst stage embryos were scored according to the standard Gardner blastocyst grading system by a single embryologist. Amplified trophectoderm biopsies were analyzed using either Illumina's 24-sure array CGH or VeriSeq NGS protocols and chromosomal profiles were compared to embryo score to determine correlates.

Main results and the role of chance: We received blastocyst morphology scores for 528 embryos (112 patients) from a single IVF clinic. All 528 embryos were subjected to PGS using aCGH or NGS platforms and chromosome ploidy was determined for all but 4 embryos (all had poor quality or no DNA post biopsy). Embryos were grouped based on their Gardner scale embryo score (3–6 for stage of hatching, A–C, for inner cell mass and trophectoderm score with A being optimal) and genotypes were compared. Embryos with the optimal A trophectoderm score exhibited a significantly higher euploid rate (79.31%) compared to those with a B (60.51%) or a C (38.66%) trophectoderm. No correlation was found between stage of hatching or inner cell mass organization and embryo ploidy at this time.
Limitations, reason for caution: Identifying a correlation between blastocyst morphology and ploidy provides an additional resource for optimal embryo transfer but does have limitations. The process is dependent on consistent scoring and grading of the blastocyst and on the judgment of the individual embryologist, which undoubtedly varies across all clinics.
Wider implications of the findings: All subjects seeking IVF treatments stand to benefit from a more accurate system of identifying suitable embryos for transfer. In cases where PGS is not used, a comprehensive analysis of embryo morphology is the most efficient method in obtaining the successful transfer of an embryo. The findings of this study may transform the assessment of embryo morphology and establish a more universal methodology in the morphological determination of embryos that are suitable for transfer.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Genesis Genetics.
Trial registration number: NA.
Keywords: PGS, IVF, embryo morphology, blastocyst

O-004 An investigation into the developmental potential of mosaic embryos

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[2]*University of Oxford, Nuffield Department of Obstetrics and Gynaecology, Oxford, United Kingdom*
[3]*Tecnobios, Bologna, Italy*

Study question: The existence of preimplantation embryos composed of a mixture of diploid and aneuploid cells has been recognised for more than two decades, but the developmental fate of such embryos has been impossible to determine. Can mosaic diploid-aneuploid blastocysts implant and lead to ongoing pregnancies and chromosomally normal births?
Summary answer: Most mosaic diploid-aneuploid blastocysts either fail to implant or miscarry after transfer, although a minority do succeed in producing chromosomally normal pregnancies. These findings confirm that diploid-aneuploid embryos have reduced developmental capacity in comparison with uniformly euploid embryos, but that some are potentially viable.
What is known already: Mosaicism – the presence of chromosomally distinct cell lines within the same embryo – is common during preimplantation development, affecting ~30% of blastocysts. Mosaicism is caused by incorrect segregation of chromosomes, most often arising during the first three cleavage divisions following fertilisation. Technical limitations have precluded detection of mosaicism in preimplantation embryos transferred to the uterus, preventing determination of its impact on clinical outcome and causing uncertainty over the clinical utility of such embryos.
Study design, size, duration: The study involved next-generation sequencing (NGS), a method capable of detecting aneuploidy in trophectoderm biopsies, even when only a minority of cells are affected. Genetic material from transferred embryos, surplus after aneuploidy screening using microarray-CGH, was reassessed using NGS, revealing mosaicism. Outcomes for mosaic and non-mosaic embryos were compared.
Participants/materials, setting, methods: 42 mosaic blastocysts were identified following reanalysis of biopsied material using NGS. All embryos had been transferred and had a known clinical outcome (i.e., implantation, miscarriage, birth). Outcomes for these embryos were compared to those for 51 blastocysts, predicted to be uniformly euploid, derived from a well-matched contemporary control group.
Main results and the role of chance: Of the blastocysts with mosaicism detected, 62% did not implant, 12% miscarried and only 26% produced ongoing pregnancies. A total of 109 mosaic abnormalities were detected, affecting almost all chromosomes, 63% of which affected whole chromosomes, the remainder involving chromosomal fragments (segmental). 30% of the mosaic embryos contained a mixture of segmental and whole chromosome abnormalities, 40% had whole chromosome errors only and 30% had segmental errors alone. Blastocysts with mosaic whole chromosome aneuploidy failed to implant significantly more often than those with mosaic segmental abnormalities (P = 0.0038). Embryos with mosaic errors affecting three or more chromosomes never produced an ongoing pregnancy. Control group outcome comparison showed that both implantation and ongoing pregnancy rates were reduced by a third when mosaic embryos were transferred.
Limitations, reason for caution: Analysis of entire mosaic blastocysts should be undertaken in order to determine the extent of euploidy and aneuploidy within them.
Wider implications of the findings: NGS succeeded in detecting mosaic abnormalities difficult to identify using previous methods. Most mosaic blastocysts failed to implant or miscarried, although a minority produced viable, euploid pregnancies. It can be concluded that mosaic embryos are usually compromised by the presence of aneuploid cells and have reduced developmental potential. However, if no entirely normal embryos are found, those with only 1–2 aneuploidies or segmental abnormalities could be considered for transfer (with appropriate patient counselling and prenatal follow-up).
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Reprogenetics UK.
Trial registration number: NA.
Keywords: embryo, mosaicism, next generation sequencing, implantation potential

O-005 Does disappearance of multinucleation from the 2-cell to 4-cell stage observed by time time-lapse correlate chromosomal normality detected by PGS microarray?

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[2]*Fakih Fertility Center, IVF Clinic, Abu Dhabi, United Arab Emirates*
[3]*Fakih Fertility Center, IVF Clinic, Dubai, United Arab Emirates*

Study question: Is multinucleation at the 2 cell and 4 cell stage of embryonic development associated with increased aneuploidy rate and reduced blastocyst formation rate?
Summary answer: Multinucleation in the 2 cell stage embryos is relatively frequent during embryonic development, even in euploid embryos. Early multinucleation could later disappear when embryos progress to 4 cell stage. This transitory event can be monitored using Time-lapse imaging system.
What is known already: Time lapse technology allows embryo development to be monitored continuously. Embryos that have multinucleated blastomeres have a greater chance of being chromosomally abnormal. Detection of multinucleated blastomeres either on day 2 or 3 is associated with poor prognosis to blastocyst formation and chromosomal status of day 3 embryos.
Study design, size, duration: This is a retrospective study including 261 embryos from 50 patients (mean age = 34.9). Embryos were monitored in a special tri-gas incubator with built-in time-lapse monitoring system (EmbryoScope™, Unisense, Danmark). Data was collected from cycles carried out between January 2014 and December 2014.
Participants/materials, setting, methods: After standard microinjection, oocytes were placed in the EmbryoScope and cultured in Global Total Medium (LifeGlobal, IVF Online) under mineral Oil (Sage). Images were acquired every 20 min from 7 different focal planes. On day 3 of embryonic development, 236 embryos were biopsied and analyzed by array-CGH technique. Multinucleation (MN) was annotated at 2-cell and 4 cell-stage. Blastocyst formation was scored by according to Gardner's grading system (Gardner, 1990). For statistical analysis Chi-square contingency test was performed using Prism (GraphPad).
Main results and the role of chance: Among 236 embryos analyzed that had multinucleation at 2-cell and 4-cell stage, 70 (29.6%) were detected as euploid. In Table 1 shows parameters and number of embryos with multinucleation at 2-cell and 4-cell stage; and also embryos that did not have multinucleation at both states.

Table 1

	Group 1 MN at only 2-cell stage	Group 2 MN at 2 and 4-cell stage	Group 3 No MN
No of embryos	87	33	116
Age	35.6 ± 6.02	36.3 ± 5.65	33.8 ± 5.84
Blastocyst Formation rate	57.4% (50/87)	51.5% (17/33)	63.7% (74/116)
Euploidy Rate	31.0% (27/87)	15.1% (5/33)	32.7% (38/116)

P-value > 0.05.

Limitations, reason for caution: Number of embryos and cycles included in the study.

Wider implications of the findings: Although not significant, embryos with multinucleation both 2-cell stage and 4-cell stage, have higher aneuploidy rate compared to those with multinucleation at only at the 2-cell stage (31.0% vs. 15.1%). Blastocyst formation rate was similar in both groups (57.4% vs. 51.5%, $p > 0.05$). These findings suggest that time lapse observation of multinucleation at both 2-cell and 4-cell stages coincides with higher aneuploidy. A larger study including higher number of embryos are needed to investigate this association.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Fakih Fertility Center, Abu Dhabi, UAE.

Trial registration number: 0

Keywords: A-CGH, multinucleation, time lapse, ICSI, blastocyst

O-006 Embryos with cell division aberrations monitored by time-lapse imaging in a PGS program: are they able to develop into euploid blastocysts?

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Study question: What is the ultimate fate of embryos with cell division abnormalities (e.g., direct cleavage, reverse cleavage and asymmetric cleavage) observed using time-lapse technology? How do affected embryos evolve throughout preimplantation development and is there evidence of aneuploidy rescue?

Summary answer: The majority of embryos with cleavage aberrations arrested. Those which developed into euploid blastocysts excluded some cells during morula compaction. Analysis of the excluded cells revealed a higher incidence of aneuploidies with respect to corresponding trophectoderm cells, providing evidence for a potential self-correction mechanism for mosaic embryos.

What is known already: Erroneous cleavage divisions (such as 1–3 cells and 2–5 cells) are considered negative morphological indicators of embryo viability and are associated with a significant reduction in implantation potential. While most anomalous cleavage divisions are expected to produce aneuploid cells, it is unknown whether this occurs in all cases. Additionally, the existence of systems for the elimination of aneuploid cells has been proposed, although thus far no mechanisms that could achieve such 'self-correction' have been described.

Study design, size, duration: 130 patients underwent PGS and 730 embryos were retrospectively morphokinetically analyzed. Cleavage aberrations were observed affecting 89 embryos and only 19 of these formed blastocysts. Chromosomal analysis was carried out to determine embryo ploidy status. Additionally, excluded cells of 11 euploid blastocysts, which developed from partially compacted morulae, were analyzed.

Participants/materials, setting, methods: Embryo development was retrospectively analyzed using a time-lapse imaging system (Embryoscope, Unisense Fertilitech). Multiple cell divisions were measured and evaluated. Embryo biopsy was undertaken on Day 5–6 and comprehensive chromosome screening performed through Array-CGH on trophectoderm and excluded cells.

Main results and the role of chance: Out of 730 embryos obtained from 130 patients (mean age 39.8 ± 4.4), 89 (60 patients, mean age 39.1 ± 4.1) showed cell division aberrations. Of those, 70 arrested or were discharged (78.7%): 47 arrested at <8/9 cell stage (67.1%), 13 at morula stage (18.6%) and 10 developed into not transferrable blastocysts (14.3%). Only 19 blastocysts out of 89 were suitable for TE biopsy (21.3%): 14 were found to be euploid (73.7%) and 5 aneuploid (26.3%). All the euploid blastocysts were derived from partially compacted morulae, whereas all the fully compacted morulae produced aneuploid blastocysts. Analysis of cells excluded by 11 chromosomally normal blastocysts while at the partially compacted morula stage revealed aneuploidy in 5 samples, apoptosis in 4 samples and euploidy only in 2 samples.

Limitations, reason for caution: Limited number of cases of the study group. Further studies are recommended.

Wider implications of the findings: This study revealed the fate of embryos displaying morphokinetic aberrations, confirming high rates of aneuploidy and developmental arrest. Furthermore, a potential mechanism of aneuploidy 'rescue' was identified. The results suggest that mosaic embryos may form partially compacted morulae in order to exclude aneuploid cells, which subsequently undergo apoptosis. Not only is this finding of great biological interest, but there are also significant clinical ramifications, especially in terms of cells chosen for biopsy during PGS.

Study funding/competing interest(s): Funding by hospital/clinic(s) none.

Trial registration number: None.

Keywords: cell division aberrations, time lapse, aneuploidy rescue, ArrayCGH, morula compaction

O-007 Immunohistochemical study of abnormal mitosis in development of human embryos

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Study question: What is the consequence of abnormal mitosis on preimplantation development? And what are the mechanisms of formation for supernumerary nuclei?

Summary answer: We demonstrate that nuclear abnormalities are frequent in early human embryos, associated with DNA damage, the presence of centromere-less chromosomal fragments, poor developmental potential, and chromosomal mosaicism. These results show that a significant portion of the genomic abnormalities in embryo development arise from post-fertilization mitosis.

What is known already: The focus in genomic abnormalities in embryo-development research has been on oocyte meiosis, although reports assert that mosaicism may frequently arise post-fertilization. It is also known that blastomeres can contain extra-nuclear DNA (i.e., micro and macro-nuclei) in pre-implantation human embryos (cleavage and blastocyst stage). Mosaicism is challenging to clinically diagnose, as multiple cells are required, and the mechanisms of their formation are still poorly defined.

Study design, size, duration: An internal review board approved, academic laboratory in-vitro research study, on consented, donated cryopreserved (2000–2010) human embryos, from an university-affiliated infertility clinic. A total of 163 cryopreserved cleavage stage embryos containing 731 blastomeres were analyzed. Mitotic abnormalities, including multiple nuclei, abnormal spindles, abnormal cytokinesis were quantified.

Participants/materials, setting, methods: Vitrified stage embryos of good or poor quality were thawed, stained for markers to study chromosome segregation (anti-centromere, pericentrin, and β-tubulin), and DNA damage (phospho-γH2ax). Confocal microscopy was used to analyze abnormal DNA segregation, cytokinesis anomalies, and distinguish spindle attachment from DNA packaging errors (nuclear, mitotic spindle, ±centromere).

Main results and the role of chance: Cleavage stage blastomeres contained multinucleation in 15.7% (115/731 blastomeres), higher in low quality arrested embryos (66%), comparing to successful developing blastocyst (5%), suggesting it adversely affects preimplantation development ($p < .0001$). Mechanistically, multinucleation arises if abnormal chromosome segregation

[pre-mitotic (chromosome breaks preventing centrosome-microtubule-spindle attachment), and mitotic errors (abnormal chromosome attachment due to intrinsic microtubule-spindle defects)], and cytokinesis, defined for embryos containing a supernumerary centrosomes and larger size blastomeres within the embryo. Moreover, cells with a high number of multinucleation exhibited failed cytokinesis, and suggest that is the consequence of abnormal chromosome segregation. We found that cells with micronuclei frequently lack centromeres (49%), and in all their DNA content analysis showed mosaicism, indicating DNA damage as the cause for micronucleation that directly correlates with chromosomal aneuploidy.

Limitations, reason for caution: The data on cytokinesis are preliminary and are further investigated. Additional molecular studies regarding the mechanism of abnormal cytokinesis will be performed.

Wider implications of the findings: This study reinforces the relevance of mitotic errors in human embryo development and demonstrates that abnormal mitosis significantly contributes to their developmental arrest. The presence of abnormally segregating mitotic chromosomes, and nucleoplasmic bridges suggest abnormalities in chromosome cohesion/segregation as the cause for abnormal nucleation. We also report that defective cytokinesis as an abnormal blastomeric development mechanism, secondary of abnormal chromosome segregation, and that centromere absence suggest pre-mitotic DNA damage as the cause for multinucleation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – The study was funded by the Naomi Berrie Diabetes Research Center.

Trial registration number: NA.

Keywords: mitosis, cytokinesis, human embryo, developmental arrest, multinucleation

O-008 Multi-nucleation in human embryos should not be used as a marker for their elimination from candidates for transfer

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Study question: Is the appearance of multinucleated blastomeres (MNBs) at 2-cell stage a marker for developmental failure of human embryos?

Summary answer: Appearance of MNBs is not always a predictor of chromosomal aneuploidy. The blastulation rate of embryos with MNBs is similar to that of embryos without MNBs. Blastocysts developed from embryos with MNBs have a full term developmental competence to healthy babies.

What is known already: MNBs are frequently observed in human embryos with poor morphology. Its appearance would be associated with low pregnancy rates following transfer probably due to chromosomal aberration or chromosome mosaicism. Although such morphological abnormalities implicate developmental failure of embryos, it remains unknown whether these properties lead to aneuploidy and implantation failure.

Study design, size, duration: This study was approved by the ethical committee of the Japan Society of Ob/Gy. We assessed relationships between the appearance of MN in human embryos and their development *in vitro* using 139 donated embryos and *in vivo* by retrospective analysis of time-lapse images of 46 embryos which were singly transferred.

Participants/materials, setting, methods: Donated pronuclear ova were injected with a mixture of mRNAs encoding EGFP-α-tubulin and mRFP1-histone-H2B. Dynamic changes of their chromosomes were monitored continuously using a confocal microscope inside an incubator for 120 h. Chromosome analysis was performed using microarray-CGH. Time-lapse images of embryos in clinical study were captured using PrimoVision.

Main results and the role of chance: Fluorescent imaging study revealed that 74% of embryos (80/108) showed MNBs at 2-cell stage after RNA injection. Nevertheless, embryos with MNBs developed to the blastocyst stage (45%, 36/80) and morphologically good blastocysts (15%, 12/80). These values were similar to those obtained from embryos without MNBs (blastulation rate 50%, good blastocyst rate 18%). The duration between first and second cleavage was 8.5–12.0 h for embryos with MNBs that developed to the blastocyst stage. Seventy percent of blastocysts developed from embryos with MNB were found to be euploid (14/20). This value was also similar to that of embryos without MNBs (75%). In clinical study, sixteen embryos (35%) with MNBs transplanted following transfer and four healthy babies were born at the present time (January 5, 2015).

Limitations, reason for caution: Multi-nucleation of embryos at 2-cell stage which had normal developmental competence appeared late at night. Thus, it is difficult to observe multi-nucleation of normal embryos based on conventional observation without time-lapse system. Further studies are required to make critical evaluation of normal and abnormal embryos showing multi-nucleation.

Wider implications of the findings: This study suggests that the appearance of MNB at first mitosis does not directly correlate with chromosomal aneuploidy and subsequent embryo development. It would be of negligible significance to assess the presence of MNBs compared with the observation of timing of mitosis.

Study funding/competing interest(s): Funding by national/international organization(s) – Japan Society for the Promotion of Science. No other competing interests are declared.

Trial registration number: Japan Society of Ob/Gy (Obstetrics and *Gynecology*) 112.

Keywords: microarray-CGH, delivering, fluorescent imaging, multi-nucleation, time-lapse imaging

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SELECTED ORAL COMMUNICATIONS

SESSION 03: PCOS: FROM LABORATORY TO CLINICAL SCIENCE

Monday 15 June 2015 **10:00–11:30**

O-009 The neurokinin B receptor antagonist AZD4901 decreases LH and testosterone secretion in women with PCOS: a randomised, double-blind, placebo-controlled clinical trial

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Study question: To test the hypothesis that Neurokinin B (NKB) receptor antagonism will reduce LH secretion and thereby decrease testosterone in women with Polycystic Ovary Syndrome (PCOS).

Summary answer: The selective NKB receptor antagonist AZD4901 reduced LH secretion and pulse frequency in women with PCOS, with a significant and sustained fall in serum testosterone.

What is known already: PCOS is characterized by elevated serum testosterone (T) concentrations, and associated with increased luteinizing hormone (LH) secretion and accelerated pulse frequency. Recent discoveries have led to the emergence of the kisspeptin-NKB-dynorphin system as the pivotal modulator of GnRH and gonadotropin secretion: manipulation of this pathway thus presents a therapeutic target to address a central pathophysiology in PCOS.

Study design, size, duration: We undertook a randomized, double-blind, placebo-controlled trial with daily oral dosing of 20, 40 or 80 mg of AZD4901 or placebo for 28 days. *Primary outcome:* Change in 8 h LH area-under-curve (AUC) between baseline (day-1) and day 7 vs. placebo. *Secondary outcomes* included changes in LH pulsatility and testosterone.

Participants/materials, setting, methods: *Key eligibility criteria:* Diagnosis of PCOS (≤6 cycles/year, hyperandrogenemia (Free T > 0.85 ULN) and

polycystic ovary morphology). *Statistical analyses:* LH AUC was calculated by trapezoid summation. LH pulsatility was analysed with blinded deconvolution. Comparisons between groups were performed using mixed effects models for repeated measures with baseline as a covariate.

Main results and the role of chance: Sixty-five women were randomized (27 ± 5 years, BMI 31.5 ± 6.0 kg/m^2, mean ± SD), with 13 to 16 patients contributing to the primary endpoint in each of the four arms. At day 7, LH AUC reduced by 52% (95% CI 30–67%) in the 80mg group (from 67.4 ± 1.6 to 36 ± 2.3 IU/L*h) compared to placebo (from 61.1 ± 1.9 to 69.8 ± 1.7 IU/L*h); $p = 0.0003$. LH pulse frequency also reduced from 5.8 ± 2.1 to 3.7 ± 2.1 pulses/8hr in this group (from 7.2 ± 2.3 to 6.8 ± 2.6); $p < 0.0001$ vs. placebo. Concurrently, testosterone decreased by 29% (14–41%) at day 7 in the 80mg group (from 2.2 ± 1.3 nmol/L to 1.6 ± 1.5) vs. placebo (from 1.5 ± 1.7 to 1.6 ± 1.9 nmol/L); $p = 0.0006$. Average T reduction was maintained in this group over the remaining study period (17% decrease from baseline vs. placebo at day 28).

Limitations, reason for caution: Larger studies with a longer duration of follow-up assessing clinical outcomes are required to fully assess therapeutic potential. Applicability of our findings to the subset of women with normo-androgenemic phenotype requires further investigation.

Wider implications of the findings: We have demonstrated the potential for Neurokinin B antagonism as a therapeutic approach to address LH and testosterone hypersecretion in PCOS. This approach addresses the central neuroendocrine pathophysiology in this common, yet unmet, medical need. Our findings are consistent with decreased LH secretion and pulse frequency observed in patients with NKB signaling defects. Neurokinin B antagonism may therefore have potential in other reproductive disorders where decreased gonadotropin secretion is of therapeutic benefit.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – AstraZeneca.

Trial registration number: Clinicaltrials.gov; NCT01872078.

Keywords: PCOS, testosterone, LH pulsatility

O-010 Randomized controlled trial of the effects of metformin versus combined oral contraceptives in adolescent PCOS women through a 24 months follow up period

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Study question: What are the effects of metformin and combined oral contraceptive pills (COC) over 24-month period on adolescent girls with PCOS presenting hyperandrogenemia and menstrual irregularities

Summary answer: Metformin and COC have comparable therapeutic effectiveness on cycle regularity and hirsutism. Metformin was associated with significant weight loss and improvement in the metabolic syndrome, while COC was associated with non-significant weight gain and deterioration of the metabolic syndrome.

What is known already: No studies for 24-month period. Follow up for 12-month only showed no difference in the therapeutic effectiveness between metformin and the COC on hirsutism and acne, but metformin treatment resulted in a reduction in fasting insulin and lower triglyceride levels than COC.

Study design, size, duration: Randomized controlled trial. The study included 119 adolescent girls. Participants were randomly divided into three groups, using computer-generated random-number tables. The duration of follow up was 24 months. Based on 0.8 power to detect a significant difference ($P = 0.05$, two-sided), 32 patients were required for each study group.

Participants/materials, setting, methods: PCOS was defined according to Rotterdam consensus workshop group, 2004. *Group A (n = 40):* received oral metformin 1700 mg/day, *group B (n = 40):* received low-dose combined oral contraceptives, and *group C (n = 39):* were followed without treatment in the outpatient clinic of Alexandria IVF/ICSI center.

Main results and the role of chance: In group B significant decline in serum testosterone reached the lowest value by the end of the 2-year (0.7 ± 0.2 vs. 1.3 ± 0.5 ug/ml). By the end of the study, group A showed a significant decline in fasting (18.6 ± 3.0 to 10.0 ± 3.0 μIU/ml) and after-load insulin levels (126 ± 43 to 64 ± 15 μIU/ml) with a significant rise in glucose/insulin ratio (GIR) from 4.1 ± 0.3 to 4.6 ± 0.5. Group B showed significant rise in fasting and after-load insulin (from 15.0 ± 3.0 mIU/ml and 103.0 ± 91.0 μIU/ml to 19.0 ± 4.0 and 187.0 ± 22.0 μIU/ml respectively) and GIR dropped significantly from 4.4 ± 0.2 to 3.1 ± 0.3. Metformin was associated with a significant loss of weight from 87.0 ± 6.0 to 72.0 ± 0.5 Kg while COC was associated with a non-significant gain in weight (from 84.0 ± 6.0 to 91.0 ± 9.0 Kg).

Limitations, reason for caution: None.

Wider implications of the findings: The choice of the proper line of therapy should be tailored for every patient, according to her age, stage in life, presenting symptoms, various personal and familial risk indices, as well as her own informed choices. This study suggests that insulin sensitizers are a proper choice for adolescent PCOS women presenting with acne and hirsutism, having a safer profile than combined oral contraceptives.

Study funding/competing interest(s): Funding by University(ies) – None.

Trial registration number: PACTR201307000567163.

Keywords: PCOS, combined oral contraceptives, adolescent, metformin, insulin resistance

O-011 Overweight and obese but not normal weight women with PCOS are in risk for pre-diabetes and type 2 diabetes mellitus: prospective population-based cohort study

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Study question: What are the respective roles of polycystic ovary syndrome (PCOS) and weight gain and/or overweight/obesity for the development of pre-diabetes [impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)] or type 2 diabetes mellitus (T2DM) by the end of reproductive life (at 46 years)?

Summary answer: PCOS was associated with an increased risk of pre-diabetes and T2DM, and PCOS in association with overweight/obesity was the strongest risk factor for both disorders, whereas the risk was not increased in normal-weight women with PCOS.

What is known already: The risk of developing pre-diabetes and T2DM has been shown to be increased in women with PCOS. However, additional prospective longitudinal studies are needed to study the respective roles of PCOS *per se*, body mass index (BMI) and weight gain on the development of pre-diabetes and/or T2DM.

Study design, size, duration: In the prospective follow-up Northern Finland Birth Cohort 1966 ($n = 5889$) questions on oligo-amenorrhea (OA) and hirsutism (H) were asked at 31 years (81% answered) and diagnosis of PCOS at 46 years (72% answered). Clinical examinations were performed at 14, 31 and 46 years and 2-hour oral glucose tolerance test (OGTT, $n = 2780$) was performed at 46 years.

Participants/materials, setting, methods: Women reporting both OA + H and/or PCOS diagnosed by practitioner were considered as having PCOS ($N = 279$). The outcomes [(IGF ($n = 101$), IGT ($n = 217$) and T2DM ($n = 313$)] were diagnosed according to OGTT. T2DM diagnosis was also asked in questionnaires (31 and 46 years) and verified from the national drug and hospital discharge registers.

Main results and the role of chance: The increase in BMI between all three time-points (14, 31 and 46 years) was significantly greater in women with PCOS who developed pre-diabetes or T2DM than in women with PCOS and normal glucose tolerance. The risks of impaired glucose metabolism [IGM (defined as pre-diabetes and/or T2DM), odds ratio (OR) 1.97, 95% confidence interval (CI) 1.32–2.95] and T2DM (OR 3.00, 95% CI 1.68–5.36) were increased in women with PCOS. The risks of pre-diabetes (OR 3.89 95% CI 1.99–7.61), T2DM (OR 13.62 95% CI 5.72–32.42) and IGM (OR 6.22 95% CI 3.64–10.62) were increased in women with PCOS and BMI ≥ 25 kg/m^2, but not in women

with PCOS and BMI < 25 kg/m^2. All results were adjusted for consumption of alcohol, smoking and socioeconomic status at age 46.

Limitations, reason for caution: The diagnosis of PCOS was based on self-reporting, which may have led to information bias. Ovarian ultrasonography was not available to aid the diagnosis of PCOS. The relatively high rate of missing data after combining the 31- and 46-year data (questionnaire and clinical examination) may have biased the results.

Wider implications of the findings: The risk of IGM was increased in women with PCOS. Overweight/obesity in association with PCOS was the strongest predictor for pre-diabetes and T2DM, whereas the risk was not increased in normal-weight women with PCOS. Moreover, weight gain during the long follow-up played a crucial role for development of pre-diabetes or T2DM in women with PCOS. These results emphasize the role of weight gain prevention during adolescence and early adulthood.

Study funding/competing interest(s): Funding by hospital/clinic(s) Funding by national/international organization(s) Finnish Medical Society Duodecim, the North Ostrobothnia Regional Fund, the Academy of Finland, the European Commission, Medical Research Council, the National Institute for Health Research.

Trial registration number: NA.

Keywords: polycystic ovary syndrome, type 2 diabetes mellitus, pre-diabetes, overweight, obesity

O-012 Metabolism alteration in follicular niche: the nexus between intermediary metabolism, mitochondrial function, and classic polycystic ovary syndrome

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Study question: What is the function of intermediary metabolism in follicular fluid and mitochondria of cumulus cell from classic PCOS patients?

Summary answer: Follicular fluid intermediary metabolic profiles provide signatures of classic PCOS ovary local metabolism and establish close link with mitochondria dyfunction of cumulus cells and highlighting the role of metabolic signal and mitochondrial crosstalk involved in the pathogenesis of classic PCOS.

What is known already: Classic polycystic ovary syndrome (PCOS) is a high-risk phenotype accompanied by increased risks of reproductive and metabolic abnormalities; however, the local metabolism characteristics of the ovaries and their effects on germ cell development are unclear.

Study design, size, duration: This study analyzed Follicular fluid (FF) and cumulus cells (CCs) collected from PCOS and matched control group at the time of oocyte retrieval.

Participants/materials, setting, methods: 95 FF of PCOS group and 55 FF of Control group were collected for the targeted metabolomics study. 80 CCs of PCOS group and 91 CCs of Control group were collected to assess mitochondrial function by transmission electron microscopy, flow cytometer,immunofluorescence, real-time-PCR and luminometer.

Main results and the role of chance: The targeted metabolomics study showed that glycolysis pathway was down regulated, BCAA catabolism pathway was up regulated, acylcarnitines was decreased, ketone bodies was increased, TCA cycle was up regulated and dysregulated, metabolites of NAD catabolism was decreased in FF of PCOS group. The mitochondria in CCs from PCOS patients showed abnormal mitochondrial structure, a decreased transmembrane potential, an aggregated distribution of mitochondria and a decreased mtDNA content with a decrease PGC-1a mRNA expression and hypermethylation of PGC-1a promoter. Follicular fluid intermediary metabolic profiles provide signatures of classic PCOS ovary local metabolism and establish close link with mitochondria dyfunction of cumulus cells and highlighting the role of metabolic signal and mitochondrial crosstalk involved in the pathogenesis of classic PCOS.

Limitations, reason for caution: The relationship of those alterations with clinical parameters of PCOS such as metabolic parameters and pregnancy rate, need further analyzed.

Wider implications of the findings: All those changes of intermediates metabolites have important epigenetic modifying roles could influence post-translational modification. Those epigenetic and PTM alterations could induce dysfunction of mitochondrial, and vice versa. The study of crosstalk between intermediary metabolism and mitochondrial function confirm the important role of epigenetic and PTM in the pathogenesis of classic PCOS, and probably bring a more integrated understanding of pathogenesis and possibly, new therapeutic opportunities for women with classic PCOS.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported in part by the Ministry of Science and Technology of China Grants (973 program; 2014CB943203 and 2011CB944504), the National Natural Science Funds for general program (31371521 and 31230047). The authors have no conflict of interest to declare.

Trial registration number: NA.

Keywords: polycystic ovary syndrome, metabolism, follicle fluid, cumulus cells, pregnant outcome

O-013 LncRNAs expression signatures of cumulus cells isolated from PCOS patients revealed by microarray

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Study question: Polycystic ovary syndrome (PCOS) is the most common and complex endocrinopathy, being found in 6–8% of women at reproductive age and accounting for about 75% of anovulatory infertility. To date there has been no study of lncRNAs expression profiles or their functional roles in PCOS.

Summary answer: Clusters of lncRNAs were aberrantly expressed in PCOS patients compared with non-PCOS patients, which revealed that lncRNAs differentially expressed in cumulus cells may exert a partial or key role in hormone abnormalities of PCOS patients and maybe impact the oocyte development.

What is known already: Long noncoding RNAs (lncRNAs) are an important class of pervasive genes involved in a variety of biological processes including reproductive and metabolic pathways of the endocrine system.

Study design, size, duration: In this study, we described lncRNAs profiles in cumulus cells isolated from ten patients (5 PCOS and 5 non-PCOS patients) by microarray.

Participants/materials, setting, methods: Seventy patients (thirty-five women with PCOS and thirty-five normal women without PCOS) referred to our center for IVF were included in this study after obtaining written informed consent. The cumulus cells isolated from ten patients (5 PCOS and 5 non-PCOS patients) were used for microarray analysis of lncRNAs and that isolated from the other sixty patients (30 PCOS and 30 non-PCOS patients) were used for an extra evaluation.

Main results and the role of chance: From the data, we found 623 lncRNAs were significantly up-regulated or down-reguated, which could be used to discriminate PCOS from non-PCOS cumulus cells (≥twofold). Of them, the up-regulated lncRNAs (n = 620) were more common than down-regualted ones (n = 3). In addition, the distribution of the differentially expressed lncRNAs was unbalance in all human chromosomes, i.e., there are 74 lncRNAs were transcribed from regions on chromosome 2 while only 7 lncRNAs were transcripts from regions on chromosome 22. Further analysis showed the differentially expressed lncRNAs were classified into three subgroups: HOX loci lncRNAs (n = 2), Enhancer-like lncRNAs (n = 87) and lincRNAs (n = 6). Five differentially expressed lncRNAs (ENST00000454271, ENST00000433673, ENST00000450294, ENST00000432431 and XLOC_011402) were selected to validate of the microarray results by quantitative RT-PCR.

Limitations, reason for caution: More work will be needed to determine whether these lncRNAs can serve as new therapeutic targets and diagnostic biomarkers in PCOS.

Wider implications of the findings: As a common endocrine and metabolic disorder in women, the occurrence or abnormal oocyte development of PCOS was proved to be regulated by a lot of lncRNAs. To reveal the functions of differentially expressed lncRNAs will provide potential targets for further treatment of PCOS and novel insights into other endocrine diseases.

Study funding/competing interest(s): Funding by national/international organization(s) – National Basic Research Program (grant 81170622 and grant 81401172).

Trial registration number: The study is not a RCT research.
Keywords: PCOS, long noncoding RNA (lncRNA), cumulus cells, microarray

O-014 Combined effect of insulin resistance condition and environmental pollutant – cadmium on cell survival and steroidogenesis in human granulosa cells from follicular fluid of IVF patients

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Study question: Environmental endocrine pollutant-cadmium (Cd) is a known risk factor for dysfunctional granulosa cell. Because insulin resistance (IR) is also involved in granulosa cell malfunctioning and is currently targeted predominantly, we questioned whether Cd along with insulin resistance is aggravating the functioning of granulosa cells ultimately leading to aberrations in follicular development.
Summary answer: Inhibition of steroidogenesis and decrease in cell survival with respect to decrease in protein expression of StAR, CYP19A1, 17β-HSD and 3β-HSD, progesterone and estradiol concentrations and increase in cleaved PARP-F2, active caspase-3 and ANNEXIN-V/PI staining respectively was observed in insulin resistant human granulosa cells treated with 32 μM Cd as compared to control.
What is known already: IR is prevalent in 60–80% of women with polycystic ovarian syndrome (PCOS). The tolerable weekly intake for Cd is 2.5 μg/Kg body weight. 32 μM Cd has been observed in follicular fluid of cigarette smoking females undergoing IVF. Cd inhibits hypothalamus pituitary ovarian axis, steroidogenic enzymes in ovary and granulosa cells, different developmental stages in rodents. Pre-clinical studies on combined exposure of Cd and IR on granulosa cells of rats revealed deleterious effect on reproductive function.
Study design, size, duration: *Control vs. treatment*: Granulosa cells were isolated from 18 donors and 24 PCOS women. IR was confirmed in PCOS granulosa cells by protein expression of IR, p-IRS-1(307), PI(3)K, p-Akt, PPAR-g. Cd dose was standardized by MTT assay. Control and PCOS-IR granulosa cells were incubated with 32uM Cd for 24 hrs.
Participants/materials, setting, methods: *Invitro* granulosa cell culture: control, control + Cd, PCOS-IR, PCOS-IR + Cd, in triplicates, repeated 3 times. Estradiol and progesterone analysis from supernatant and protein expression analysis of StAR, CYP11A1, CYP19A1, 17b-HSD, 3b-HSD, cleaved PARP-F2, active caspase-3 from cell lysate. ANNEXIN-V/PI staining by FACS and Confocal microscopy for cell death.
Main results and the role of chance: In PCOS-IR significant decrease in protein expression of IR ($p < 0.01$), PI(3)K ($p < 0.05$),increase in p-IRS-1(307) ($p < 0.001$), p-Akt ($p < 0.01$) and PPAR-g ($p < 0.01$) as compared to control and PCOS-non IR granulosa cells. MTT revealed that 32 μM Cd revealed significant decrease in granulosa cell viability as compared to control ($p < 0.001$). In PCOS-IR + Cd group significant decrease in protein expression of StAR ($p < 0.001$, $p < 0.05$, $p < 0.05$), CYP19A1 ($p < 0.01$, $p < 0.01$, ns), 17β-HSD ($p < 0.001$, ns) and 3b-HSD ($p < 0.01$, $p < 0.01$, ns), progesterone ($p < 0.05$, ns, $p < 0.001$) and estradiol concentrations ($p < 0.001$, ns, $p < 0.001$). In cell death parameters increase in protein expression of cleaved PARP-F2 ($p < 0.001$ and $p < 0.05$), active caspase-3 ($p < 0.01$ and $P < 0.05$) and a positive ANNEXINV/PI staining as compared to control, PCOS-IR and Cd group was observed indicating granulosa cell death through apoptosis. (ns = non significant).
Limitations, reason for caution: Insulin resistance was confirmed at cellular level because of unavailability of serum samples from the patients.
Wider implications of the findings: Although individual parameters were variedly affected at protein level in different groups, overall effects seemed to me more deleterious in PCOS-IR + Cd group. Increase in apoptosis and decrease in steroidogenesis would affect the normal developmental programme of the follicle by depriving nutrients required for the achievement of a healthy oocyte and its release ultimately compromising fertility at the level of preconception. Research on IR and pollutants needs to be emphasized for better understanding of idiopathic infertility.
Study funding/competing interest(s): Funding by national/international organization(s) – CSIR-SRF.

Trial registration number: Registration number is not required for this study.
Keywords: insulin resistance, cadmium, human granulosa cell, apoptosis, steroidogenesis

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SELECTED ORAL COMMUNICATIONS

SESSION 04: TESTIS FUNCTION: DIAGNOSIS AND RISKS

Monday 15 June 2015 **10:00–11:30**

O-015 Opioid receptors are present in male germ cells and modulate meiosis

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Study question: To analyze the expression and distribution of the three types of opioid receptors in male germ cells and analyze its role during the spermatogenesis
Summary answer: Male germ cells express active mu-opioid receptor (MOR), delta-opioid receptor (DOR) and kappa opioid receptor (KOR) in mice and describe its function as a modulator of mice spermatogenesis
What is known already: Numerous studies have demonstrated the presence of endogenous opioid peptides in different testicular cell types, providing evidences that the opioid system participates in an important way in the regulation of testicular function. However, the exact role of the opioid system during spermatogenesis has not been clarified since the presence of the three receptors: MOR, DOR, KOR remain unknown.
Study design, size, duration: Transillumination-assisted microdissection technique we used to obtain the stage-specific segments of seminiferous tubules for qRT-PCR and Immunoblotting thecniques. Isolated total testis cells were treated for 1 h and 24 h, and used in functional experiments, immunocytochemistry and FACS analyses. Treatments: morphine, DPDPE and U-50488 as the MOR, DOR and KOR selective agonist respectively.
Participants/materials, setting, methods: Testis from 100 adult healthy *Swiss* male mice. qRT-PCR, Immunoblotting, Immunofluorescence and FACS approches.
Main results and the role of chance: We have evidenced for the first time that active MOR, DOR and KOR are present in male germ cells by qRT-PCR, Immunoblotting and Immunofluorescence techniques. By using flow cytometry and qRT-PCR approaches we found that the three receptors act as a modulator of spermatogenesis, being the response time-exposure dependent. Our results suggest that the three opioid receptors are involved in meiosis of the mice spermatogenesis by modifying the expression of synaptonemal complex proteins SYCP1 and SYCP3.
Limitations, reason for caution: *In vitro* studies.
Wider implications of the findings: The presence of opioid receptors in mice spermatogenic cells, contribute to resolve several long-standing issues concerning the role of opioid receptors in spermatogenesis and open up novel avenues of research of the opioid system as a biochemical tool for the diagnosis and treatment of male infertility.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s) – This work was supported by grants from by a grant from the Basque Government (GIC 12/173).) IM and MG (Zabalduz) was supported by fellowship from Basque Government (Zabalduz). IU was supported by fellowship from University of the Basque Country and HE was supported by fellowship from Gangoti Barrera Foundation. The authors have no conflicts of interest to declare. The authors have no conflicts of interest to declare.
Trial registration number: No trial registration number.
Keywords: Opioid receptors, spermatogenesis, fertility, meiosis

O-016 Environmental exposure to selected endocrine disrupting chemicals adversely influences reproductive hormone levels in men

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Study question: Does environmental exposure to bisphenol A (BPA), phthalates and alkylphenols affect reproductive hormone levels (follicle stimulating hormone (FSH), luteinising hormone (LH), total testosterone (T), sex hormone

binding globulin (SHBG), oestradiol (E) and inhibin B) in men attending reproductive medicine clinic?
Summary answer: Selected endocrine disrupting chemicals (EDCs), as phthalates and their metabolites, can adversely affect the levels of reproductive hormones measured in serum of men attending reproductive medicine clinic, however, there was no evidence confirming the adverse effects of BPA or alkylphenols.
What is known already: Reproductive toxicities of BPA, phthalates and alkylphenols have been extensively studied in laboratory animals. A number of recent epidemiologic studies have also suggested toxic effects of BPA and certain phthalates on human male reproductive system, but the results of these are not conclusive, since there is considerable amount of contradicting data. There are no epidemiologic studies to investigate the influence of environmental exposure to alkylphenols on human health.
Study design, size, duration: Prospective cohort study included 140 men of subfertile couples seeking fertility treatment at university-based tertiary centre. Data was collected from February 2011 until June 2012. Single-spot urine samples were obtained to measure EDCs and creatinine concentration to account for urinary dilution. Non-fasting blood samples were taken to measure hormone levels.
Participants/materials, setting, methods: Gas chromatography/mass spectrometry was used to measure: BPA, nonylphenol (NP), octylphenol (OP), di(2-ethylhexyl)-phthalate (DEHP), dibuthyl-phthalate (DBP), diethyl-phthalate (DEP) and their metabolites. Additionally to the measured hormones, free androgen index (FAI), inhibin B/FSH and T/LH ratios were calculated to evaluate Sertoli and Leydig cell function, respectively.
Main results and the role of chance: BPA was detected in 98% of all measured samples. Phthalates with their metabolites were detected in >95% of the urine samples, OP was detected in 90% and NP was detected in 79% of samples with 0.3 ng/mL limit of detection. After adjusting for confounding factors using linear regression models, DEHP and its metabolites showed negative association with T (primary metabolite mono-(2-ethyl-5-oxohexyl)phthalate b = -0.61, 95% CI -1.13 to -0.10), inhibin B (DEHP β = -12.24, 95% CI -23.06 to -1.44) and with inhibin B/FSH ratio (DEHP β = -0.19, 95% CI -0.32 to -0.05). DEP has shown inverse correlation with LH in univariate analysis, however this was insignificant in multivariable model (β = -0.05, 95% CI -0.11 to 0.01). There was no influence of BPA or alkylphenols on any of the observed outcomes.
Limitations, reason for caution: Urinary EDC exposure measurements are subject to temporal variability. EDCs can also cause negative reproductive effects by other pathways that are not reflected in altered serum hormone levels. Many studied outcomes provide a potential for incidental correlations.
Wider implications of the findings: We have shown that phthalates can adversely influence male reproductive hormone levels. This is in agreement with the previous laboratory and some epidemiologic studies showing negative effects on male reproductive function. BPA and alkylphenols do not seem to have a comparable effect, which suggests their minor relevance at environmentally present concentrations compared to phthalates. Still, our study was comprised of men attending fertility clinic and the results may not be representative of the general population.
Study funding/competing interest(s): Funding by national/international organization(s) – Slovenian Research Foundation (P3-334-0327).
Trial registration number: NA.
Keywords: endocrine disrupting chemicals, bisphenol A, phthalates, alkylphenols, male reproductive hormones

O-017 FSH treatment improves sperm DNA damage in men with idiopathic infertility carriers of the FSH receptor p.N680S homozygous N genotype: an interim analysis

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Study question: To assess whether in men with idiopathic infertility, the sperm DNA fragmentation (sDF) improves depending on the FSH receptor (FSHR) genotype as assessed by the non-synonymous polymorphisms (SNP) rs6166 (wild type or p.N680S).
Summary answer: FSH treatment improves sDF in a subgroup of idiopathic infertile men, although 40% of these men do not show any significant improvement. The response of sDF, a surrogate marker of sperm quality, together with the evaluation of FSHR SNP p.N680S might be useful to predict the response to FSH treatment.
What is known already: FSH is fundamental for spermatogenesis and is empirically used to treat male idiopathic infertility. Several studies suggest that sDF could be a candidate predictor of response to FSH treatment, in terms of probability to conceive. Furthermore, it is widely accepted that the FSHR SNP p.N680S influences ovarian response in women and testicular volume in men.
Study design, size, duration: Multicenter, longitudinal, prospective, open-label, two-arms clinical trial. Subjects enrolled were idiopathic infertile men and received 150 IU of recombinant FSH (Gonal f®) every other day for 12 weeks and were then followed-up for further 12 weeks after FSH-withdrawal. Patients were evaluated at baseline and at the end of the two phases.
Participants/materials, setting, methods: Eighty-eight men with idiopathic male infertility carrier of the homozygous FSHR p.N680S N or S genotype, FSH < 8 IU/L and sDF > 15%, were enrolled. 66 patients completed the sDF analysis. sDF was centrally evaluated by TUNEL/PI assay coupled to flow cytometry, resolving two different sperm populations, namely: PIbrighter and PIdimmer.
Main results and the role of chance: Thirty-seven men (56%) were carriers of the p.N680S homozygous-N and 29 (44%) of the homozygous-S genotype, respectively. Total sDF (PIbrighter + PIdimmer) was significantly lower at the end of the study in patients carriers of the p.N680S-N allele than patients carriers of p.N680S-S allele ($p = 0.008$). Only in patients carriers of the p.N680S-N allele, total sDF decreased significantly from baseline to the end of the study ($p = 0.021$) and this decrease was entirely sustained by the sperm population containing vital sperms (i.e., PIbrighter fraction) ($p = 0.008$). PIdimmer fraction, including only non-vital sperms, was significantly higher in patients carriers of the p.N680S-S allele than in carriers of N allele ($p = 0.018$). Total sDF was inversely related to total sperm number ($p = 0.020$) and progressive sperm motility ($p = 0.014$).
Limitations, reason for caution: The statistical power of the results obtained so far is 86.9%, with alpha-error 0.05. This is an interim-analysis.
Wider implications of the findings: The study suggests that FSH treatment induces a significant improvement of total sDF in men carriers of the p.N680S homozygous N allele. This sDF decrease awaits confirmation, since the study will be completed by June 2015.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – The study was supported by unrestricted grant by Merck Serono.
Trial registration number: EudraCT number 2010-020240-35.
Keywords: FSH treatment, male infertility, Sperm-DNA fragmentation

O-018 Meiotic studies of ejaculate-derived spermatocytes in Robertsonian translocations and small supernumerary marker chromosomes carriers using a novel immunocytogenetic technique

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Study question: What are the meiotic behaviors of the chromosome abnormalities in carriers of Robertsonian translocations (ROBs) and small supernumerary marker chromosomes (sSMCs)?

Summary answer: Carriers of ROBs and sSMCs showed decreased recombination, impaired synapsis, and an association of chromosome abnormalities with the XY body, as well as the improper segregation of uninvolved chromosomes.
What is known already: ROBs and sSMCs are associated with infertility and increased sperm aneuploidy. Chromosome segregation studies in ROBs have found variability in the percentage of unbalanced sperm produced. Reorganized chromosomes are shown to associate with the transcriptionally silenced XY body, which is suggested to cause spermatogenic arrest. Studies of reciprocal translocations have found evidence of transcriptional silencing at the breakpoints of rearrangements. The majority of sperm studies have shown that the segregation of sSMCs is variable.
Study design, size, duration: The ejaculate sperm was studied in three carriers of chromosome abnormalities: one sSMC only carrier, one ROB only carrier, and one sSMC plus ROB carrier. Control testicular sperm was retrieved from the testicular tissue of five normospermic fertile men with normal karyotypes who were undergoing vasectomy reversals.
Participants/materials, setting, methods: Meiotic recombination, synapsis and meiotic inactivation in ejaculate spermatocytes were investigated using immunostaining. The chromosomal origin of the sSMC was assessed by Multiplex Fluorescence In Situ Hybridization (M-FISH). The segregation of the ROB and sSMC in the sperm and incidence of an interchromosomal effect (ICE) were examined by FISH.
Main results and the role of chance: The sSMC-only and ROB-only carriers showed decreased global recombination compared to controls ($P < 0.05$), although impaired synapsis and an association of the chromosome abnormality with sex chromosomes were also observed. In the sSMC-only and sSMC plus ROB carriers, the sSMC was found in 13.5% and 11.5 % of sperm, respectively. Segregation analysis of the sSMC plus ROB case showed that 91.2% of the sperm were normal/balanced and 8.8% were unbalanced. ICE involving the sex chromosomes were greater in both sSMC carriers. The frequency of XX/YY disomy for the sSMC plus ROB case was higher than controls ($P < 0.0001$). In the sSMC-only case, total aneuploidy ($P = 0.0176$), XX/XY disomy ($P = 0.0003$), and XY disomy ($P = 0.0268$) were higher than controls.
Limitations, reason for caution: We compared ejaculate-derived spermatocytes to control testicular-derived spermatocytes. The differences observed may be a result of the spermatocyte source. However, our preliminary date comparing ejaculate and testicular derived spermatocytes in the same individuals saw no statistical differences in the meiotic characteristics examined in this study.
Wider implications of the findings: We observed impaired meiotic recombination, synapsis, and XY body association in carriers of ROB and marker chromosomes, marking the first such study in sSMC carriers. We report the first immunofluorescent study of meiotic defects on ejaculate-derived spermatocytes, providing an exciting new opportunity to study meiosis in infertile men. Furthermore, FISH studies on infertile carriers of marker chromosomes support previous observations that this population tends to produce sperm with markers at frequencies lower than theoretically expected.
Study funding/competing interest(s): Funding by national/international organization(s) – Canadian Institutes of Health Research.
Trial registration number: NA.
Keywords: male infertility, interchromosomal effects, meiotic segregation, small supernumerary marker chromosomes, Robertsonian translocation

O-019 Infertile men have frequently leydig cell dysfunction: study on hypogonadism, vitamin d and bone mass in 5,177 subjects

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Study question: Are infertile men at risk of Leydig cell dysfunction?
Summary answer: Hypogonadism and low vitamin D levels are very frequent in infertile males. Both conditions, caused by Leydig cell dysfunction, are implicated in the frequent low bone mass seen and osteoporosis seen in these patients.
What is known already: Male factors are responsible for half of the cases of couple infertility. Whatever the cause, spermatogenic disruption is clinically and hormonally recognized by low sperm count and Sertoli cell markers (FSH levels). However, recent evidence showed that Leydig cell impairment is also frequent in subjects with primary testicular damage, as evidenced for example by reduced INSL3 and 25(OH)-vitamin D levels. The latter is caused by reduced expression of CYP2R1, a major enzyme involved in 25-hydroxylation of cholecalciferol, and lower 25(OH)-vitamin D levels are well known cause of low bone mass. Furthermore, testosterone (T) production by the Leydig cells might be also impaired in men with primary spermatogenic damage. These aspects have not been comprehensively analyzed in large number of subjects.
Study design, size, duration: Prospective cohort study on subjects referred to our tertiary Universitary Centre for semen analysis during the period January 2011–June 2014.
Participants/materials, setting, methods: We evaluated the presence and type of hypogonadism, 25(OH)-vitamin D status and bone mass in men who had semen analysis (*n*: 11,516), complete andrological program [including semen culture (*n*: 10,394), history and physical examination (*n*: 7,527), hormone analysis (FSH, LH, T, 25(OH)-vitamin D; *n*: 5,884), and ultrasound of the testes (*n*: 5,177)]. Men with total sperm count <10 million/ejaculate (*n*: 2,583) underwent also genetic analysis (karyotype, Y chromosome microdeletions, CFTR mutations; *n*: 2273) and DEXA (*n*: 855).
Main results and the role of chance: Azoospermia was present in 9.3% of cases (*n*: 481/5,177), oligozoospermia (with or without reduced motility and/or normal sperm morphology) in 40.6% (*n*: 2302), asthenozoospermia in 12.2% (*n*: 632), and normozoospermia in 34.5% (*n*: 1787). Main causes or risk factors were varicocele (28%), genetics (15%), obstruction/sub-obstruction of seminal tract (12%), cryptorchidism (6%), infections/iatrogenic causes/ejaculation disorders/prior surgery (14%) and idiopathic forms (25%). Primary hypogonadism (T < 10.4 nmol/L, LH > 8 IU/L) was found in 25.7% of cases, secondary hypogonadism (T < 10.4 nmol/L, LH < 1.5 IU/L) in 1.3%, subclinical hypogonadism (T > 10.4 nmol/L, LH > 8 IU/L) in34.2%. Men with all forms of hypogonadism have frequently insufficient (48.5%) or deficient (25.4%) 25(OH)-vitamin D levels and higher risk of low bone mass, osteoporosis (16.8%) and osteopenia (31.5%). The role of chance is limited by the high number of subjects studied.
Limitations, reason for caution: Longitudinal studies are necessary to confirm these data.
Wider implications of the findings: This study, performed in a very large cohort of subjects, showed that hypogonadism, low vitamin D levels, and low bone mass are very frequent in infertile males. Metabolic and other clinical conditions associated with low T and low vitamin D levels need therefore to be accurately evaluated in these subjects, and treatment should consider also these aspects other than specific treatment only of infertility.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Department of Medicine.
Trial registration number: NA.
Keywords: male infertility, hypogonadism, vitamin D, osteoporosis, testosterone

O-020 Early diagnosis of testicular tumours in non-obstructive azoospermia: a comparative study

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Study question: In men with non-obstructive azoospermia (NOA) undergoing testicular sperm extraction (TESE) for fertility treatment, is immunohistochemical detection of intratubular germ cell neoplasia (IGCN) in AgarCytos, made of the remnants of the TESE specimen, equally accurate as in a standard testicular biopsy?
Summary answer: No cases were encountered in which the standard testicular biopsy was positive for IGCN, while the AgarCyto was negative. Therefore, the standard testicular biopsy did not have added value in this study.
What is known already: Infertile men are at higher risk for testicular cancer compared to the general population. IGCN can be detected by immunohistochemistry in standard testicular biopsies and, albeit less accurate, in semen using PLAP and OCT3/4. Previously we have shown that IGCN can also be successfully detected by immunohistochemical evaluation of AgarCytos, made of the TESE remnants. The observed incidence of a germ cell (pre)malignancy in NOA patients undergoing TESE for fertility treatment was 4.4%.
Study design, size, duration: Between January 2013 and May 2014 a prospective cohort study was conducted at a Dutch University Hospital. All males with NOA (*n* = 197) undergoing a urological work-up followed by a diagnostic

TESE (n = 303) for fertility treatment were included. Simultaneously a standard testicular biopsy was performed.
Participants/materials, setting, methods: After cryopreservation of sperm, if present, an AgarCyto was made of the remnants of these TESE biopsies. Sections of the AgarCyto and standard testicular biopsy were stained with hematoxylin-eosin for pathological examination as well as PLAP and OCT3/4 for immunohistochemistry to detect IGCN.
Main results and the role of chance: Six men (3.0%) were diagnosed with a germ cell (pre)malignancy. In three cases TESE and a (partial) orchidectomy were performed in the same setting because of a suspect ultrasound (n = 2) or inguinal located testis (n = 1). One of them had a negative AgarCyto but partial orchidectomy revealed IGCN, a subsequent TESE AgarCyto was positive for IGCN. Microscopic evaluation including immunohistochemical analysis of the AgarCytos diagnosed three (1.5%) more cases of a germ cell (pre)malignancy compared to scrotal ultrasound alone. Two patients opted for radiation without histological confirmation of a positive AgarCyto, in one case the standard biopsy was negative and in another case it was lost. In the other four patients result of the AgarCyto were either confirmed by the standard biopsy and/or by orchidectomy.
Limitations, reason for caution: Although very unlikely based on the test-characteristics of the immunohistochemical markers, the chance of a false-positive AgarCyto is not completely ruled out in two cases. Because of the lack of a standard biopsy when an orchidectomy was performed simultaneously with TESE, possible superiority of the AgarCyto could not be established.
Wider implications of the findings: Men undergoing TESE because of NOA should be offered simultaneous screening for IGCN because of the increased incidence of germ cell (pre)malignancies. The principal advantage of using the TESE remnants is that all available testicular tissue can be used for both sperm recovery and pathological evaluation, increasing the yield of spermatozoa as well as the chance to find (pre)malignant cells. Limiting the number of biopsies from the testis decreases the possibility of complications after TESE.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study was (partially) funded by Merck Serono (Schiphol-Rijk, the Netherlands), but there are no conflicting interests to disclose.
Trial registration number: NA.
Keywords: infertility, TESE, intratubular germ cell neoplasia, screening, immunohistochemistry

SELECTED ORAL COMMUNICATIONS

SESSION 05: BASIC SCIENCE IN FEMALE INFERTILITY

Monday 15 June 2015 **10:00–11:30**

O-021 High-resolution imaging of meiosis in live human oocytes

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Study question: How do live human oocytes develop into fertilisable eggs?
Summary answer: By imaging chromosomes and the microtubule spindle at high resolution over the entire course of meiosis in live human oocytes, we were able to determine the stages of meiosis and to gain insights into the causes of aneuploidy in human eggs.
What is known already: Chromosome segregation errors during meiosis in human eggs are the leading cause of pregnancy loss and human aneuploidy. Studies of chromosome segregation in live human oocytes that could reveal the causes of aneuploidy are currently missing.
Study design, size, duration: A long-term study involving the systematic collection and quantitative high resolution imaging of immature human eggs obtained from patients enrolled in the *in vitro* fertilization programme in Bourn Hall Clinic (Cambridge, UK) between September 2012 and November 2014. A total of 374 cells were analysed in this study.
Participants/materials, setting, methods: A total of 140 women (aged 23–43, mean age 33.5 ± 4.5 years) undergoing ovarian stimulation for intracytoplasmic sperm injection (ICSI) took part in this study. Donated immature eggs were collected for in depth analysis by high resolution live imaging of meiosis on automated confocal microscopes combined with immunofluorescence labelling.
Main results and the role of chance: We established methods that allowed us for the first time to study chromosome segregation and spindle assembly in live human oocytes. We labelled chromosomes and microtubules in immature human oocytes and used high resolution confocal microscopy to follow their maturation into fertilisable eggs. Quantitative image analysis of the time-lapse videos revealed the stages of meiosis in human oocytes and provided insights into the causes of egg aneuploidy.
Limitations, reason for caution: Immature human oocytes included in this study were developmentally delayed and would have been rejected for ICSI procedures and discarded, because they had not yet reached the required stage for sperm injection at the time of the fertility treatment.
Wider implications of the findings: Most of our knowledge about the development of eggs in mammals comes from studies in mouse oocytes. Our work generated the first comprehensive data set on the mechanism of spindle assembly and chromosome segregation in live human eggs. This work not only sheds light on a fundamental process at the beginning of our life, but it also provides a basis to improve and develop fertility treatments in the future.
Study funding/competing interest(s): Funding by national/international organization(s). Medical Research Council. European Research Council (grant agreement no. 337415). The Lister Institute of Preventive Medicine.
Trial registration number: NA.
Keywords: female infertility, egg aneuploidy, live imaging, meiosis, chromosome segregation

O-022 Whole genome sequencing reveals the complex etiology of primary ovarian insufficiency

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Study question: Our primary goal was to assess the level of genetic complexity underlying primary ovarian reserve disorders such as primary ovarian insufficiency (POI) and diminished ovarian reserve (DOR).
Summary answer: Our analysis identified 10,350 novel and known deleterious single nucleotide variants (SNVs) among patients in a POI cohort. While this is the first indication that many of these variants may be predictors of human ovarian reserve, of the 668 common to all patients, 94 are among genes that regulate apoptosis, estrogen-regulated inflammation, primordial follicle progression, and meiosis.
What is known already: A number of studies have linked individual genetic loci with the development of ovarian reserve phenotypes such as POI and DOR. While many of these loci have been characterized in mouse models and detected in different patient cohorts, the targeted nature of these studies means the full genomic landscape underlying ovarian reserve mechanisms in humans remains largely unexplored.
Study design, size, duration: Our cohort study included women with idiopathic POI diagnosed before 38 years of age and utilized blood samples collected between November 2012 and February 2014. Women with a natural parity of ≥1, history of gynaecological surgery, cancer treatment, genetic syndromes or major illness or trauma that would compromise fertility, were excluded.
Participants/materials, setting, methods: After obtaining IRB consent, blood samples were collected from all patients and whole genome sequencing performed on extracted DNA using Next Generation Sequencing platforms. Bioinformatics tools were then applied to report variant calls, novel and deleterious variants, and those shared across samples.
Main results and the role of chance: Of the variants we identified in the whole genome sequences of our POI cohort, 10,350 (including 223 novel) were predicted to be deleterious due to loss of function or protein structural changes. Additionally, many SNVs shared among our cohort were within conserved non-coding regions. We used an in-house, fertility specific annotation tool to filter genes according to their impact on reproductive potential. Thus, 94 deleterious variants common to all of our POI patients were identified among genes known to regulate processes such as primordial follicle progression and meiotic chromosome stability, as well as a number of maternal effect genes. Notably, featured among these genes were a number of NLRP family members, many

orthologues of which have been well characterized for their role in oogenic processes in animal models.
Limitations, reason for caution: The short read length used in our whole genome sequencing makes the detection of complex structural variants and repetitive regions more challenging.
Wider implications of the findings: The identification of rare, exonic and intronic genetic variants among our cohort highlights the power of whole-genome sequencing in the identification of variants that associate with altered human ovarian reserve. Our data emphasizes the complex, polygenic nature of ovarian reserve disorders and the likelihood that loci reportedly associated with these disorders represent only a small fraction of coding and non-coding loci regulating ovarian reserve.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Celmatix Inc.
Trial registration number: NA.
Keywords: POI, ovarian reserve, next generation sequencing, genetics, infertility

O-023 Sirt2-mediated BubR1 regulation promotes oocyte maturation

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Study question: Does the sirtuin family member, Sirt2, positively influence oocyte maturation and what is its mechanism of action?
Summary answer: Sirt2 over-expression stabilizes BubR1 in mouse oocytes, promotes the formation of attachments between chromosomes and spindle microtubules and accelerates progression through meiotic maturation.
What is known already: Sirtuins (Sirt1–7) are a family of NAD^+-dependent deacetylases with diverse functions and potent anti-aging properties. In somatic cells, Sirt2 modulates a key cellular regulator known as BubR1 (for Budding uninhibited by benzimidazole-Related 1). BubR1-depletion disrupts meiotic progression in oocytes and the formation of attachments between chromosomes and spindle microtubules. It is unknown whether BubR1 is regulated by Sirt2 in oocytes and if this could be important for meiotic control.
Study design, size, duration: In order to examine the effect of increased Sirt2 levels, we engineered a transgenic mouse model of the C57BL/6J strain that over-expresses Sirt2 (hereafter referred to as Sirt2Tg). Fully-grown germinal vesicle (GV)-stage oocytes ($n > 30$ per group in triplicate) were isolated from hormonally-primed wild-type (WT) and Sirt2Tg mice.
Participants/materials, setting, methods: The timing of first polar body extrusion – which marks the completion of meiotic maturation – was assessed. High resolution confocal microscopy was used for detailed analyses of spindle assembly, chromosome alignment and sub-cellular localization of BubR1. Protein expression was quantified using Western blotting.
Main results and the role of chance: We used immunoblotting to compare Sirt2Tg oocytes with WT oocytes and confirmed that Sirt2Tg oocytes specifically over-expressed Sirt2 but not Sirt1. We found that Sirt2Tg females produced twice as many fully-grown oocytes as age-matched wild-type animals ($P < 0.05$). Significantly, Sirt2Tg oocytes expressed ~threefold higher levels of BubR1 than wild-type oocytes consistent with Sirt2-dependent BubR1 stabilization. Furthermore, at the sub-cellular level, BubR1 was enriched on spindle microtubules in Sirt2Tg oocytes. Importantly, spindle-localized BubR1 in transgenic oocytes was associated with accelerated formation of stable attachments between chromosomes and microtubules suggestive of increased meiotic efficiency. Entirely consistent with this, Sirt2Tg oocytes completed meiotic maturation marked by first polar body extrusion ~2 h in advance of WT oocytes.
Limitations, reason for caution: Although Sirt2 over-expression was associated with increased BubR1 and the observed effects inSirt2Tg oocytes are consistent with potentiated BubR1 function, Sirt2 could influence additional non-BubR1 targets that could also contribute to our findings.
Wider implications of the findings: Increased Sirt2 levels were associated with increased BubR1 stability and with effects consistent with potentiated BubR1 function, notably accelerated chromosome-microtubule attachment formation. Intriguingly, a recent paper examined the effect of Sirt2-depletion in mouse oocytes and reported impaired attachment formation between chromosomes and microtubules entirely in line with our results. Permeable small molecule Sirt2 agonists provide a novel prospect for impacting BubR1 function to improve oocyte quality.
Study funding/competing interest(s): Funding by University(ies). Funding by national/international organization(s) – UNSW MREII Grant. Ramaciotti Establishment Grant.
Trial registration number: NA.
Keywords: oocyte, meiosis, oocyte maturation, sirtuins, BubR1

O-024 Reproductive and metabolic effects of exogenous administration of irisin versus physical activity in high-fat diet-fed female mouse model

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Study question: Would the effects of physical activity and exogenous administration of irisin be similar or different on parameters related with reproduction and metabolism in the high-fat diet-induced obesity model of the female C57BL/6J mice? We hypothesized that exogenous administration of irisin would have similar affects as physical activity.
Summary answer: Exercise promotes weight-loss and healthy metabolism. It seems that irisin administration provides similar results compared to exercise in a female mouse model. Reproductive and metabolic parameters in blood showed similar improvements between exercise and irisin groups compared to controls. Moreover, ovarian histology presented comparable improvements after exercise and irisin administration.
What is known already: Obesity affects every aspect of reproduction in females, while exercise promotes healthy metabolism and protects against metabolic disorders like obesity. It has been documented that exogenously administered irisin (FNDC5), which is a new polypeptide hormone regulated by PGC1-alpha, induces the browning of subcutaneous fat and thermogenesis, and it presumably could be prepared and delivered as an injectable polypeptide. Indeed, increased formation of brown fat has been shown to have anti-obesity effects in adult humans.
Study design, size, duration: 60 female C57BL/6J mice were gathered at approximately 5–6 week of age and were divided into three groups. They were fed with a high-fat diet. Control group remained sedentary. Irisin group remained also sedentary but intravenously received 10^{10} FNDC5-expressing adenovirus after 20 weeks. Exercise group performed treadmill after 12 weeks.
Participants/materials, setting, methods: The study was carried out in an university hospital. Mice were killed at 22wk and had their ovaries excised for histological assessment. Blood was obtained by cardiac puncture. E2, FSH, LH, AMH, BMP, ANP, BNP, FGF21, ghrelin, insulin, leptin, adiponectin, resistin, kisspeptin, RBP4, visfatin levels were measured in blood.
Main results and the role of chance: Final weight, blood levels of estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-mullerian hormone (AMH), bone morphogenetic proteins (BMP), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), fibroblast growth factor 21 (FGF21), ghrelin, insulin, leptin, adiponectin, resistin, kisspeptin, retinol binding protein 4 (RBP4), visfatin were statistically similar between exercise and irisin groups ($p > 0.05$). Final weight, blood levels of BMP, FGF21, ghrelin, insulin, resistin, kisspeptin, visfatin were significantly lower in exercise and irisin groups compared to controls ($p < 0.05$), while LH/FSH ratio, ANP, BNP, RBP4 levels were significantly higher in the controls ($p < 0.05$). AMH level was significantly higher in exercise and irisin groups ($p < 0.05$). Ovaries of mice in exercise and irisin groups manifested different stages of follicular development, whereas the ovaries of mice in control group were inactive.
Limitations, reason for caution: Data of this study revealed that short treatments of obese female mice with irisin improved reproductive and metabolic parameters and caused a small weight loss. Whether similar or longer treatments with irisin and/or higher doses would cause similar or more weight loss in human subjects remains to be determined.
Wider implications of the findings: The therapeutic potential of irisin is apparent. Exogenously administered irisin induces the browning of subcutaneous fat and thermogenesis, and it presumably could be prepared and delivered

as an injectable polypeptide. After comprehensive studies on human subjects, pharmacological induction of irisin may be of interest to promote weight loss and improve reproductive and metabolic parameters that were impaired due to obesity in patients who cannot benefit from the favorable effects of exercise.
Study funding/competing interest(s): Funding by University(ies) – Scientific Research Projects Coordination Unit of Istanbul University.
Trial registration number: NA.
Keywords: obesity, irisin, exercise, female infertility

O-025 Serum five-miRNA panel as potential therapeutic targets for POI

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Study question: Our objective was to evaluate the levels of miRNA expression in serum samples of premature ovarian insufficiency (POI) patients to explore whether circulating miRNAs in serum can be used as therapeutic targets for POI.
Summary answer: These novel results may further indicate a physiological involvement of miR-21, miR-132, miR-145, miR-181a and miR-599 during follicle selection and ovulation in the ovary. miRNA profiling in serum has potential as a novel method for POI detection in the Chinese Han population. The five-miRNA panel in serum may serve as potential non-invasive therapeutic targets in POI patients.
What is known already: miRNAs are endogenous, nonprotein-coding, regulatory RNAs with important roles in health and disease. miRNAs are present in the circulation in a stable form and their levels are altered in diseases. The wide range of sources of circulating miRNAs makes it possible for circulating miRNAs to reflect every aspect of human physiological status and therefore, provides an advantage for them to serve as better biomarkers than other circulating molecules.
Study design, size, duration: We present the miRNA expression profiles in pooled serum of nine POI samples compared with normal volunteer samples by microarray, a panel of candidates (miR-21/132/145/181a/599) with highest expression patterns were selected. The differentially expressed miRNAs were further assessed in large cohorts of forty POI patients and twenty healthy volunteers in individual samples by qRT-PCR.
Participants/materials, setting, methods: *Paticipants:* Chinese Han women presenting with POI and normal volunteer. *Setting:* University affiliated hospital. *Methods:* We present the miRNA expression profiles in pooled serum of nine POI samples compared with normal volunteer samples by microarray, a panel of candidates (miR-21/132/145/181a/599) with highest expression patterns were selected. The differentially expressed miRNAs were further assessed in large cohorts of forty POI patients and twenty healthy volunteers in individual samples by qRT-PCR.
Main results and the role of chance: *Main results:* We present the miRNA expression profiles in pooled serum of nine POI samples compared with normal volunteer samples by microarray, a panel of candidates (miR-21/132/145/181a/599) with highest expression patterns were selected. The differentially expressed miRNAs were further assessed in large cohorts of forty POI patients and twenty healthy volunteers in individual samples by qRT-PCR. The large cohort also witnessed an increase compared with healthy volunteers (miR-21 (p = 0.0198)/miR-132 (p = 0.0429)/miR-145 (p = 0.0295)/miR-181a (p = 0.002)/miR-599 (p = 0.0165)). *The role of chance:* Further systematic analysis was used to validate these miRNAs involved in follicular cell survival (miR-181a and miR-145), proliferation (miR-599) and estradiol production (miR-21 and miR-132) in granular cells. It is promising to use circulatory microRNAs as therapeutic targets for POI. Evidence from *in vitro* studies in our lab also suggests specific roles for these miRNAs in regulating ovarian granular cell function.
Limitations, reason for caution: Serum miRNAs tests in POI patients have less time and money benefits compared with the FSH and AMH tests, but may witness great importance as therapeutic targets in Chinese Han population.
Wider implications of the findings: This finding suggests a phenotypic overlap expression of miRNAs in two cohorts of POI patients. These novel results may further indicate a physiological involvement of miR-21, miR-132, miR-145, miR-181a and miR-599 during follicle selection and ovulation in the ovary. Future research of microRNAs in physiologic and dysfunctional ovulation may offer new diagnostic and treatment strategies for POI.
Study funding/competing interest(s): Funding by national/international organization(s) – 973 Project No. 2010CB945104.
Trial registration number: Reg No. in ChiCTR:20100002.
Keywords: premature ovarian insufficiency, miRNA, therapeutic targets

O-026 Periostin a new non-invasive parameter in addition to the morphologic criteria for evaluating oocyte/blastocyst quality and its impact on endometrial receptivity

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Study question: Does the periostin (POSTN) content of follicular fluids (FFs), embryo wash-out or serum represent an additional non-invasive parameter to evaluate the oocyte/blastocyst quality and predict good implantation and pregnancy outcome?
Summary answer: POSTN expression in serum, FFs and embryo wash-out was significantly correlated with blastocyst quality. According to our data, POSTN levels combined with morphological criteria were able to predict pregnancy outcomes more than the morphological evaluation alone. Therefore POSTN could be used as an innovative non-invasive biomarker to improve IVF-outcome prediction.
What is known already: Embryo-endometrium cross-talk represents the limiting factor to have a good implantation and achieve pregnancy. Several extracellular matrix (ECM) proteins such as laminin, fibronectin and osteopontin have been already identified as key regulators of this interaction. Experimental data recently demonstrated that POSTN, a secreted protein belonging to the ECM family, may play a central role in regulating the embryo-endometrium cross-talk. Specifically, POSTN expression was able to predict endometrial receptivity and pregnancy outcome in spontaneous cycles.
Study design, size, duration: This is an experimental study with prospective collection and evaluation of 200 individual FF samples, collected from 50 female patients undergoing ICSI. A total of 50 wash-out medium derived from an equal number of transferred blastocysts were collected. From each patient, a serum sample at pick-up day was withdrawn.
Participants/materials, setting, methods: POSTN content was firstly verified in a random pool of samples by western blot (WB) and then measured by ELISA in 200 individual FFs, in 50 wash-out medium samples of transferred blastocysts and serum samples of patients. The relationship between POSTN levels, morphological blastocyst quality and pregnancy outcome was evaluated.
Main results and the role of chance: POSTN levels decreased with increasing patient's age and in case of poor ovarian reserve and of recurrent abortions history both in FF and serum. POSTN levels were significantly higher in women who got pregnant (17/50) than in those who did not both in FFs (63.45 ± 10.05 vs. 29.02 ± 14.47 ng/mL) and in serum (306.23 ± 41.40 vs. 120.92 ± 38.10). In FFs, levels were directly related to the blastocyst morphological quality and pregnancy outcome. POSTN levels were significantly higher in type A blastocysts and, in particular, in those giving pregnancy both in FF and wash-out (95.93 ± 18.20 vs. 41.83 ± 8.98 ng/mL; 10.74 ± 3.68 vs. 1.09 ± 2.95 ng/mL; in FF and wash-out medium of blastocyst resulted or not in pregnancy, respectively).
Limitations, reason for caution: Confirmation of our preliminary data on a large population may be required and on endometrial samples.
Wider implications of the findings: POSTN evaluation in individual FF samples might represent a new non invasive biomarker of embryo quality to use as a supplemental tool to predict embryo quality during IVF. Since this protein is a marker of embryo-endometrial cross-talk, POSTN concentration in FFs, wash-out or serum may give also information on the efficiency of endometrial receptivity and embryo implantation.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck-Serono S.p.A.
Trial registration number: No trial registration number.
Keywords: periostin, embryo-endometrial cross talk, embryo implantation, blastocyst quality, endometrial receptivity

SELECTED ORAL COMMUNICATIONS

SESSION 06: ENDOMETRIAL RECEPTIVITY

Monday 15 June 2015 **10:00–11:30**

O-027 Endometrial biopsy prior to assisted reproductive techniques (ART) does not improve treatment outcome in unselected patients

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Study question: Does a timed mid-luteal phase endometrial biopsy (EB) affect the chances of a clinical pregnancy in an unselected population of women undergoing ART in the next menstrual cycle?
Summary answer: EB performed in the mid-luteal phase of the menstrual cycle does not increase clinical pregnancy rates in an unselected group of women undergoing ART.
What is known already: A growing number of studies employing different methodologies and assessing different populations of infertile patients indicate a possible beneficial effect of EB on ART outcome. This was mainly observed when women with recurrent implantation failure (RIF) were included in the studies, but little is known about that effect on women undergoing their first cycle or frozen embryo transfers (FET). The cause of the beneficial effect of the biopsy is unclear and is under investigation.
Study design, size, duration: The design was that of a prospective, randomized, non-blinded controlled study with 1:1 allocation. The target population of this UK-based study was 160 women based on recruitment period. All participants were screened and recruited from a university affiliated infertility clinic from January 2013 to January 2015.
Participants/materials, setting, methods: Women <49 years of age with no major uterine anomalies undergoing treatment using own oocytes were eligible. Research ethics committee approval was obtained. Pipelle EB was conducted on LH + 7–9 or on days 18–23 of cycle preceding ART when no LH surge. No intervention was performed in the control group.
Main results and the role of chance: The outcomes of 76 women in the EB group and 75 in the control group are known. No differences in age, BMI, smoking status, cause of infertility, number of previous live births, ovarian reserve, embryo quality, or day of transfer were observed. There were no significant differences in clinical pregnancy rates between the biopsy and control groups on an intention-to-treat basis [56.9% (41/72) versus 48.5% (33/68); RR 1.17 (95% CI = 0.85–1.61); $P = 0.319$] or per embryo transfer [65.1% (41/63) versus 55% (33/60); RR 1.18 (95% CI = 0.88–1.58); $P = 0.254$]. No differences were observed in the rates of biochemical pregnancy or first trimester miscarriage. Subgroup analysis of first cycle patients and treatment types showed no difference in outcome variables. One woman did not tolerate the EB and vasovagal episodes were experienced by another four women. There were no infectious or haemorrhagic complications.
Limitations, reason for caution: Lack of blinding can be a potential source of bias, however due to the nature of the intervention, blinding was not possible. Live-birth data is being collected and will be reported when available. Only 14 FER cycles were included which prevents any definitive conclusions in this group.
Wider implications of the findings: The findings of our study suggest that EB performed in the cycle preceding ART does not increase clinical pregnancy rates in an unselected population. Based on these results, routine EB in this population prior to ART should not be carried out. The procedure is well-tolerated, feasible and associated with minimal risks. Further research is needed to assess the effect of EB in selected women with RIF,uterine pathology and/or in women undergoing FET only.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – The funding for this study was provided by University of Nottingham and Nurture Fertility.
Trial registration number: The study was prospectively registered on ClinicalTrials.gov (NCT01882842).
Keywords: endometrial biopsy, ART, clinical pregnancy, randomised controlled trial

O-028 Local endometrial injury: a treatment strategy to improve implantation rates: a systematic review and meta-analysis

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Study question: This is a systematic review and meta-analysis of RCTs comparing the efficacy of endometrial injury as compared to no intervention in women with recurrent implantation failure and an unselected group comprising patients with previous failed cycles and those embarking on their first IVF cycle.
Summary answer: Clinical pregnancy rate was significantly improved following local endometrial injury in women with recurrent implantation failure (OR 3.50, 95% CI 2.04–6.01, $p < 0.00001$) and points towards a benefit in women embarking on their first cycle but does not reach statistical significance (OR 1.32, 95% CI 0.93–1.86, $p = 0.12$).
What is known already: This is the first systematic review and meta-analysis to include only randomized controlled studies and the first meta-analysis to review the effect of local endometrial injury on women with recurrent implantation failure and an unselected group comprising patients with previous failed cycles and those embarking on their first IVF cycle.
Study design, size, duration: Systematic review of RCTs to September 2014 comparing the efficacy of endometrial injury as compared to no intervention in women with recurrent implantation failure and an unselected group comprising patients with previous failed cycles and those embarking on their first IVF cycle. 7 RCTs and 907 participants were included.
Participants/materials, setting, methods: Participants included women with recurrent implantation failure and patients with previous failed cycles and those embarking on their first IVF cycle. MEDLINE, EMBASE, Cochrane Library, National Research Register, ISI Conference Proceedings, ISRCTN Register and Meta-register were searched for RCTs to September 2014. 7 RCTs and 907 participants were included.
Main results and the role of chance: Meta-analysis showed that clinical pregnancy rate was significantly improved after local endometrial injury in patients with at least 1 previous failed cycle (OR 3.5 [95% CI 2.04 to 6.01, $P < 0.00001$]. The evidence points towards a benefit for local endometrial injury in the unselected population but does not reach statistical significance (OR 1.32, 95% CI 0.93–1.86, $p = 0.12$). In the unselected group the power of the analysis is diluted by including first cycle patients. This is probably where the difference lies. Inter-study variation is avoided by including randomized controlled studies only. Two reviewers assessed the quality of the studies independently.
Limitations, reason for caution: In the unselected group the power of the analysis is diluted by including first cycle patients. Further studies are required to examine the effect of local endometrial injury in first cycles only.
Wider implications of the findings: The evidence is strongly in favour of inducing local endometrial injury in the cycle preceding IVF in patients experiencing recurrent implantation failure. This study is clinically very relevant as it determines the group of patients most likely to benefit from this novel procedure. Further studies are required to determine the effect of local endometrial trauma in the cycle preceding ovarian stimulation in patients embarking on their first IVF treatment cycle.
Study funding/competing interest(s): Funding by hospital/clinic(s) – NA.
Trial registration number: 21463.
Keywords: endometrium, implantation

O-029 Endometrial injury: reflections about bias in meta-analyses

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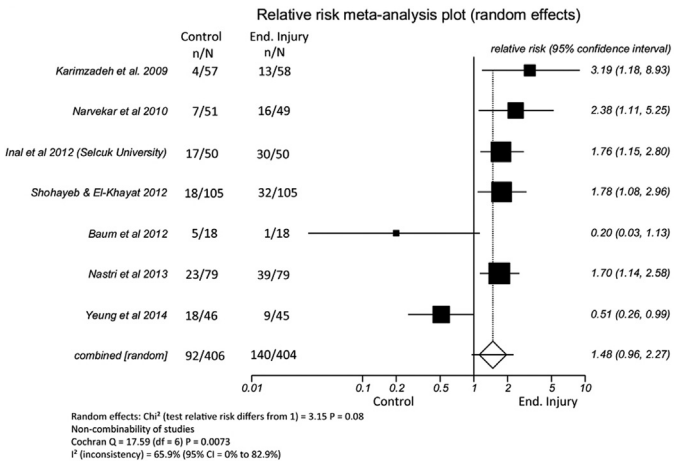
Study question: Do the data currently available from meta-analyses confirm that endometrial injury (EI) can truly improve clinical pregnancy rates (CPRs) in patients with implantation failure (IF) without any possibility of damage?
Summary answer: At present, EI has no definitive support in evidence-based medicine for use in ART.
What is known already: Meta-analyses are of primary importance as a source of findings for evidence-based medicine, but the quality is strictly dependent on the quality/quantity of available randomised controlled trials (RCTs). In 2012, three meta-analyses that examined EI showed beneficial effects on CPRs in patients with IF. However, these results were based on a few RCTs, which used restricted populations. In addition, further RCTs did not confirm this beneficial effect and suggested harmful effects, specifically in repeated IFs.
Study design, size, duration: A systematic review based on electronic searches of databases up to November/2014 was conducted to identify RCTs that compared any type of intentional EI in a cycle prior to the cycle of embryo transfer in ART cycles with no intervention or with a simulated procedure that could not cause EI.
Participants/materials, setting, methods: Seven RCTs (810 patients) were included as targets for data extraction and meta-analysis. The primary outcome was CPRs in patients with IF. Data management and analysis were conducted using StatsDirect statistical software. The measure of heterogeneity was evaluated using Cochran's Q and I^2 tests.
Main results and the role of chance: A random-effects model was used in the meta-analysis because of high heterogeneity between studies: Cochran's Q: 17.59, $P = 0.0073$; $I^2 = 65.9\%$. A chi-squared test statistic was used with its associated probability that the pooled Relative Risk was equal to 1. The general pregnancy rate did not differ between the group of patients submitted to EI (34.6%, 140/404) and the group not submitted to EI (control group: 22.7%, 92/406) ($P = 0.08$; RR = 1.47, 95% CI = 0.96–2.27). Figure 1 summarises the results.

Figure 1. Random-effect model. Forest plot for clinical pregnancy rate.



Limitations, reason for caution: Robust randomised trials comparing a standardised protocol of endometrial injury with no intervention are still needed before definitive conclusions can be reached.
Wider implications of the findings: Methodological problems caused by clinical heterogeneity (differences in inclusion criteria and in interventions performed) and insufficient power (low sample size) cause difficulties in drawing inferences from meta-analyses. Accordingly, meta-analyses must be analysed with extreme caution and not considered source of absolute truth. For EI, even a few new studies can change the initial expectations about the procedure. The observed heterogeneity challenges the validity of EI, indicating that it can, at least potentially, jeopardise the outcomes of ART.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Centre for Human Reproduction Prof. Franco Jr. Paulista Center for Diagnosis Research and Training.
Trial registration number: Not applicable. The study was authorised by the local ethics committee.
Keywords: evidence-based medicine, endometrial injury, meta-analysis, implantation failure, pregnancy

O-030 Endometrial receptivity evaluation in a natural cycle preceding IVF can predict the pregnancy success

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Study question: We assessed to what extent the endometrial receptivity status diagnosed with the Win-Test in a natural cycle preceding the IVF cycle can predict the IVF success?
Summary answer: Women with a receptive endometrium in the natural cycle preceding their IVF cycle, double their pregnancy rate after a fresh transfer following ovarian stimulation compared to patients which were evaluated with a non receptive endometrium.
What is known already: Implantation failures caused by endometrial receptivity problem is a major issue for IVF laboratories. Endometrial receptivity status can be assessed by the Win-Test (Patent EP10305561.2; PCT/EP2011/058757). This test allows personal care management by determining the cycle day where endometrium is the most receptive to identify the appropriate timing for embryo replacement. In which measure the endometrial receptivity status under a natural cycle can predict the success of a subsequent IVF attempt remains a full question.
Study design, size, duration: We conducted a mono-centric prospective study. All patients were treated at clinic OVO, Montréal, Canada. Women were recruited from February until December 2014.
Participants/materials, setting, methods: Fifty women undergoing ART with autologous eggs at clinic OVO were recruited. The cycle before their IVF, an endometrial biopsy was performed during the participant's implantation window. RNAs were extracted from the endometrial samples and 11 biomarkers of endometrial receptivity were assessed by RT-qPCR.
Main results and the role of chance: Among the 50 collected endometrial biopsies, 12 were discarded due to inappropriate timing of the endometrial sampling collection outside the theoretical implantation window. Thirty-eight endometrial biopsies were assessed to determine the prevalence of receptive endometria under natural cycle. Then, according to the endometrial receptivity status in natural cycle, the pregnancy outcome following IVF was analyzed in 18 patients which had an embryo transferred. We evaluated that 73% of the patients had a receptive endometrium during the implantation window in natural cycle. For the receptive patients ($n = 11$), 91% had a biochemical pregnancy and 82% had a clinical pregnancy (one pregnancy with early miscarriage), whereas only 43% of patients which were diagnosed as non-receptive ($n = 7$) had a clinical pregnancy following their embryo transfer.
Limitations, reason for caution: Further investigations with a larger number of patients are recommended.
Wider implications of the findings: The evaluation of endometrial receptivity status the cycle preceding the ovarian stimulation can predict IVF success, opening new perspective in the patient care management. Embryos from patients diagnosed as non-receptive in natural cycle should be cryopreserved for a later transfer once the best timing of the endometrial receptivity will be determined using the Win-Test.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Clinic OVO.

Trial registration number: NA.
Keywords: endometrial receptivity, IVF, win-test

O-031 Role of maximum endometrial thickness in prediction of clinical pregnancy rate in assisted reproduction treatment cycles

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Study question: Is maximum endometrial thickness at fresh embryo transfer a useful predictor of clinical pregnancy rate?
Summary answer: Maximum endometrial thickness (MET) in fresh embryo transfer cycle is a clinically significant and independent predictor of clinical pregnancy and can be used for counselling patients and deciding on fresh embryo replacement or freezing all embryos and deferring transfer to frozen embryo replacement cycle.
What is known already: A systematic review of studies on role of endometrial thickness in prediction of clinical pregnancy has shown no predictive capacity yet reporting a good discriminatory capacity at lower cut off values of endometrial thickness. The risk of bias was considered high and there were no adjustment for important confounders as number and quality of embryos transferred.
Study design, size, duration: This is an observational cohort study of 24649 assisted reproduction cycles between 2008 till 2013 from all UK CARE (Centres for Assisted Reproduction Clinics).
Participants/materials, setting, methods: All patients having Assisted reproduction cycles and fresh embryo transfer in the period from January 2008 till December 2013 in CARE clinics were included. The maximum endometrial thickness (MET) recorded during ovarian stimulation at the time of HCG administration was recorded and used for the analysis.
Main results and the role of chance: Logistic regression analysis for prediction of clinical pregnancy rate (CPR) has shown that MET is an independent significant predictor of CPR in fresh embryo transfer cycles (OR 1.02,95% CI 1.01–1.03). MET has modest performance as a sole predictor of CPR (Area under receiver operating curve (AUROC) = 0.56). When used in combination with other predictors as woman's age, previous live birth, number of oocytes retrieved, number of embryos transferred, and embryo transfer day, the predictive performance is improved (AUROC = 0.68). Subgroup analysis by the day of embryo transfer, number of embryos transferred and transfer of euploid embryos after aneploidy screening of embryos has shown that MET is still a significant predictor. A multilevel Likelihood ratio (LR) analysis has shown that the likelihood ratio and 95% confidence intervals when MET is below the 5thcentile (<7 mm),3rdcentile (<6 mm) and 1stcentile (<5 mm) are 0.55 (0.49–0.66), 0.37 (0.27–0.50), 0.18 (0.09–0.35), respectively, corresponding to CPRs of 26.9%, 19.3%, and 10.6% respectively. At the upper end of MET, no adverse impact on CPR was detected with multilevel likelihood ratios and 95% CIs at 95thcentile (>15 mm), 97thcentile (>17 mm), 99thcentile (>18 mm) being 1.14 (1.03–1.26), 1.09 (0.92–1.28), 1.05 (0.84–1.31), respectively, with corresponding CPRs of 42.6%, 41.5%, and 40.6% respectively.
Limitations, reason for caution: This is a retrospective analysis of data which although including large number of patients and using statistical adjustment for confounding variables as age, number of oocytes, number and quality of embryos transferred could not adjust for all potential confounders e.g., as previous uterine surgery, infection, endometrial scarring or use of adjuvant treatment to correct thin endometrium.
Wider implications of the findings: With a current lack of any reliable endometrial receptivity test in ART cycles a simple test as MET has the potential to give clinically useful information. Knowledge of clinical pregnancy rate (CPR) at various cut-offs of MET can better inform patients and practitioners and may help them to make informed decisions regarding cancellation of fresh embryo transfer and freezing all embryos. This study has shown that MET is a significant independent predictor of CPR but its overall performance was modest. This is expected due to the complexity of the implantation process and involvement of multiple maternal (endometrial) and embryonic factors. The likelihood of clinical pregnancy is significantly reduced when the MET is below 7 mm. This may be clinically significant and impact on the decision of clinicians/ patients to proceed with embryo transfer.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Birmingham Women's hospital/Academic department of obstetric and Gynaecology in Birmingham university-United kingdom.
Trial registration number: NA.
Keywords: endometrium, embryo transfer, endometrial thickness, clinical pregnancy rate

O-032 Effect of long term combined pentoxifylline and tocopherol administration on the endometrial proliferation: a prospective cohort study of 368 cases of thin endometrium

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Study question: Does long term administration of combined Pentoxyfylline and Tocopherol improve endometrial proliferation (thickness and volume) in infertile patients with a documented thin unresponsive endometrium?
Summary answer: Long term combined Pentoxifylline and Tocopherol administration significantly promotes endometrial proliferation and increases endometrial thickness and endometrial volume.
What is known already: Previous Meta-analysis reported that the probability of clinical pregnancy in patients with thin endometrium (<7 mm) is significantly lower compared with cases presenting an appropriate endometrial proliferation. Its Treatment remains a real challenge since repeated failure to achieve adequate endometrial development may request gestational surrogacy. Long term combined Pentoxyfylline and Tocopherol treatment has been previously reported to improve the endometrial growth, but no study has evaluated their long term endometrial effects on a large prospective cohort of infertile patients during the implantation window.
Study design, size, duration: We are presenting a prospective cohort study including 368 women with thin endometrium. Thin endometrium was defined as an endometrial volume below 2ml or an endometrial thickness below 7 mm during the mid luteal phase of a non conceptional cycle. Patients were included between 2012 and 2014.
Participants/materials, setting, methods: Patients were evaluated during mid luteal phase twice three months apart: once before, then after the introduction of the combined treatment including 800 mg of Pentoxifylline and 1000 UI of Tocopherol administered daily. An endometrial ultrasonic evaluation in two and three dimension defined the endometrial thickness and volume.
Main results and the role of chance: 15% of the included patients did not improve their endometrial volume, 67% normalised their volume while 18% improve their endometrial volume but without a complete normalisation after 3 months of treatment. The mean endometrial volume and thickness increased significantly from 1.6 ml (±0.4) and 4.7 mm (±1.6) before, to 2.1 ml (±0.3 ml) and 7.1 mm (±1.8) respectively after 3 months of treatment ($p < 0.001$, $p = 0.001$).No change was observed regarding Doppler of the uterine artery or the endometrial vascularization under treatment.
Limitations, reason for caution: Only a randomized trial using a placebo may definitely prove the effectiveness of a long term combined treatment with Pentoxyfylline and Tocopherol to improve thin unresponsive endometrium.
Wider implications of the findings: Approximately 80% of the replaced human embryos fail to implant. Multiple factors may contribute to this failure, but the majority of these failures are linked to a poor endometrium conjugated with a poor embryo quality. Even if an ideal thickness has never been able to predict pregnancy, deficiency of the endometrial proliferation is clearly associated with a poor prognosis for implantation and placentation. Preventive treatment optimizing the endometrial proliferation may be useful to increase implantation rates.
Study funding/competing interest(s): Funding by hospital/clinic(s). Hopital Pierre Rouqués – Les Bluets, Service de PMA, Paris, France. No conflict of interest.
Trial registration number: No trial registration number.
Keywords: thin endometrium, embryos implantation failures, echo 3D, pentoxifylline – tocopherol, *in vitro* fertilization

SELECTED ORAL COMMUNICATIONS

SESSION 07: SAFETY FOR ART CHILDREN: BIRTH AND BEYOND

Monday 15 June 2015 **10:00–11:30**

O-033 Perinatal outcomes following stimulated versus natural cycle IVF: analysis of 90,980 singleton live births following stimulated and unstimulated IVF

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Study question: Does ovarian stimulation affect perinatal outcomes such as preterm birth (PTB) and low birth weight (LBW) following IVF treatment.
Summary answer: There was no increase in the risk of adverse perinatal outcomes of PTB, early PTB, LBW and very LBW following acceptable limits of ovarian stimulation (≤20 oocytes retrieved) compared to unstimulated IVF treatment.
What is known already: Pregnancies resulting from assisted reproductive treatments (ART) are associated with a higher risk of pregnancy complications compared to spontaneously conceived pregnancies. The possible reason of adverse obstetric outcomes following ART has been attributed to the underlying infertility itself and embryo specific epigenetic modifications due to the *in vitro* fertilisation techniques. It is of interest whether ovarian stimulation that is routinely used in IVF to optimise live birth rates, affects perinatal outcomes.
Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of assisted reproduction treatment (ART) in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Data from 1991 to 2012 involving at total of 90,980 singleton live births (78,761 following stimulated fresh IVF cycles and 12,219 following unstimulated IVF cycles) were analysed.
Participants/materials, setting, methods: Data on all women undergoing either a stimulated fresh IVF treatment cycle or an unstimulated IVF cycle during the period from 1991 to 2012 were analysed to compare perinatal outcomes of PTB, early PTB and LBW and very LBW among singleton live births. Occurrence of a live birth at <37 weeks gestation is defined as a PTB and at <32 weeks gestation as early PTB. Birth weight <2500 g is defined as LBW and <1500 g as very LBW. Logistic regression analysis was performed adjusting for female age, year of treatment, previous IVF cycles, previous live birth, number of oocytes (≤20 or >20) and day of embryo transfer (cleavage or blastocyst stage).
Main results and the role of chance: The unadjusted odds of PTB (OR 1.41, 95% CI 1.34, 1.49), early PTB (OR 1.57, 95% CI 1.44 to 1.71), LBW (OR 1.82, 95% CI 1.72 to 1.92), and very LBW (OR 1.83, 95% CI 1.61, 2.08) were significantly higher with stimulated compared to unstimulated IVF. However there was no significant increase in the risk of adverse perinatal outcomes following stimulated IVF after adjusting for the potential confounders: PTB (adjusted odds ratio (a OR) 0.92, 95% CI 0.80, 1.05), early PTB (a OR 0.93, 95% CI 0.74, 1.16), LBW (a OR 1.11, 95% CI 0.97, 1.28) and very LBW (a OR 1.07, 95% CI 0.79, 1.45).
Limitations, reason for caution: Although the analysis was adjusted for a number of important confounders, the dataset had no information on confounders such as smoking, body mass index (BMI) and the medical history of women during pregnancy to allow adjustment.
Wider implications of the findings: Analysis of this large dataset suggests that there is no increased risk of adverse perinatal outcomes following IVF involving ovarian stimulation within acceptable safe limits compared to unstimulated IVF. These findings support the use of safe ovarian stimulation aiming to retrieve ≤20 oocytes to maximise the chances of IVF success compared to unstimulated IVF without concerns of adverse effects.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding: none.
Trial registration number: NA.
Keywords: stimulated IVF, natural cycle IVF, perinatal outcomes

O-034 A multivariate analysis of large-for-gestation risk factors in IVF and naturally conceived singletons

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Study question: Does the frozen-thawed embryo transfer (FET) procedure act as a significant independent risk factor for a birth of a large for gestational age (LGA) singleton?
Summary answer: FET procedure was identified as significant independent risk factor for the birth of a LGA singleton in a larger group of singletons, born after FET, fresh ET and after natural conception.
What is known already: Pregnancy outcome after FET seems reassuring, but higher risk for birth of LGA singletons after FET has been revealed. The birth of a LGA neonate represents a higher risk for maternal and perinatal morbidity and mortality, therefore the reasons must be investigated. Moreover, there is a potential concern that LGA birth weight could derive from underlying epigenetic disturbances.
Study design, size, duration: In retrospective case-matched study the risk factors for LGA were analysed in 4508 singleton pregnancies and births after FET, fresh ET and natural conception. The IVF procedures were performed in the time period between January 2004 and December 2011 at University Medical Centre Ljubljana.
Participants/materials, setting, methods: For identification of LGA risk factors, 1127 IVF singleton pregnancies and births (211 after FET, 916 after fresh ET) and 3381 naturally conceived controls (for each IVF pregnancy 3 pregnancies, matched by maternal age, parity and hospital) were included into a logistic regression model.
Main results and the role of chance: Smoking, hypertension, multiparity, BMI, gestational diabetes and IVF procedure were included into the LGA logistic regression model. Smoking, hypertension and double embryo transfer reduced the risk for LGA, while multiparity, higher BMI and gestational diabetes raised the risk for LGA.
FET was found to be a significant independent risk factor for LGA ($p = 0.032$; OR 1.697 with 95% CI 1.047–2.752). Fresh ET and ICSI procedures did not influence the LGA rate at all, whereas the transfer of 2 embryos slightly reduced the LGA risk.
BMI between 25 and 30 was another significant independent risk factor for LGA in both IVF pregnancy groups: FET ($p = 0.041$, OR 2.460 with 95% CI 1.030–5.857) and fresh ET ($p = 0.003$; OR 2.188 with 95% CI 1.297–3.691).
Limitations, reason for caution: In our study 95% of embryos were transferred at the blastocyst stage on day 5, so influence of embryo development stage to the LGA birth in IVF groups was not performed.
Wider implications of the findings: FET and higher BMI are the main risk factors for LGA birth among IVF conceived singletons. The most obvious maternal and IVF reasons for higher LGA rate after FET procedure are being excluded, and it seems that more subtle reasons for this phenomenon exist. Besides speculating on epigenetic disturbances, we should continue to search for other, not yet revealed, causes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No special funding needed from Division of Gynaecology, University Medical Centre Ljubljana.
Trial registration number: No trial registration number needed.
Keywords: IVF, singletons, large-for-gestation, frozen embryo transfer, birthweight

O-035 Is peak estradiol during the follicular phase associated with neonatal birthweight in singleton pregnancies conceived after fresh or frozen embryo transfer? Analysis of 3600 pregnancies

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Study question: Do singletons resulting from frozen thawed embryo transfer (FRET) have an increased birthweight compared to those after fresh embryo transfer and could this difference be associated with the estradiol (E2) serum concentrations during early stages of implantation?
Summary answer: Singletons born after FRET have an increased birthweight compared to those born after fresh embryo transfer and this seems to be associated with peak estradiol concentrations during the late follicular phase.
What is known already: Most studies report that singleton pregnancies resulting from FRET seem to have an increased neonatal birthweight compared to those from fresh embryo transfer. The different hormonal environment and especially the E2 serum concentrations during the late follicular phase between stimulated cycles with fresh transfer and FRET cycles has been hypothesized to be one of the main explanations for this finding. However, evidence directly linking late follicular phase E2 with birthweight are currently lacking.
Study design, size, duration: Singleton pregnancies resulting from FRET (n = 747) were retrospectively compared to those from fresh embryo transfer (n = 2885), during the period 1990–2013 at the Fertility Clinic of the Erasmus hospital of the French-speaking Free University of Brussels.
Participants/materials, setting, methods: The neonatal birthweight of singleton pregnancies after frozen (n = 747) or fresh embryo transfer (n = 2885) leading to a delivery ≥21 gestational weeks was compared. Generalized estimating equation (GEE) analysis was used in order to assess associations while accounting for the non-independence of data and also adjusting for important confounders.
Main results and the role of chance: A singleton delivered after a FRET cycle had a significantly higher birthweight by 102 grams (95% CI: 59.5–144.9, $p < 0.05$) even after adjusting for maternal age at delivery, parity, BMI, smoking status and gestational age at delivery. However, when the peak estradiol level at the end of the follicular phase was added to the regression analysis model, the birthweight difference between frozen and fresh embryo transfer cycles was not significant while for every 1000 pg/mL increase in the concentration of E2 the birthweight was reduced by 28.4 grms (95% CI: -49.7 to -7.01).
Limitations, reason for caution: The study was not adjusted for the number of embryos transferred or the type of culture media used. Although this study links E2 concentration and birthweight, the underlying mechanism remains to be elucidated. Furthemore, other factors might contribute to the difference in birthweight between pregnancies after fresh or FRET cycles.
Wider implications of the findings: Our findings confirm the observation that singletons born after fresh ET have a lower birthweight than those born after FRET. Furthermore, they support the hypothesis that this difference seems to be related to the altered endocrine environment following ovarian stimulation for IVF. Whether this altered endocrine environment during early embryo development is associated with other health aspects of the neonate is a question that warrants further investigation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Erasme Hospital, Brussels, Belgium.
Trial registration number: None.
Keywords: FRET, IVF, ICSI, estradiol, outcomes

O-036 Academic performance in adolescent children at 9th grade of primary school born after assisted reproductive technology (ART) – a national controlled cohort study

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Study question: Is academic performance, measured as test scores after ninth grade of primary school, different in ART than in spontaneously conceived (SC) adolescents aged 15–16 years? Do mean ninth grade test scores differ in ART singletons vs. ART twins?
Summary answer: Higher crude ninth grade mean test scores were observed in both ART singletons and twins compared to SC singletons and SC twins. The difference disappeared after adjustments for child sex, maternal age, birth weight, gestational age and socio-economic status. Similar mean test scores were observed in ART-singletons and ART-twins.
What is known already: Only few and smaller studies with selected control populations have explored IQ in ART children and no previous studies have included ninth grade test scores from a standardized national test in a complete national ART cohort of 15–16-year old adolescents. Most studies in younger aged children have found similar IQ in ART and SC offspring. One previous Danish study found similar test scores in the SC twins and singletons.
Study design, size, duration: National register-based controlled cohort study including all ART children (n = 8,251; n = 4,991 singletons and n = 3,260 twins) born in Denmark from 1995–2000 and two control populations conceived after spontaneous conception; (1) All twins born in Denmark (n = 10,833) and (2) A randomly selected singleton population two-times the ART singleton population (n = 10,052).
Participants/materials, setting, methods: Data on test scores and maternal socio-economic status were obtained in Statistics Denmark. All ninth grade students (age 15–16 years) complete a general test of academic achievements, scored on a scale from -3 to 12 (average 4.6). Ninth grade test scores were compared by student's t-test and multivariate linear regression analysis.
Main results and the role of chance: In the unadjusted analyses, ART singletons achieved higher test scores than SC singletons. Mean test score in ART singletons was 7.13 (SD 2.38) vs. 6.71 (SD 2.42) in SC singletons ($P < 0.001$). ART singletons achieved 0.213 (95% CI: 0.156–0.271) points higher grades than SC singletons. When adjusting for maternal age, birth weight, gestational age, sex and socioeconomic status, no difference was found. ART twins achieved a higher mean test score than SC twins 7.19 (SD 2.27) vs. 6.78 (SD 2.45)($P < 0.001$). Adjusted, ART twins achieved 0.098 (95% CI: 0.031–0.165) points higher grade than SC twins. ART twins vs. ART singletons had similar mean test scores in the unadjusted analyses, but in the adjusted analyses ART singletons achieved 0.36 (95% CI 0.187–0.538) points lower grades than ART twins.
Limitations, reason for caution: Adjustments were made for important confounders like child sex, maternal age and socio-demographic status. However, the risk of residual confounding from factors like family stability, parental educational length, etc. will be addressed in further analyses.
Wider implications of the findings: Even though ART children have higher risks of preterm birth and low birth weight, ART adolescents have similar school performance as SC adolescents in a compulsory national test performed by all students both from public and private schools in Denmark. Further ART singletons and twins had comparable scores hence the higher obstetric risk in twins does is not associated to poorer academic performance in adolescence. These findings are very important information for the infertile patients.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s) – Hvidovre Hospital, Copenhagen University Hospital: Danish Medical Association in Copenhagen (KMS).
Trial registration number: NA.
Keywords: school performance, ART, spontaneous conception, singletons, twins

O-037 Risk of cancer in children born after assisted reproductive technology in Norway

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Study question: Do children born to mothers who underwent assisted reproductive technology (ART) have an increased risk of cancer compared to children born to mothers not exposed to ART?
Summary answer: Although this population based cohort study did not observe overall increased cancer risk for children born to women after ART as compared to children born to women who conceived without ART, there were elevated risks of leukemia and Hodgkin lymphoma.

What is known already: The causes of childhood cancer are still unknown. Procedures used during ART have been speculated to cause epigenetic modifications in early fetal development that later could increase cancer risk. However, it has also been suggested that parental infertility itself could be a contributory factor. Several studies have reported increased risks of cancer in children conceived by ART, most notably for leukemia, although results have been inconsistent.
Study design, size, duration: The study cohort consisted of all children born between 1984 and 2011, registered in the Medical Birth Registry of Norway (MBRN). Cancers were identified by linkage to the Cancer Registry of Norway (CRN). Study subjects were followed from birth, until the first cancer, death, emigration, or 31st December 2011.
Participants/materials, setting, methods: Out of 1 628 677 children, 25 782 were born to mothers after ART. Hazard ratios (HR) and 95% confidence intervals (CI) were used to compare risk of cancer in ART children to non-ART children, adjusting for maternal age, birth order, calendar period, gender, birth weight and gestational age.
Main results and the role of chance: 51 cancers in the ART group comprised: 17 leukemias (33%), 12 cancers of the central nervous system (CNS) (24%), 5 soft tissue cancers (10%), 3 Hodgkin lymphomas (6%), 14 cancers at other sites (25%). 4503 cancers in the non-ART group included: 1021 leukemias (33%), 1020 CNS cancers (33%), 308 soft tissue cancers (7%), 258 Hodgkin lymphomas (6%), 1896 cancers at other sites (42%). Median age at diagnosis was 3.2 years in the ART group and 8.8 in the non-ART group. Median follow-up time was 6.9 and 13.7 years, respectively. HR for overall cancer in ART children compared to non-ART children was 1.21 (95% CI 0.90–1.63). Significantly increased HRs, however, were observed for leukemia (1.67, 95% CI 1.02–2.73) and Hodgkin lymphoma (3.63, 95% CI 1.12–11.72).
Limitations, reason for caution: The study may be underpowered due to few cancer cases in the ART group. This was especially true for Hodgkin lymphoma, which has not previously been described as elevated among ART children. In addition, our follow-up time, especially for ART children, was relatively short.
Wider implications of the findings: The demonstrated increased risk of leukemia in children conceived by ART is consistent with previous findings. An association between maternal subfertility and childhood cancer has also been found. The present study cannot determine whether it is the ART or the subfertility that may cause increased risk, and further research is necessary to make this differentiation. ART children should also be monitored in the future to ascertain whether their increased cancer risk persists into adulthood.
Study funding/competing interest(s): Funding by national/international organization(s). The study was funded by the Norwegian National Advisory Unit on Women's Health. All authors claim no competing interests.
Trial registration number: Not applicable, the study is not a clinical trial.
Keywords: ART, IVF, leukemia, Hodgkin lymphoma, childhood cancer

O-038 Risk of cancer in children and adolescents conceived by assisted reproductive technologies

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Study question: The aim of this study is to investigate whether offspring conceived by assisted reproductive technologies (ART) has an increased cancer risk, compared with the general population and with naturally conceived offspring from subfertile parents.
Summary answer: In preliminary analyses, after a median follow-up of 14.4 years no significantly increased risk of cancer was found in offspring conceived by ART compared with naturally conceived offspring from subfertile parents.
What is known already: It is estimated that more than 5 million ART-babies have been born worldwide. Each phase of the ART procedure is different from natural conception and there is growing evidence that ART procedures could perturb epigenetic processes during the pre-implantation period. Few studies examined cancer risk in ART-conceived offspring. Although the results of most studies are reassuring, recent studies showed slightly increased risks. However, most studies had a short follow-up and a small number of cases.
Study design, size, duration: The study population consists of all offspring of a nationwide historic cohort of subfertile women treated with ART between 1983 and 2000. The cohort includes ≈ 44,000 live-born children, of whom 26,000 were ART-conceived and 18,000 were naturally conceived. The median follow-up time is estimated at 18 years.
Participants/materials, setting, methods: Data on type of subfertility treatment and maternal risk factors are available from Study questionnaires and medical records from the mothers. Cancer incidence has been ascertained through linkage with the Netherlands Cancer Registry. Cancer risk is compared between ART-conceived offspring and naturally conceived offspring using multivariable Cox regression.
Main results and the role of chance: Since data preparation is still ongoing analyses could only be performed on the more recently born offspring of the cohort (5,184 ART-conceived and 4,526 naturally conceived). After a median follow-up 14.4 years, 32 cancers were observed of which 17 were observed in the ART offspring and 15 in the naturally conceived offspring. No significantly increased risk of cancer was found in the ART-conceived offspring when compared with naturally conceived offspring (hazard ratio = 1.45, 95% confidence interval 0.69–3.07). Adjusting for maternal age and birth weight did not materially change the risk.
Limitations, reason for caution: Since data preparation is ongoing, current results are based on a subgroup of the cohort. Therefore, results must be interpreted with caution. Results based on the whole cohort (≈44,000 children with 18 years follow-up time) are available in May 2015 and can be presented at the conference.
Wider implications of the findings: Despite the increasing use of ART techniques, information about the possible health risks for children conceived by these techniques is essentially lacking. This means that there is inadequate knowledge for physicians to advice parents who consider ART about potential health risks for ART-conceived children. Furthermore, pediatric oncologists caring for ART-conceived children/adolescents with cancer need evidence-based information about the presence or absence of a (causal) association between infertility treatment and risk of cancer.
Study funding/competing interest(s): Funding by national/international organization(s). Foundation Children Cancer Free.
Trial registration number: NA.
Keywords: ART, childhood cancer, epidemiology, cohort study, subfertility

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INVITED SESSION

SESSION 08: PCOS: CLINICAL ASPECTS

Monday 15 June 2015 **11:45–12:45**

O-039 Polycystic ovary syndrome in adolescent girls: Assessment and health-related quality of life

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The polycystic ovary syndrome (PCOS) remains a conundrum, not least because of its evolution during adolescence and difficulties in making the diagnosis. Many of the symptoms of PCOS occur naturally during adolescence, namely erratic menstrual cycles and acne. Furthermore the ultrasound appearance of the ovary can have a characteristic multicyctic or polycystic pattern – another topic of much debate and some disagreement. There are few studies that look at normative ranges of endocrine profiles or ovarian ultrasound parameters in the adolescent population, or for that matter how they may change over time in different populations. Consensus opinion suggests that PCOS should not be diagnosed until at least 2 years after the menarche, even though some individuals may have clear pointers to the likely diagnosis. At all ages obesity will amplify the expression of PCOS and increase the difficulties in management. Obesity also has the greatest impact upon quality of life, followed by signs of androgen excess and menstrual irregularity – concerns about fertility come later, but are often expressed by worried mothers. Management focuses on lifestyle interventions, where possible and anti-androgen therapy, often in the form of the combined oral contraceptive pill. Metformin therapy appears to confer minimal benefit as indeed is the case for adult women with PCOS.
Keywords: PCOS, adolescence, anovulation, hyperandrogenism, polycystic ovary

O-040 Aromatase inhibitors vs. clomiphene citrate for induction of ovulation in PCOS

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For the past 60 years clomiphene citrate (CC) has been the first line treatment for those with absent or irregular ovulation but who have normal basal levels of endogenous oestradiol (almost all of whom have PCOS). The action of CC is by blocking hypothalamic oestrogen receptors, signaling a lack of circulating oestrogen to the hypothalamus and inducing a change in the pattern of pulsatile release of gonadotrophin releasing hormone (GnRH) and consequently a discharge of FSH. Although CC restores ovulation in approximately 80% of patients it results in pregnancy in only about 35–40%, a gap thought to be mainly due to the anti-oestrogen effect of CC on the endometrium. Aromatase inhibitors are non-steroidal compounds that suppress oestrogen biosynthesis by blocking the action of the enzyme aromatase which converts androstendione to oestrogens. Letrozole, the most widely used aromatase inhibitor, is given orally in a dose of 2.5 mg–5 mg/day, is almost free of side effects and does not affect the endometrium. Theoretically, it has several advantages over CC. These were confirmed in an RCT from Legro and colleagues in which 750 women with anovulatory PCOS were randomized to receive either CC or letrozole. A 44% increase in pregnancy rate was achieved by letrozole over CC (27.5% vs. 19.5%). Twinning rate was non-significantly higher in those who received CC (7.4% vs. 3.2%) with no significant difference in the rate of congenital abnormalities. The conclusion would seem to be that letrozole can be regarded as a serious competitor to CC for first-line therapy for induction of ovulation. More is the pity that letrozole is still deemed to be 'off label' due to spurious data, subsequently disproved, linking it with a possible teratogenic effect.

Keywords: clomiphene, letrozole, PCOS

INVITED SESSION

SESSION 09: DATA REPORTING SESSION (PGD CONSORTIUM)

Monday 15 June 2015 **11:45–12:15**

O-041 Data from the ESHRE PGD consortium

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[6]*University of Athens St Sophia's Children's Hospital, Dept. of Medical Genetics, Athens, Greece*
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Introduction: The ESHRE PGD Consortium was set up in 1997 and from that time has been collecting data on PGD and PGS. In addition, the PGD Consortium has created different Working Groups (WGs) over the years to look at important aspects of PGD. Current WGs include the development and implementation of an on-line database for the annual data collections, retrospective data mining, a survey of new technologies introduced into PGD practice, evaluation of the clinical utility of PGD for HLA compatibility testing, evaluation of collaborative working practices between IVF and genetic teams and finally interactive webinars for PGD consortium members.

Methods: So far, 15 retrospective data collections, representing cycles performed until 2012 with babies born until 2013 have been analysed. All data collections were based on a Filemaker Pro database submitted via email by each centre. The data includes all aspects of PGD/PGS cycles and data curators include the ESHRE Scientific Officer along with volunteers from the PGD Consortium Steering Committee and other experts in PGD. Currently there are 125 registered centres worldwide, including from Europe, Argentina, Australia, Brazil, Canada, Egypt, India, Israel, Japan, Korea, Pakistan, Russia, Singapore, South Africa, Taiwan, Thailand, United Arab Emirates and the USA.

Results: Data from over 58,000 PGD/PGS cycles have been included in the ESHRE PGD database. As such, it comprises the world's largest collection of PGD / PGS data providing a valuable resource for data mining and for following trends in PGD practice. As a result of a huge increase in the number of reported cycles each year, the Steering Committee have found it very difficult and time consuming to mine the data and produce accurate tables. Moreover, the nature of PGD/ PGS treatments has changed significantly over the last years and today we face complexity in IVF cycle management and genetic analysis techniques. Therefore, the Steering Committee have found it timely to rejuvenate the data collection and make it more fit for purpose. To do so, we have invested time in strategic initiatives, first to determine what technologies are being used or are being introduced into genetic diagnosis and also how IVF cycles are being managed for PGD. This information has allowed us to restructure data collection and mining and has led to the setup of a new online PGD database. The design of the new database will allow centres to input and analyse their own data in real time. Now that the database is ready our focus will be to inspire and encourage all PGD centres to submit data and find out more about the advantages of the online database: Add your data prospectively from oocyte retrieval to analysis, embryo transfer and pregnancy / live birth. Keep track of your fresh and cryopreserved PGD / PGS cycles. Audit your centre's pregnancies and live births according to ART and genetic analysis techniques. And last but not least, network with PGD practitioners, discuss trends and identify good practice.

Discussion: The ESHRE PGD Consortium continues its activities as an important forum for PGD practitioners to share data and exchange experiences. The information extracted from the data collections helps to monitors quality issues in PGD and survey the introduction and effectiveness of new PGD technologies and methods. The WG activities of the Consortium are focused towards supporting PGD centres to continue their provision of high quality PGD services. The SC acknowledges the contribution of all active PGD centres to the aims of the Consortium.

Keywords: ESHRE PGD consortium, PGD data collection

INVITED SESSION

SESSION 10: FERTILITY SOCIETY OF AUSTRALIA EXCHANGE LECTURE

Monday 15 June 2015 **11:45–12:15**

O-042 Embryo morphokinetic development is the same for patients with and without polycystic ovaries and is not impaired by ivm treatment

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AIM: To determine if polycystic ovarian syndrome (PCOS) or *in vitro* maturation (IVM) treatment affect embryo development events and morphokinetic parameters after time lapse incubation.

Method: This paper represents a prospective case-control study. The study involved 83 participants who underwent 83 treatment cycles. Cycles were completed between November 2012 and July 2014. Participants were recruited for the study at the Fertility Specialists of WA and Fertility Specialists South, Perth, Western Australia. Of the PCOS diagnosed patients, 32 underwent IVM treatment (PCOS-IVM) and 23 had standard ICSI treatment (PCOS-ICSI). There were 38 patients without PCOS who underwent standard ICSI treatment comprising the control group (control-ICSI). All embryos were cultured in an embryoscope (Unisense Fertilitech) time-lapse incubator. Morphokinetic annotations were performed retrospectively. All normally fertilised embryos were included in the analysis of abnormal events and early embryo arrest; however, only embryos with annotations completed to the blastocyst stage (tB) were included in the final morphokinetic analysis.

Results: The PCOS-IVM group showed significantly more embryos with multinucleated two cells ($p = 0.041$), multinucleated four cells ($p = 0.001$) and uneven two cells ($p = 0.033$) compared with the control-ICSI group, but not the PCOS-ICSI group. There were no significant differences in the rates of any abnormal events between the PCOS-ICSI and control groups. Embryo arrest

between day two to three was higher in the PCOS-IVM and PCOS-ICSI groups compared to the controls ($p < 0.001$ and $p = 0.001$). Embryo arrest from day three to four was higher in the PCOS-IVM group compared to both the PCOS-ICSI and Control groups ($p < 0.001$). There were no differences in embryo arrest rates across all three groups at the compaction or blastulation stages. Cumulative rates of embryo arrest, from time tPB2 to time tB, result in a decreased proportion of useable PCOS-IVM blastocysts compared to the PCOS-ICSI and Control groups; however, of the embryos remaining, there was no significant difference in morphokinetic development between the three groups.
Conclusions: This study to compares the time-lapse analysis of IVM to standard ICSI for patients with and without PCOS. This allows for a more detailed and specific timeline of events from embryos generated using this approach for patients diagnosed with PCOS and shows that embryos generated from IVM have an increased rate of early embryo arrest, however; morphokinetic development is not impaired in embryos which progress to the useable blastocyst stage.
Keywords: IVM, ICSI, PCOS, morphokinetics

INVITED SESSION

SESSION 11: PARAMEDICAL INVITED SESSION: LABORATORY

Monday 15 June 2015 **11:45–12:45**

O-043 Artificial gametes: biology, implications and patient perspectives

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Reproductive medicine is awaiting novel Artificial Reproductive Techniques (ARTs) that no longer require couples with a wish for a child to have functional male and female gametes in order to conceive a child, which is genetically related to both intended parents. These ARTs will use 'artificial gametes', generated by manipulation of the intended parents' stem cells. Artificial gametes will fundamentally change the type of cells used by reproductive medicine and the patient groups that they can help conceive a genetically related child. Proactive consideration of the biological progress, the implications of clinical application and patients' interest is important and timely. To study the biological progress on artificial gametes, we conducted a systematic literature review. This review summarizes the findings of studies reporting on a priori defined starting-points and end-points including gamete formation, fertilization, and the birth of offspring. To study the implications of clinical application of artificial gametes we conducted another systematic review. This review summarizes reflections on the implications of clinical application of artificial gametes, reported on by all stakeholder groups (e.g., biologists, ethicists) and in all types of papers published in peer-reviewed journals (e.g., editorials, opinion papers). To study patients' interests, an anonymous survey questioning hypothetical treatment preference and important treatment characterizes was send to all 921 couples confronted with non-obstructive azoospermia and treated with TESE-ICSI in Dutch fertility clinics in the past 5 years. The review including 70 biological studies identified nine potential pathways to conceive offspring with the aid of artificial gametes. For seven of them, the formation of artificial sperm and/or artificial oocytes resulted in the birth of animal offspring. Animal offspring has even been born from artificial sperm from a female donor animal and fertilization has been achieved with artificial oocytes from a male donor animal. To date, no study has reported the birth of human offspring from artificial gametes. Nevertheless, the creation of human sperm and/or human oocytes was reported using six pathways to create artificial gametes. One of these pathways even resulted in fertilization. Human artificial sperm has also been created from women, while the formation of artificial oocytes from men has not been reported. Although these studies proof that artificial gametes can be generated, efficacy and long-term safety has not been unambiguously proven. The review including 84 papers on stakeholders reflections, identified the following eight overall objectives to be safeguarded during clinical application of artificial gametes: (i) timing the implementation of new treatments correctly, (ii) meeting 'plausible demands of patients', (iii) improving and safeguarding public health, (iv) promoting the progress of medical science in the interest of future patients, (v) providing treatments that are morally acceptable for the general public, (vi) controlling medical practice, (vii) offering treatments that allow acquisition of informed consent, and (viii) funding treatments fairly. The patient survey showed that the vast majority (88.5%) of couples confronted with non-obstructive azoospermia would opt for an artificial gamete based treatment as either a first or a last resort option. The most important treatment characteristics were safety for the child, pregnancy rates, and treatments ability to permanently cure infertility. Other treatment characteristics of influence are costs, burden, naturalness, advancedness of technology, *in vivo* versus *in vitro* conception, and moral acceptability. Achieved biological progress, ongoing preclinical research on effectiveness and safety, reflections on potential implications and patients' interest demonstrate the urgency of defining minimal conditions for clinical application. Given the broad scope of the identified objectives to be safeguarded during clinical application of artificial gametes, several (societal) stakeholders should be consulted during pre-implementation processes to define the minimal criteria to be met prior to clinical application.
Keywords: artificial gametes, biology, ethics, patient perspectives

O-044 Human semen quality in the new millennium: prospective studies of semen quality in Europe and other countries

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The debate whether semen quality in general has decreased re-emerged in 1992 when a meta-analysis showed that semen quality had decreased by 50% during a 50 years period. Several publications followed, and some detected a temporal trend whereas other did not. The overall conclusion that semen quality had declined is mainly based on retrospective, historic data, which limits their significance, and the topic has been controversial. On this background standardised and coordinated studies of semen quality of young men not selected due to fertility status and partners of pregnant women (i.e., fertile men) have been undertaken in various countries. Some of the studies of the young men, which have been ongoing since the late 1990s, have shown a somewhat heterogeneous temporal pattern. A decrease or more than 20% in total sperm counts and sperm concentration has been shown for Finnish men between 1998 and 2006, and a decrease of approximately 15% has also been indicated for young Spanish men during the recent decade whereas no changes were observed between 2000 and 2010 for Swedish men. In contrast, an increase in total sperm count and sperm concentration (approximately 14% and 12%, respectively) among Danish men in the period 1996–2010 was observed, however, still compatible with a pronounced reduction in both total sperm counts and sperm concentration when compared to the early 1940s. A French study corroborated the previously indicated decreasing trend among French men. Several studies have shown that chances of conception increase when sperm concentration increases towards 40–60 mill/mL and the number of spermatozoa with normal morphology increases to 9–12%. According to the current WHO guidelines a sample is classified as normal if the sperm concentration is 15 million per mill or more, the number of morphologically normal spermatozoa is 4% or more and more than 32% have good motility. There is little doubt that many men with semen parameters at that level or lower may be infertile with a need of fertility treatment if they want to reproduce. On the other hand, there is no guarantee that a man with better semen quality, including higher sperm counts has normal fecundity (ability to reproduce). A striking feature is the high frequency of young men with poor semen quality in all examined countries. When interpreted against studies describing associations between pregnancy chances and sperm counts and frequencies of morphologically normal spermatozoa it seems like only approximately 25% of men have an optimal semen quality, that 20–30% may be at risk of prolonged waiting time to pregnancy if they want to become fathers, and another 10–15% may have so low sperm counts that they may be at risk for need of fertility treatment. Humans are globally exposed to many classes of chemicals with endocrine disrupting potential via a variety of mechanisms. Exposure during fetal life may compromise testicular development, leading to reduced semen quality in adulthood, increased risk of testicular cancer and potentially also reduced capacity for testosterone production, besides an increased risk of being born with cryptorchidism and hypospadias as described by the Testicular Dysgenesis Syndrome (TDS) hypothesis. Since the first description of TDS hypothesis more and more evidence has emerged to support this hypothesis. Adverse effects caused by prenatal exposures do not exclude that exposures in adulthood may also alter testicular function; however, less focus has been on this aspect. Similarly,

limited attention has been on the effect of life-style factors. However, recent publications have also indicated a possible contribution from such factors to the impaired human testicular function.

Keywords: semen quality, general population, European countries

INVITED SESSION

SESSION 12: DEBATE: EMBRYO CULTURE SYSTEM: SINGLE STEP VERSUS SEQUENTIAL CULTURE MEDIA

Monday 15 June 2015 **14:00–15:00**

O-045 Single step media

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University of Hull, Centre for Cardiovascular and Metabolic Research, Hull, United Kingdom

O-046 Sequential media

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Clinical embryologists currently enjoy a wide selection of commercially available components for the construction of their preferred culture system, to include embryo culture media. These media carry regulatory approval, both of the products and of the manufacturing facilities, and the literature makes clear that most, if not all, of these media can produce stellar clinical outcomes, at least in the right hands. The ability to produce viable human blastocysts, ones that implant at significantly higher rates than cleavage-stage embryos, is a crucial adjunct, not only in the strategy of expanding single embryo transfer with accompanying high pregnancy rates, but also in the melding of ART with molecular diagnostics and in cryobiology. It was historically the use of sequential culture systems that fostered blastocyst production and transfer in human ART as earlier versions of monoculture, meaning those in use prior to the advent of KSOMAA and its current derivatives, fell short in this respect. Without a doubt, contemporary single-step media derived from KSOMAA, of which there are several commercial variations, can generate viable human blastocysts at rates equivalent to sequential pairs. But the question of "is one better than the other" remains elusive for a number of reasons. First, it becomes clear that arguing "is one better than two" seems absurd as one views the breadth of intermediary metabolism. Both are compromises that average across the requirements of hundreds of pathways so that a more accurate question may be "which is least harmful?". Second, although there are many comparative studies in the literature, a number are flawed by a failure to optimize both medium systems so that managerial issues cloud results. Others present subpar clinical outcomes that reduce the sensitivity of the study to discriminate between the variables of medium composition/presentation. Still others are conducted over such an extensive period of time that isolating even the laboratory contributions to outcome is difficult. Lastly, we are debating a snapshot in time, one where we culture embryos on a two-dimensional, polystyrene surface under conditions developed originally for anchorage-dependent somatic cells. Will there be culture platforms of the future that reproduce, in a temporally correct fashion, the unique chemical and physical environments of the infundibulum, ampulla and endometrium, complete with nutrient gradients? Likely, yes and if so, it is certain that multiple media will best support such a culture system.

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Keywords: embryo culture, sequential

INVITED SESSION

SESSION 13: ARE YOU SURE IT IS ENDOMETRIOSIS?

Monday 15 June 2015 **14:00–15:00**

O-047 Adolescent endometriosis

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Endometriosis, histologically defined as functional endometrial glands and stroma developing outside of the uterine cavity, is a common gynecologic disorder. Pathogenesis of endometriosis is enigmatic and remains controversial, even if retrograde menstruation seems the most probable mechanism for the development of the disease. There three types of endometriotic lesions: peritoneal superficial endometriosis (SUP); ovarian endometrioma (OMA); deep infiltrating endometriosis. The economic burden of endometriosis is considerable notably because of repeated absenteeism from work, numerous medical (hormonal and non hormonal) treatments, repetitive surgery and long delay in diagnosis. If endometriosis is usually diagnosed in adults, the disease has its roots in adolescence. Clinical questioning is simple, cost-effective and essential for the diagnosis of endometriosis. A link exists between certain perimenarchial symptoms and the diagnosis of endometriosis, especially DIE. Markers at adolescence associated with the development of endometriosis are the following: family history of endometriosis (especially in first-degree relatives); severe primary dysmenorrhea; absenteeism from school during menses; dysmenorrhea resistant to nonsteroidal anti-inflammatory drug treatment; noncontraceptive use of oral contraceptives to treat severe dysmenorrhea. Questioning patients about their adolescent history can identify markers associated with endometriosis. A better awareness of these markers could help in singling those youngsters who are more prone to develop endometriosis. Today it can only be speculated that a prompter and more thorough handling of severe dysmenorrhea could lead to an earlier diagnosis and a better management. Studies are necessary to precise the relationship between endometriosis and oral contraceptive use, to determine whether we will have to reconsider the management of severe pelvic pain in adolescent and to be able to precise exactly when the disease starts.

Keywords: endometriosis, adolescent, diagnosis, oral contraceptive pills

O-048 The need to have a laparoscopic diagnosis of endometriosis before starting treatment

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Endometriosis is a common disease, affecting women mostly during their reproductive phase. The prevalence is estimated to range between 5–10%, however, due to the predominantly intra-abdominal presence of endometriotic lesions, a lack of clinically robust biomarkers and imaging techniques it remains unclear how common the disease really is. While adolescent girls typically present with dysmenorrhea, older women often also suffer from non-cyclical pain, deep dyspareunia, dyschezia and dysuria as well as fatigue. In addition, 30–50% of women with endometriosis experience fertility problems. There is no place for hormonal treatment in women trying to conceive naturally, however, it is one of the options for women suffering predominantly from pain symptoms. Laparoscopic identification, ideally coupled with the histological confirmation of endometriotic lesions, is generally considered the diagnostic gold standard. In the right clinical setting with sufficient surgical expertise this diagnostic procedure is then combined with surgical excision and/or ablation of endometriotic lesions. However, despite its wide use and years of experience since its introduction into the clinic, laparoscopy, as any surgery, is an invasive procedure associated with potential morbidity and, rarely, mortality. This lecture will discuss the potential pros and cons of empirical treatment, i.e., treatment without a surgical diagnosis of endometriosis assuming the presence of endometriotic disease. It will cover all aspects of diagnostic and therapeutic risks and aims to making a recommendation based on the existing evidence.

Keywords: endometriosis, laparoscopy, diagnosis

INVITED SESSION

SESSION 14: THE END OF THE END? TELOMERES IN HUMAN REPRODUCTION

Monday 15 June 2015 **14:00–15:00**

O-049 Normal telomere shortening and elongation

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Telomeres cap the ends of eukaryotic chromosomes, preventing the recognition and repair of the natural chromosomal terminus by the DNA damage response apparatus. As a consequence of end-replication losses, telomeres shorten with on-going cell division. Short telomeres can elicit a p53-dependent cell cycle arrest, referred to as replicative senescence, that provides a stringent tumour suppressive function. However, in the absence of a functional DNA damage checkpoint response, on-going cell division results in continued telomere erosion and ultimately the loss of the end-capping function. Telomeres can then be subjected to DNA repair activities, resulting in telomere fusion, the formation of dicentric chromosomes and the initiation of cycles of anaphase-bridging, breakage and fusion. The ensuing cellular crisis leads to a strong selection pressure for the up-regulation of telomere maintenance mechanisms, principally telomerase activity, which facilitates the escape from crisis. This process is considered a key mutational mechanism that drives genomic instability and clonal evolution during malignant progression. Our approach has been to develop high-resolution, single-molecule technologies, to study telomere length and fusion in detail; these methods have provided a level of clarity that was hitherto impossible to achieve in human cells. Our work has focused on understanding the mechanisms underlying telomere dynamics and the fusion of short dysfunctional telomeres in human cells. Importantly, this work has provided tools that have allowed us to translate our understanding of telomere dynamics into clinical situations resulting in the development of powerful prognostic markers that are likely to have broad utility in both solid and haematological malignancies. In addition to the role that telomeres play in replicative senescence and genomic instability in somatic tissues, telomere maintenance is also required in the germline to ensure that functional telomeres are passed between the generations. Despite this requirement, we have documented telomeric deletion events in the male germ-line that create extremely short telomeres. These events have the potential to drive large-scale chromosomal mutation events in the male germ line. Our work concerning telomere dynamics and dysfunction in replicative senescence, tumour progression and in the male germ line will be presented.
Keywords: telomere, senescence, DNA repair

O-050 Telomeres and reproductive aging

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Aneuploidy condemns a large proportion of human embryos to implantation failure or miscarriage, and it underlies the aging effect on reproduction in women. Why so many human oocytes and embryos succumb to aneuploidy remains an enigma. Understanding how age increases non disjunction in oocytes and pre implantation embryos is an urgent priority because women increasingly delay childbearing. We have proposed a Telomere Theory of Reproductive Aging. Unlike male germ cells, oocytes and early embryos lack appreciable levels of telomerase activity. Moreover, late ovulating oocytes have traversed more cell cycles during fetal oogenesis and spend longer time in the ovary, exposed to the inevitable effects of reactive oxygen. We have tested this theory in mice and women, and shown that telomere shortening in oocytes drives oocyte aging. Experimental telomere shortening in mice, which in most strains have long telomeres and only modest oocyte aging, recapitulates the reproductive aging phenotype observed in women, including chromosome abnormalities, reduced chiasma and synapsis, spindle abnormalities, pre implantation embryo arrest and apoptosis. Telomere length in the polar body correlates highly with that in the oocyte, so it provides an approach to assess telomere length in oocytes and resulting embryos without perturbing them. Studies in women undergoing IVF show that short polar body telomeres are associated with embryo fragmentation, blastocyst aneuploidy, and failed IVF cycles. Telomere attrition in circulating leukocytes also has been associated with aging, recurrent pregnancy loss and aneuploid offspring. We employed a robust single cell qPCR based assay to measure telomere length in polar bodies and qPCR to measure telomere length in leukocytes from the same women. Telomere lengths in the female germ line and somatic cells showed minimal correlation. Moreover, oocyte but not leukocyte telomere length reflected ovarian function. Finally, experiments in mice show that long term administration of a potent antioxidant and the SIRUIN agonist resveratrol can mitigate the effects of reproductive aging and conserve telomere reserve in the ovary.

SELECTED ORAL COMMUNICATIONS

SESSION 15: NURSING INTERVENTIONS IN CLINICAL PRACTICE

Monday 15 June 2015 **14:00–15:00**

O-051 Training of assisted reproduction nurses in 3D folliculometry and individualized learning

A. Rodríguez[1], R. Vassena[1], A. Blázquez[1], J. J. Guillén[1], S. Franci[1], O. Coll[1], V. Vernaeve[1]
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Study question: Can nurses with no previous experience in transvaginal ultrasound learn to perform folliculometry with 3D transvaginal ultrasound (3DTVUS) during ovarian stimulation cycles (OSC) in assisted reproductive techniques (ART)?
Summary answer: Nurses with no previous experience in transvaginal ultrasound can learn 3D folliculometry during OSC, and they achieve competence within a reasonable timeframe.
What is known already: 3D folliculometry has been shown to be more accurate than conventional 2D ultrasound, producing less intra- and inter-observer variability. Qualified paramedical personnel such as nurses could play an important role in ART by performing specialized technical acts. As LC-CUSUM has been already applied to evaluate learning procedures in folliculometry during OSC in ART physicians, we applied this statistical methodology to nurses, in order to perform tailored training and assess their learning curve in 3D folliculometry.
Study design, size, duration: Prospective study including 8 nurses with more than 2 years experience in ART, carried out between February and September 2014. Study duration was determined by the scan number necessary to achieve competence by each nurse as assessed by LC-CUSUM curves. Fifteen gynecologists participated in the study as experts.
Participants/materials, setting, methods: 3DTVUS scans were performed in oocyte donors from the 8th day of OSC by the expert and the nurse. LC-CUSUM curves were used to assess learning. Success of each nurse measurement was defined as a deviation from the expert's of ≤3 follicles ≥10 mm and ≤2 follicles ≥14 mm measured with 3DTUVS.
Main results and the role of chance: Six of the eight nurses achieved competence in 3DTVUS folliculometry after 68, 106, 141, 153, 185 and 194 ovarian scans, respectively. Two of them did not achieve competence after 200 scans. Customized individual training is being offered outside the study to these two trainees in order to improve their learning and achieve competence. There was significant variability in the number of scans needed to achieve competence by the nurses; however, they were all within a reasonable timeframe. Scans were performed in oocyte donors, a relatively homogeneous population, thus eliminating the possibility of subject characteristics to influence the learning curve.
Limitations, reason for caution: This study cannot evaluate whether interrupting the training will result in longer learning curves, therefore, the results apply only to centers with high patients flow and continuous learning options. LC-CUSUM is an individualized approach to training; the curve must be monitored closely due to the variability in number of scans needed to achieve competence.
Wider implications of the findings: Easiness in achieving competence in 3DTVUS folicullometry in nurses, as shown by this study, may play an important role in standardizing performance in ART units not only in physicians

but also in nurses. No previous experience in ultrasound scanning is needed to become proficient in 3D folliculometry, making 3DTVUS a promising tool to improve the professional reach of nurses with beneficial implications for both the personnel and the ART center.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.
Keywords: 3D ultrasound, nurses training, folliculometry

O-052 Hospitalisation for ovarian hyperstimulation syndrome: prevalence and risk factors in a monocentric cohort study

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[1]*Hopital Saint Joseph, Service de Medecine et de Biologie de la Reproduction, Marseille, France*

Study question: How important is the prevalence and risk factors of hospitalisation for ovarian hyperstimulation syndrome (OHSS) in women undergoing induction of ovulation for *in vitro* fertilization in an IVF centre?
Summary answer: Results showed a low prevalence (17/867 patients) of hospitalization for OHSS with a short mean duration 4.75 ± 3.10 in patients undergoing induction of ovulation for *in vitro* fertilization. All cases with hospitalization, moderate capillary leak syndrome, ascites with or without hydrothorax were recorded. Neither hepatic, cardiac or thrombosis complications were observed.
What is known already: OHSS is a complication of hormonal IVF treatment, two clinical entities are known, early and late OHSS. Early OHSS that is the most iatrogenic can be predicted based on patient risk factors (e.g., polycystic ovary syndrome) and adapted ovulation stimulation or delayed transfers can be proposed to these patients. Late OHSS occurs after embryonic implantation and is prevented by single embryo transfer. Despite the precaution taken, severe OHSS cases were recorded.
Study design, size, duration: It was a 2-year observational study including 867 patients who benefited IVF treatment in a French Center. Cycles with hospitalization (n = 17) for OHSS were included in the "cases group" and were compared with cycles without hospitalization (n = 1082) based on following criteria: E2 > 900 pg/mL and number of oocyte at pick up >4.
Participants/materials, setting, methods: Database was obtained using medical records. Each patient was monitored at least 5 weeks after treatment, and longer when pregnancy occurred. Descriptive analysis of the population was performed and Khi2, fisher-exact-test or student-test analysis used to compare hospitalized for OHSS and non hospitalized cases.
Main results and the role of chance: There were 1099 cycles with ovarian follicles pick up, 17 patients (1.54%) were hospitalised for OHSS. These patients were younger (p = 0.017) and displayed more dysovulation (p = 0.015) than others. They have more frequently received a recombinant FSH than urinary FSH (p = 0.010). Number of oocytes were significantly more important in hospitalised cases (p < 0.001). There is no agonist short protocol in the cases group, neither statistical difference using long agonist protocol. The mean number of transferred embryo is less important in cases group (p < 0.001). Pregnancy rate per transfer was not significantly different. All patients hospitalized presented pain and ascites. Four of them were hospitalised for pleural effusion. Risk factors of OHSS were identified for three patients; one for previous uncontrolled response, and the others for polycystic ovary syndrome.
Limitations, reason for caution: The risk factors of OHSS in women undergoing induction of ovulation for IVF are consistent with literature, but a multicentric study is required to confirm the role of urinary FSH in limiting hospitalization. Increasing number of participant would allow evaluating the implication of body mass index in OHSS severity.
Wider implications of the findings: For patient with risk factors, we propose short ovarian stimulation protocols, single embryo transfer or postponed transfer to limit hospitalisation. The development of vitrification had a key role to play in the management of patients at risk of OHSS either by embryo freezing or by oocyte vitrification. The next step is to define the best strategy: oocyte or embryo preservation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hopital Saint Joseph.
Trial registration number: NA.
Keywords: hospitalisation, OHSS, IVF, risk factors

O-053 Fertility awareness in the Flemish population: optimism can be disadvantageous

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[2]*KHLim University College, Nursing/Midwifery, Hasselt, Belgium*
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[5]*Uppsala University, Public Health, Uppsala, Sweden*

Study question: Do Flemish adolescents, students and people of reproductive age have sufficient fertility-related knowledge in order to make informed decisions?
Summary answer: There is a lack of fertility-related knowledge within the Flemish population. Adolescents, students and people of reproductive age overestimate both their fertility and the efficacy of assisted reproduction.
What is known already: Similar studies have been performed in student-populations. Our results confirm earlier findings. Our study is the first in the Flemish (Belgian) population and the first study including results of adolescents and people of reproductive age.
Study design, size, duration: This study has a cross-sectional design. We included 989 adolescents (mean age 15 years), 348 students (mean age 23 years) and 374 persons of reproductive age (mean age 35 years, range 25–45 years).
Participants/materials, setting, methods: Adolescents were recruited at school (different education types). Students were recruited at their University College or at their University. People of reproductive age were randomly addressed in hospitals, sports facilities, play courts and shops. The Swedish Fertility Awareness Questionnaire (Lampic, 2006) was translated and adapted according to the study group.
Main results and the role of chance: The majority of Flemish adolescents, students and people of reproductive age believe that female fertility starts to decline after the age of 35 years. More than 50% of Flemish adolescents believe that there is a marked decrease in female fertility after age 50. Thirty-five percent of Flemish students believe there is a marked decrease in female fertility at age 40–44, 12% think there is a marked decrease at age 45–49 and 20% think there is only a marked decrease after the age of 50. Similar results are found in people of reproductive age. The majority of the Flemish population believes that there is a 40–100% chance to become pregnant in one ART-cycle.
Limitations, reason for caution: Schools were randomly selected, but participating schools were located in only two (out of five) provinces of Flanders. The difficulty-level of the questionnaire was possibly too high for the adolescents.
Wider implications of the findings: These results indicate that more education is needed in fertility-related factors. Increased knowledge is desired in order to allow informed decisions in family planning. Lack of knowledge may contribute to the tendency to postpone parenthood in Western countries.
Study funding/competing interest(s): Funding by national/international organization(s) – Praktijkgericht Wetenschappelijk Onderzoek (PWO).
Trial registration number: NA.
Keywords: fertility awareness, knowledge, maternal age

O-054 Nursing use of failure mode and effects analysis (FMEA) to review incident reports in an infertility clinic

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Study question: The objective of the study is to review and analyze, using FMEA, all incident reports submitted to the hospital safety reporting database primarily focusing on ART lab /nursing communication.
Summary answer: Thirty-three percent of the incident reports were related to failure in communication between nursing and laboratory staff. Eight percent of the incidents were classified as severe; however, the majority were classified as mild.
What is known already: Communication is key to the delivery of safe, quality care in a medical setting. The complexity of a IVF clinic together with its rapidly changing technology, can complicate the exchange of information among different care providers. FMEA is a risk assessment tool designed to identify failure that may occur in a process in order to understand how and why mistakes happen.
Study design, size, duration: Review of incidents reports submitted between December 2013 and November 2014. Seventy-three incident reports filed.

Participants/materials, setting, methods: Members of the clinic report incidents that may compromise care. This could include any deviation from protocols, documentation, and transcription errors. All reports submitted to the safety reporting database between December 2013 and November 2014 were divided between those pertaining to ART lab and nursing communication and all others.
Main results and the role of chance: Seventy-three incidents were submitted in the period of December 2013 and November 2014. Twenty four (24) of the reports (33%) were related to communication between nursing and ART lab. These reports were classified according to FMEA. They were divided into 3 categories: minor, moderate, and severe. Fifteen (63%) were minor, two (8%) were severe and the remaining seven were moderate (29%). The majority of the incidents covered issues such as consents, transcription errors, physicians orders and documentation. None of these incidents caused harm to the patient. Chance is not a factor.
Limitations, reason for caution: There is no bias or confounding factors in this study.
Wider implications of the findings: The findings of this study are in line with other studies about communication and its complexity in healthcare. By improving communication many of these errors could be eliminated. The streamlining of paperwork and obtaining an electronic medical records (EMR) could also reduce communication errors.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: nursing, communication, ART lab, quality

SELECTED ORAL COMMUNICATIONS

SESSION 16: EMBRYO CULTURE SYSTEMS AND OUTCOME

Monday 15 June 2015 **15:15–16:30**

O-055 The beneficial effect of autologous endometrial cells in an *in vitro* coculture system on human early embryo development: a randomized study

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Study question: What is the influence of autologous endometrial cells in a coculture system on blastocyst rate at day 5/6 (D5/6) compared with conventional culture medium in *in vitro* fertilization (IVF).
Summary answer: Autologous endometrial cells in an *in vitro* coculture system significantly improved the available blastocysts (D5/6) for single fresh embryo transfer and cryopreservation compared with conventional extended culture medium.
What is known already: *In vitro* culture conditions, including culture medium affect early embryo quality. For mimicking the microenvironment of early embryo development under IVF conditions, autologous endometrial coculture (ECC) system using the patient's own endometrial cells (EC) has been reported.
Study design, size, duration: This interventional, monocentric, randomized, double-blind controlled trial was conducted at our clinic in Canada, from April 2013 to November 2014 and is still ongoing. Forty-four IVF couples were enrolled into one of two embryo culture systems: conventional culture medium (26 controls) or on a coculture system of EC (18 coculture).
Participants/materials, setting, methods: For each patient, an endometrial biopsy was performed during luteal phase of the cycle prior to IVF. For the coculture group, EC were isolated from biopsies and grown on the day after ovulation triggering. At day 2, top quality embryos were placed either on ECC or in conventional culture medium.
Main results and the role of chance: At day 2, a total 193 and 108 top quality embryos were compared between control and coculture (mean ± SD per patient 7.4 ± 3.7 and 6.0 ± 2.2 respectively). At days 5 or 6, the blastulation rate was 34.7% (67/193) versus 60.2% (65/108) in the control and coculture groups respectively (P =0.00002). Consequently, the proportion of useful blastocysts (for fresh replacement or cryopreservation) in the coculture group was significantly higher (25.5 %) compared to the control group.
Limitations, reason for caution: The cohort size is low and the study is still ongoing at our institution.
Wider implications of the findings: The data revealed that a coculture system on endometrial cells improves the percentage of blastocysts for fresh transfer or cryopreservation compared with conventional culture medium. The coculture system of endometrial cells could potentially lead to a reduction of IVF cycles by increasing the cumulative implantation and clinical pregnancy rates. This hypothesis remains to be demonstrated by further analysis.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Genbiotech supplied the ECC freezing/culture kits free of charge. There were no conflicts of interests to be declared.
Trial registration number: ClinicalTrials.gov ID: NCT01886118.
Keywords: autologous coculture, endometrial cells, *in vitro* fertilization, embryo quality, blastocyst

O-056 Single step versus sequential culture medium: effects on embryo development, genetic and clinical outcomes

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Study question: What is the impact of performing embryo culture in sequential or single step culture medium on embryo development, genetic and clinical outcomes?
Summary answer: Single medium culture system has a positive effect on embryo development. In particular, embryos cultured in this system show an increased blastocyst developmental rate as compared to embryos cultured in sequential media. No significant differences were found in aneuploidy rate and clinical outcomes between the two groups.
What is known already: The embryo culture has an important role to determine IVF success. Two approaches have been proposed. The first, defined "back to nature" is based on sequential culture media, which mimic *in vivo* conditions. The second, defined "let the embryo choose" is based on a single medium, which contains all the components needed during embryo development. No clear evidences are available to understand which is the most efficient approach.
Study design, size, duration: Prospective randomized cohort study conducted from September 2013 to September 2014. Patients were randomly assigned to Group A (embryo cultured in sequential medium; SAGE, USA), or Group B (single step medium without refresh on day 3; IrvineScientific, USA). Blastocyst formation rate, aneuploidy rate and clinical outcomes were compared between the two groups
Participants/materials, setting, methods: A total of 3.652 embryos were cultured either in a bench-top incubator (MINC, Cook, USA) or in the Embryoscope (Unisense, Denmark). 1.617 reached blastocyst stage, among which 1.007 blastocysts underwent also comprehensive aneuploidy screening on trophectoderm biopsy. Logistic regression analysis was used to control for confounding factors.
Main results and the role of chance: Blastocyst developmental rate was 43.0% (766/1782) for group A, and 45,4% (877/1930) for group B ($p < 0.05$). Logistic regression analysis adjusted for female age, sperm quality, stimulation protocol, incubator type, aneuploidy rate and clinical outcomes confirmed this association (OR = 0.86, 95% CI = 0.74–0.98). Blastocyst formation rate was significantly different according to the incubator type, 48,2% (519/1077) and 42.6%(1098/2575) for embryoscope and bench-top incubator respectively (OR1.36, 95% CI = 1.17–1.57; $p < 0.001$). Aneuploidy rate was similar between the two groups after adjustment for female age (58.6% and 61.8% for group A and B, respectively P = NS). Biochemical (group A = 3.3% and group B = 6.6%, OR = 0.59, p = NS), miscarriage (group A = 8.0% and group B =7.0%, OR = 0.89, p = NS) and ongoing implantation rates (group A = 46.4% and group B = 42.2%, OR = 1.15, p = NS) were comparable between the two groups.
Limitations, reason for caution: Clinical outcomes analysis should be extended to cumulative live birth rate, including all the thawed embryo transfers of the involved patients, to have more evidences about the culture systems efficiency. From this study design it cannot be excluded that the advantages observed in the use of single media system is also due to the reduced manipulation of embryos.

Wider implications of the findings: Optimal *in vitro* conditions to support blastocyst development is crucial in the modern embryology. Most of the new approaches for embryo selection and evaluation require extending the culture to blastocyst stage. The possibility to increase the relative blastocyst formation rate up to 20% by adjusting some simply laboratory protocols, excluding any negative effect on clinical outcomes, is crucial to continue the progression of embryology.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: embryo culture, human embryo development, single medium, sequential medium, clinical outcomes

O-057 Effect of oxygen tension on embryo development and live birth in clinical *in vitro* fertilization (IVF) – the reproductive medicine network physiologic oxygen (PhOx) study

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Study question: Does a more physiologic oxygen tension (5% pO_2) improve live birth and embryo development rates in clinical IVF?
Summary answer: In fresh IVF cycles, in spite of an increase in implantation rate with the use of 5% pO_2, there is no difference in live birth rates between 5% and 20% pO_2. However, culture in 5% pO_2 results in significantly increased numbers of blastocysts available for transfer and cryopreservation.
What is known already: Embryos *in vivo* are exposed to substantially lower partial oxygen tension than in air. Elevated pO_2 increases free radicals leading to DNA damage and even short term oxidative stress is detrimental to gametes and embryos. In animal studies a 5% pO_2 is preferable, but human studies have been more difficult to interpret either showing improvement in development and/or live birth in good prognosis patients or demonstrating no significant benefit.
Study design, size, duration: Prospective, randomized, multicenter, double blind clinical trial evaluating the use of 5% vs. 20% pO_2 for the culture of human embryos. Primary outcome: Live birth. Secondary outcomes: Embryo development and utilization rates, implantation rate, pregnancy loss rate.
Participants/materials, setting, methods: 840 couples undergoing their first or second fresh, autologous IVF cycle with female partner ≤42 years of age. Sample size calculated to detect a 10% absolute difference in live birth rate between 5% and 20% pO_2 with 80% power and a 2-sided Type I error of 5%.
Main results and the role of chance: "There was no significant difference between live birth rates following embryo culture in 5% vs. 20% pO_2 (40.4% vs. 38.3%; $p = 0.551$). However, culture in 5% pO_2 resulted in a lower rate of day 3 and a higher rate of day 5/6 transfers ($p = 0.004$), a higher implantation rate regardless of day of transfer (32.4% vs. 27.7%; $p = 0.044$) and a higher blastocyst utilization rate (20.2% vs. 14.8%; $p < 0.001$) and frozen blastocyst per retrieved egg rate (7.7% vs. 5.7%; $p < 0.001$). There were no differences in pregnancy loss rates, neonatal birth weights or the incidence of low birth weight between the two groups. Although singletons in both groups delivered at or close to term (38.4 ± 2.2 vs. 39 ± 1.5 weeks), this difference favored embryos cultured in 5% pO_2 ($p = 0.045$).
Limitations, reason for caution: This study was powered to evaluate overall differences in live birth rates between 5% vs. 20% pO_2. No conclusions can be drawn as to whether there may be subgroups of couples whose delivery rate following IVF may benefit from a reduced pO_2. Nevertheless, the statistically significant results presented are intriguing.
Wider implications of the findings: Although live birth rates per embryo transfer procedure for embryos cultured in 5% vs. 20% pO_2 do not differ, the highly significant improvement in embryonic development and the increased number of generated and cryopreserved blastocysts support the conclusion that the more physiologic 5% pO_2 is the preferred oxygen tension for the culture of human embryos in clinical IVF at the present time.
Study funding/competing interest(s): Funding by national/international organization(s). Eunice Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA
Trial registration number: NCT01010386.
Keywords: IVF, oxygen tension, embryo development

O-058 Should we consider Day-2 embryo quality before Day-5 transfer when blastocysts reach a similar good quality?

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Study question: Does Day-2 embryo morphology have an impact on the outcome of good-quality blastocysts transfers (BT)?
Summary answer: The present investigation failed to find any significant difference in the clinical pregnancy rates (CPR) between the transfers of good-quality blastocysts derived either from good or poor-quality embryos on Day-2.
What is known already: Recently, extended culture strategy has considerably spread, as a consequence of advances in culture media. BT improves implantation rates, as a result of the synchronization between embryonic stage and endometrial receptivity, and the possibility to improve embryo selection. Actually, the efficiency of this strategy has been demonstrated in good prognosis population. However, nothing is known upon the impact of Day-2 embryo morphology on the outcome of BT having equivalent good quality.
Study design, size, duration: We retrospectively analyzed 124 BTs performed on Day-5 between January 2012 and September 2014. Inclusion criteria were: (i) female age <37 years; (ii) IVF/ICSI rank ≤2; (iii) transferred blastocyst quality: blastocoele B3/4, inner cell mass A/B, trophectoderm A/B, according to Gardner and Schoolcraft classification; (iv) known implantation data.
Participants/materials, setting, methods: Embryo quality on Day-2 was reported and sorted into «good» or «poor» quality for each transferred blastocyst. Day-2 good-quality embryos were defined by the presence of 3–5 adequately-sized blastomeres and <20% fragmentation with no multinucleation. CPR were compared between groups of transferred blastocyst(s) derived from "good" or "poor Day-2 quality" embryo(s).
Main results and the role of chance: Patients' characteristics were similar between the «good» and «poor Day 2 quality» groups. Overall, women's mean age was 30.3 ± 4.1 and 30.2 ± 4.2 years, respectively. In addition, in both groups, mean attempt rank was 1.2, and an average of 1.1 blastocyst was transferred. Finally, in group «good Day-2 quality», 51 clinical pregnancies were obtained following 99 BTs (51.5%), whereas 12 clinical pregnancies out of 25 transfers (48.0%) were reported in the «poor Day-2 quality» group. The difference between those two rates was not significant.
Limitations, reason for caution: The main weakness of this work is the low number of analyzed cycles. A further prospective randomized study enrolling a larger number of patients is required to confirm these present data. Moreover, the analysis of live birth rates among both groups would be relevant.
Wider implications of the findings: Taking those results into account, good quality blastocyst transfer should be performed irrespective of embryo quality on Day-2, since it may not compromise success rates in our good prognosis population.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Jean Verdier University Hospital.
Trial registration number: NA.
Keywords: good quality blastocyst, blastocyst transfer, Day-2 embryo quality interest

O-059 Comparison of development and ploidy status obtained in 127 sibling embryos, cultured in one-step or two-step medium with time-lapse technology in preimplantation genetic screening cycles

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Study question: The aim of this study was to evaluate possible differences among blastocyst formation rate, ploidy status and time-lapse morphokinetics

in 127 sibling embryos, cultured in one-step or two-step media from the same brand, in Preimplantation Genetic Screening (PGS) cycles performed with trophectoderm biopsy and array comparative genomic hybridization (aCGH).
Summary answer: A statistically higher percentage of blastocyst was obtained and biopsied in one-step compared to two-step medium. In addition, the blastocysts started to blastulate faster in one-step than in two-step medium. However, the percentage of euploid blastocysts was comparable in the two groups.
What is known already: Some reports showed that the choice of culture medium could have a significant effect on early embryonic and fetal development. The choice of the best culture medium is still controversial. Different culture media can lead to morphologically identical blastocysts, suggesting that preimplantation embryos have high capability to adapt to different culture conditions. To date, most studies comparing different media have not considered their impact on ploidy status of the derived blastocysts.
Study design, size, duration: The development of 127 sibling embryos, obtained in 16 PGS cycles performed from November to December 2014 with trophectoderm biopsy and aCGH, were analyzed. Mean female age was 36.1 ± 4.46 years old. All embryos were cultured in a time-lapse incubator, allowing the morphokinetic parameters to be analyzed.
Participants/materials, setting, methods: The oocytes from each patient were randomly incubated in 1:1 ratio in one-step or two-step media (SAGE, ORIGIO, USA). After denudation, all mature oocytes were injected and cultured until the blastocyst stage, maintaining the same culture medium. The obtained blastocysts were biopsied and vitrified waiting for the genetic analysis.
Main results and the role of chance: In both group, 85 oocytes were injected, obtaining equivalent fertilization rates of 77.6% ($N = 66$) and of 82.4% ($N = 70$) in one-step versus two-step medium, respectively (NS). The embryos obtained on day-3 were 63/66 = 95.5% and 64/70 = 91.4% in one-step and two-step medium, respectively (NS). The number of blastocysts obtained was higher in one-step (73.0%, $N = 46$) compared to two-step medium (50.0%, $N = 32$; $p = 0.0106$). Forty and twenty-five of them were biopsied in one-step and in two-step groups, respectively. The percentages of euploid blastocysts were 40.0% ($N = 16$) in one-step and 52.0% ($N = 13$) in two-step groups (NS). All the morphokinetic parameters were analyzed: the only significant difference observed between the two groups was the starting blastulation time, being faster in one-step compared to two-step medium (107.42 ± 11.55 vs. 100.74 ± 9.46 h, $p < 0.01$).
Limitations, reason for caution: There is still a considerable disagreement regarding which morphokinetic parameters are useful to predict blastocyst formation, implantation potential and ploidy status of the embryos. More data, including clinical pregnancy, implantation and take home baby rates, are necessary, in order to choose the best culture conditions.
Wider implications of the findings: Although it seems that employing single-step medium can lead to a higher number of blastocysts available for genetic analysis and cryopreservation, the final percentage of euploid transferable ones is comparable to that obtained with sequential-medium. It remains to be established whether faster and more numerous blastocysts will lead to improved clinical outcomes, especially in some categories of patients that usually have a high cycle cancellation rate, as poor responders or severe male infertility.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No specific funding was obtained for this study. None of the authors have any competing interests to declare.
Trial registration number: NA.
Keywords: embryo culture, culture media, blastocyst formation rate, time-lapse technology, blastocyst biopsy

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SELECTED ORAL COMMUNICATIONS

SESSION 17: ASSESSMENT OF OVARIAN RESERVE AT YOUNG AGE

Monday 15 June 2015 **15:15–16:30**

O-060 Diabetes mellitus (DM) during pregnancy is associated with maternal hyperandrogenism and elevated anti-Müllerian hormone (AMH) levels in the female offspring

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Study question: Is DM associated with maternal hyperandrogenism during pregnancy and elevated AMH levels in the female offspring?
Summary answer: Hyperandrogenism is observed in pregnant women with DM during the 2nd trimester and at time of delivery (TOD), which is associated with higher insulin levels. Daughters of women with DM have higher AMH levels suggesting that DM during pregnancy affects ovarian function of the offspring.
What is known already: Non-pregnant women with DM have higher androgen levels compared to healthy women, but it is not known whether hyperandrogenism is observed during pregnancy in diabetic women and the effect of maternal DM on the ovarian function of the female offspring
Study design, size, duration: Prospective study of pregnant women with Type 2 DM (mT2D, $n = 20$), Gestational Diabetes (mGD, $n = 27$) and control (mC, $n = 22$) carrying a female fetus. Assessment of the ovarian function of daughters of women with mT2D (dT2D, $n = 20$), mGD (dGD, $n = 27$), and mC (dC, $N = 22$).
Participants/materials, setting, methods: Blood sample of the mother was obtained: 24–28 weeks, 32–34 weeks and at TOD. A venous blood cord sample (VBCS) of the offspring was obtained at TOD. Androgens, insulin, IGF-1 HbA1c, and AMH levels were measured. Data analysis: ANOVA and Dunnett post test and Pearson's r correlation coefficient.
Main results and the role of chance: mT2DM and mGDM group had higher testosterone and insulin levels than mC at 32–34 weeks (1.0 ± 0.1 ng/ml; 0.7 ± 0.1 ng/m vs. 0.5 ± 0.1, $P < 0.001$; 28.4 ± 7.1 uUi/ml; 17.2 ± 4.4 uUi/ml vs. 9.5 ± 1.1 ng/ml; $P = 0.03$, respectively) and at TOD (1.1 ± 0.1 ng/ml; 0.8 ± 0.1 ng/ml vs. 0.7 ± 0.1 ng/ml; $P = 0.001$; 18.5 ± 2.6 ng/ml; 13.9 ± 2.3 ng/m vs. 10.2 ± 1.5 ng/ml; $P = 0.04$, respectively). Maternal T levels had a positive correlation with insulin ($r = 0.2$; $P = 0.04$) and IGF-1 levels ($r = 0.3$; $P = 0.01$). dT2DM and dGDM had higher AMH levels than dC (2.5 ± 0.7 ng/ml; 2.2 ± 0.9 ng/ml vs. 0.6 ± 0.3 ng/ml; $P = 0.04$.
Limitations, reason for caution: The size of our study may have limited the power of the study to detect an association of metabolic control with testosterone levels in pregnant women with DM.
Wider implications of the findings: This is the first study that shows that hyperandrogenism is observed in pregnant women with T2DM and GDM during the 2nd half of pregnancy. Diabetic environment during pregnancy affects the ovarian function of the female offspring at TOD, as shown by elevated AMH levels in these newborns. Long term follow up of the offspring is needed to evaluate their predisposition to develop Polycystic Ovary Syndrome is needed.
Study funding/competing interest(s): Funding by University(ies) – FONDECYT grant No 11121460 to Claudio Villarroel.
Trial registration number: None.
Keywords: diabetes mellitus, fetal programming, ovarian function of the offspring, hyperandrogenism, anti-Müllerian hormone

O-061 The influence of *in vivo* exposure to nonylphenol ethoxylate 10 (NP-10) on the ovarian reserve

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Study question: To determine the effect of environmental exposure to nonylphenol ethoxylate 10 (NP10, Tergitol), a non-ionic detergent, on the ovarian reserve in a controlled mouse model.
Summary answer: Exposure to NP-10 in the drinking water delayed the expected age-related decline in the ovarian response to gonadotropins, most probably by inhibiting primordial follicle recruitment.
What is known already: NP-10 is a common non-ionic detergent and is a wide spread environmental polluent. It penetrates the cellular and mitochondrial membranes and was recently shown *in vitro* to inhibit respiratory chain complex I activity. Its impact on the ovarian reserve is unknown.
Study design, size, duration: Female CB_6F_1 mice were maintained in a plastic free environment from 3 to 7 weeks. They drank HPLC grade water

supplemented with 4 μmol/L NP-10. Controls drank HPLC water. Untreated control groups were 3-weeks and 7-weeks old mice. At the endpoint they were stimulated, mated and embryos were *in vitro* cultured.
Participants/materials, setting, methods: Identically treated mice were sacrificed without ovarian stimulation. Their ovaries were examined for follicular population composition, apoptosis and proliferation. The enzymatic activities of respiratory chain complexes I and IV (cytochrome *c* oxidase) and citrate synthase (control enzyme) and reactive oxygen species (ROS) production was determined in brain and liver mitochondria.
Main results and the role of chance: Untreated 7-week old mice produced less oocytes (23.3 ± 3.1 vs. 66 ± 15.9) and embryos (15.3 ± 3.5 vs. 60 ± 15.5) than untreated 3-week old mice. 7-week old mice exposed to NP-10 produced more oocytes (34.2 ± 8.5 vs. 21.7 ± 8) and embryos (26.7 ± 6.5 vs. 5 ± 3.9) than the 7-week old controls drinking HPLC grade water. In the histological cross sections, significantly more primordial and primary follicles were counted in the ovaries of 7-week old NP-10 treated mice, than in the ovaries of the 7-week old controls (drinking HPLC grade water or untreated). In liver and brain mitochondria, the respiratory chain activities in citrate synthase, as well as ROS production, were not significantly different in NP-10 treated mice in comparison to controls.
Limitations, reason for caution: Our findings may be limited to the specific animal model studied.
Wider implications of the findings: In-vivo exposure to NP-10, in an animal model, ameliorated the age related decline in the ovarian response to gonadotropins by inhibiting recruitment of primordial follicles, without any detrimental effect on the cellular respiratory chain in the brain or liver. To the best of our knowledge this is the first reported successful inhibition of physiologic ovarian aging *in vivo*. The mechanism underlying this process is yet to be investigated.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hadassah-Hebrew University Medical Centre, Beilinson Hospital, Rabin Medical Centre, affiliated to the Sackler Faculty of Medicine, Tel Aviv University.
Trial registration number: NA.
Keywords: ovarian reserve, nonylphenol ethoxylate 10, primordial follicles, mouse model

O-062 Longitudinal and cross sectional assessment of the relationship between serum testosterone at age 15 and AMH levels at ages 7, 9, 11, 13 and 15

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Study question: Do girls in the upper end of testosterone range have higher anti-Müllerian Hormone (AMH) levels at age 7, 9, 11, 13 and 15?
Summary answer: Girls with relatively high testosterone at age 15 had relatively higher AMH levels at ages 7, 9, 11 and 15.
What is known already: Longitudinal studies assessing the trends in AMH levels from childhood up to puberty are scanty. They are needed to establish a wider predictive value for ovarian reserve and testosterone levels in girls. There is evidence to a strong intra-individual tracking in AMH levels between ages 7 and 9 and 9 and 11 in girls. A recent report found strong correlation between AMH and testosterone levels at age 16 and future PCOS and hyperandrogenism.
Study design, size, duration: A retrospective study on a prospective European birth cohort. The study includes longitudinal assessment of the relationship between AMH levels at age 7, 9, 11, 13, and 15 and the testosterone, SHBG and FAI at age 15–16 years in 486 girls from the ALSPAC birth cohort.
Participants/materials, setting, methods: AMH assayed in blood samples collected age 7, 9, 11 and 13 in 486 girls. AMH, Testosterone and SHBG assayed age 15–16 in 1770 girls including the previous 486. Correlational statistical analyses were conducted to assess the relationship between AMH (at different ages) and testosterone, SHBG and FAI at age15–16.
Main results and the role of chance: There was a statistically significant relationship between testosterone and SHBG levels at age 15–16 and AMH levels at ages; 7 ($P < 0.001$), 9 ($p < 0.001$), 11 ($p < 0.001$), 13 ($p < 0.001$) and 15–16 ($p < 0.001$). There was a negative correlation between AMH at age 15–16 and SHBG at the same age ($p < 0.001$). The relationship between AMH and testosterone and SHBG remained statistically significant after adjusting for age of menarche, body mass index and total body fat. We conclude that AMH measured in childhood from age 7 years onwards has the potential to predict testosterone levels after menarche.
Limitations, reason for caution: The relatively small number that limits the proportion of girls with high testosterone level may limit establishing a reliable cut off points to assess AMH value as an early predictor of testosterone levels and potentially PCOS risk in early adulthood.
Wider implications of the findings: The results of the study suggest that the ovarian testosterone production is decided from as early as 7 years of age and potentially earlier. This is encouraging that in a larger samples we would be able to establish cut off points of AMH in childhood to predict accurately future testosterone levels thence the risk of related conditions such as PCOS and potentially cardiovascular risk. This should help guide health care measures.
Study funding/competing interest(s): Funding by national/international organization(s) – Wellcome Trust.
Trial registration number: WT089549.
Keywords: anti Müllerian hormone, testosterone, birth cohort

O-063 Ovarian reserve screening in young women

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Study question: Do we have the capability to detect young women with a poor ovarian reserve through the ultrasonographic and hormonal assessment of its ovarian reserve (OR)?
Summary answer: Using 3D-ultrasound technology and serum anti-Müllerian hormone (AMH) concentrations we are capable of identifying women with a diminished OR at an early age.
What is known already: OR is generated by the net result of the original magnitude of primordial follicle endowment. The size of this reserve, evaluated by antral follicle count (AFC), seems to be decidedly variable between individuals of similar age. Currently, AFC can be accomplished by two different methods: ovarian ultrasonography and measurement of endocrine markers, as both have a notable agreement with histologically assessed primordial follicle number and the response to gonadotropin stimulation in IVF treatment.
Study design, size, duration: A prospective, population-based, cross-sectional study conducted in our Department of Obstetrics, Gynaecology and Reproductive Medicine, from January 2009 to January 2015. The study was approved by the institutional review Board and all participants gave informed consent for the trial.
Participants/materials, setting, methods: *Study group*: 653 healthy women (18–29 years) recruited from students at University of Valencia. *Positive control group*: 12 women with high risk of premature ovarian insufficiency. *Negative control group*: 15 women with BCRA mutation undergoing prophylactic salpingo-oophorectomy (PBSO). 3D-ultrasound AFC and AMH level were determined on days 2–5 of menstrual cycle.
Main results and the role of chance: *Positive control group*: Young women in high risk of premature ovarian insufficiency presented significant low levels of AMH as well as reduce AFC. *Negative control group*: BCRA mutation carriers with normal AMH and AFC presented obviously undetectable AMH/AFC following PBSO. *Study group*: The prevalence of young women with poor ovarian reserve under both ultrasonographic and hormonal test was 4.74%. On the other hand, 14.69% of the participants presented a high ovarian reserve as they fulfilled both ultrasonographic and hormonal features of polycystic ovary syndrome. Therefore, 80.57% of the participants presented a mainstream OR. The mean AMH levels and AFC in women with poor OR in the study group were comparable to those detected in the group of patients in high risk of premature ovarian insufficiency.
Limitations, reason for caution: We cannot exclude potential selection bias due to undetermined differences between our sample (tertiary educated women) and background community.
Wider implications of the findings: A later start of childbearing is a prominent feature of recent fertility trends in developed countries. This postponement of motherhood has resulted in couples having children in a period when women's fecundity is already in decline. OR screening at a young age gives women the possibility to plan their childbearing. Consequently, those women presenting

a poor OR have the opportunity of anticipating maternity or preserving their fertility trough oocyte vitrification.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – University of Valencia, Spain, Hospital Clinico Universitario, Valencia, Spain.

Trial registration number: None.

Keywords: ovarian reserve, screening, 3D-ultrasound, anti-Müllerian hormone, young women

O-064 Clinical assessment of ovarian function in long-term female survivors of childhood cancer: results of the Dutch nationwide DCOG LATER-VEVO study

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Study question: What is the effect of anti-cancer treatment on the ovarian function of long-term female survivors of childhood cancer (CCSs)?

Summary answer: Female CCSs are at an increased risk of a diminished ovarian function as measured by FSH, AMH, Inhibin B, and antral follicle count (AFC). Radiotherapy to the abdomen and/or pelvis, total body irradiation, as well as the use of alkylating agents are treatment factors associated with this increased risk.

What is known already: Over the past decades major improvements in the treatment of childhood cancer have resulted in high survival rates. However, among female CCSs a compromised reproductive system is an important and frequently encountered late effect. Anti-cancer treatment, often consisting of a combination of radiotherapy and chemotherapy, may reduce fertile life span and induce premature menopause, since therapy may deplete or accelerate the decline of the non-renewable pool of primordial follicles in the ovaries.

Study design, size, duration: The study is part of the DCOG LATER-VEVO study, a nationwide retrospective cohort study on female fertility of Dutch CCSs. The control group consisted of sisters of survivors and females from the general population. Data collection took place between January 2008 and May 2014.

Participants/materials, setting, methods: The study population consisted of female 5-year CCSs who were between 1963 en 2002 in The Netherlands, and who were at least 18 years at inclusion. Of the 1,108 CCSs and 819 controls who participated in the study, 633 (57%) and 433 (53%), respectively, provided blood and/or ultrasound data.

Main results and the role of chance: In general, median (interquartile range) FSH values were higher for CCSs compared to controls, whereas AMH, Inhibin B, and AFC were significantly lower (see Table).

	FSH (U/l)		AMH AMH (μg/l)		Inhibin B (ng/l)		AFC (no.)	
	Surv	Contr	Surv	Contr	Surv	Contr	Surv	Contr
Age 18–30 years (n = 606)	5.5 (2.4)	5.5 (2.1)	2.7 (3.5)	3.4 (3.8)	72.3 (50.4)	77.7 (54.8)	13.0 (7.0)	20.0 (13.0)
	ns		p = 0.01		p = 0.02		p < 0.001	
Age 31–40 years (n = 317)	6.3 (3.3)	6.1 (2.7)	1.2 (2.3)	1.5 (2.6)	53.5 (70.6)	73.8 (51.4)	9.0 (8.0)	13.0 (11.0)
	p = 0.02		p = 0.004		p < 0.001		p < 0.001	
Age ≥ 41 years (n = 138)	14.7 (36.1)	9.3 (16.2)	0.1 (0.2)	0.2 (0.8)	5.0 (34.1)	28.7 (61.5)	3.0 (7.0)	5.0 (6.0)
	ns		p = 0.002		p = 0..002		ns	

CCSs were at risk of having elevated FSH levels (>10 U/l), low AMH levels (<0.5 μg/l), low Inhibin B levels (<50 ng/l), as well as a low AFC (no. ≤5) (all p < 0.001). Treatment with radiotherapy to the abdomen and/or pelvis, total body irradiation, and alkylating agents, were significant independent predictors of reduced ovarian function.

Limitations, reason for caution: Treatment for childhood cancer often involves multimodal therapies. The effect of interaction of different treatment modalities as well the effect of doses and age at time of treatment remains to be established and will be evaluated in the near future.

Wider implications of the findings: The results of the study greatly improve the physician's ability to counsel female CCSs on family planning. However, not only female CCSs will potentially benefit from this study. Also females who are about to undergo cancer therapy may benefit since proven high risk populations can be offered fertility preservation timely. All with the aim of maintaining or improving the quality of life of these women.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the Dutch Cancer Society (grant no. VU 2006-3622) and by Foundation Children Cancer Free. None of the authors report a conflict of interest.

Trial registration number: NTR2922 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=2922.

Keywords: ovarian function, childhood cancer survivors, ovarian reserve, reproductive endocrinology

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SELECTED ORAL COMMUNICATIONS

SESSION 18: PREDICTOR FACTORS IN FEMALE INFERTILITY

Monday 15 June 2015 **15:15–16:30**

O-065 Chlamydia trachomatis serostatus is an independent predictor of pregnancy and pregnancy outcome

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Study question: Does seropositivity to *Chlamydia trachomatis* (Ct) predict pregnancy and pregnancy outcome among infertile women with documented tubal patency?

Summary answer: Even in the presence of tubal patency, anti-Ct IgG3 seropositivity is associated with a lower likelihood of pregnancy and live birth. Anti-Ct IgG3 seropositive women have up to 3 times the risk of ectopic pregnancy. Women on letrozole who are seropositive for anti-Ct IgG3 experience the greatest reduction in fertility.

What is known already: Ct antibody titers have been shown to predict tubal patency as documented by laparoscopic chromopertubation or

hysterosalpingography. However, specificity and reproducibility of results of commercial Ct antibody assays have been questioned, and few studies have examined the ability of Ct antibody assays to predict pregnancy without ART.
Study design, size, duration: We conducted an analysis of serum samples obtained from 1251 women enrolled in one of two randomized controlled trials: PPCOS II, a randomized controlled trial comparing clomiphene citrate and letrozole for treatment of PCOS, and AMIGOS, a randomized controlled trial comparing gonadotropins, clomiphene citrate, and letrozole along with intrauterine insemination in the treatment of unexplained infertility.
Participants/materials, setting, methods: All participants had documented patency of at least one fallopian tube. Sera were analyzed for anti-Ct IgG1 and IgG3 antibodies using a research Ct elementary body (EB)-based enzyme linked immunosorbent assay. OD_{405} readings ≥0.35 and ≥0.1 were considered positive for IgG1 and IgG3, respectively. Primary outcomes included pregnancy, live birth, and ectopic pregnancy. Log linear regression was used to determine the relative risk after adjusting for age, race, treatment, smoking status, and current alcohol use. Interaction terms were used to determine whether the association was modified by medication or number of patent tubes.
Main results and the role of chance: 243 (19%) women were seropositive for anti-Ct IgG3. They tended to be non-White and smokers. Anti-Ct IgG3 seropositive women were significantly less likely to conceive (RR 0.65, 95% CI 0.52–0.83) or to have a live birth (RR 0.59, 95% 0.43–0.80); these associations were weakened after adjusting for number of HSG-documented patent tubes (RR 0.73, 95% CI 0.56–0.97) and (0.73, 95% CI: 0.50, 1.04). Anti-Ct IgG3 seropositive women who conceived had 2.7 (95% CI: 1.40–5.34) times the risk of ectopic pregnancy. The association between anti-Ct IgG3 seropositivity and treatment outcome was not modified by number of patent tubes but was modified by treatment type; women on letrozole who were seropositive for anti-Ct IgG3 had a significant reduction in risk of pregnancy (RR 0.47, 95% CI: 0.32–0.71). Seropositivity for anti-Ct IgG1 antibodies was not associated with any of the outcomes.
Limitations, reason for caution: This is a secondary analysis. Although no women received ART, all women received treatment, thus these results cannot be generalized to predict spontaneous pregnancy among infertile women. The Ct EB ELISA used in this study is not commercially available.
Wider implications of the findings: Women who are seropositive for anti-Ct IgG3 have a significantly lower chance of conceiving, even in the setting of documented tubal patency; this may be due to underlying damage to fallopian tubal architecture. Future assessment of infertile women may include both an assessment of anti-Ct IgG3 seropositivity and hysterosalpingography.
Study funding/competing interest(s): Funding by national/international organization(s) – National Institutes of Health (NIH)/Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).
Trial registration number: NCT00719186 and NCT01044862.
Keywords: Chlamydia, pregnancy prediction, ectopic

O-066 Anti-Müllerian hormone did not predict time-to-pregnancy in 301 spontaneously conceived pregnancies in women of reproductive age

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Study question: Is anti-Müllerian hormone (AMH) serum levels associated with time-to-pregnancy (TTP) in spontaneously conceived (SC) pregnancies; and does serum-AMH concentration decrease in early second trimester of pregnancy?
Summary answer: AMH measured in pregnancy was not associated with TTP in SC pregnancies in younger women. Fecundability estimated by TTP declined with female age, nulliparity, and oral contraceptive (OC) use prior to conception. Circulating serum-AMH concentration decreased slightly but significantly with gestational week (GW).
What is known already: AMH is a sensitive marker of the ovarian reserve and the response to ovarian stimulation, and to some extend AMH predicts ongoing pregnancy rates in ART. TTP is a well-established measure of fecundability. Few studies have investigated the correlation between AMH and TTP in natural conception and it remains uncertain whether AMH can predict TTP in fertile women. AMH-levels are known to decrease during pregnancy, but knowledge of the changes in second trimester is still limited.
Study design, size, duration: A cross sectional study of 301 couples with a spontaneous conception. Participants were residents in the Capital Region of Denmark and recruited at the time of the nuchal translucency scan between 2012 and 2014. Women were aged 21–42 years and pregnant at inclusion.
Participants/materials, setting, methods: TTP was reported retrospectively in an online questionnaire. AMH was measured within 4 weeks of inclusion. AMH z-scores defined as the deviation from the mean AMH for the specific GW were calculated. Data were analysed by discrete-time survival-analysis adjusted for female age, AMH z-score and sperm concentration.
Main results and the role of chance: The mean (SD) female age was 31(3.6) years and the median(range) TTP was 2(1–32) months. Fourteen (4.7%) women had a TTP >12 months. The median(range) AMH was 23.0(<3.0;144) pmol/l. Median(range) GW at blood sampling was 13(11–19). In a linear regression analysis, AMH decreased with GW (0.93, 95% CI: 0.88–0.97). AMH z-score and TTP were unrelated in the unadjusted survival analysis (OR: 0.91, 95% CI: 0.78; 1.07). In the adjusted survival analysis, TTP remained unrelated to AMH z-score (OR: 0.87, 95% CI: 0.73; 1.03) and sperm concentration (OR:1.05, 95% CI: 0.92; 1.20), whereas TTP decreased with female age (OR: 0.94, 95% CI: 0.89; 0.99), preconception OC-use (OR: 0.61, 95% CI: 0.43; 0.86), and nulliparity (OR: 0.66, 95% CI: 0.44; 0.97). AMH remained an insignificant predictor if crude AMH-levels were used instead of AMH z-scores. Six women had a very low AMH (<5 pmol/l), of whom five conceived within 2 months.
Limitations, reason for caution: The retrospective design implies a risk of recall bias. No data on pre-pregnant AMH-levels were available. Few had an AMH <5 pmol/l and 85% were aged between 26 and 35, limiting our ability to conclude whether very low AMH-levels in women of advanced reproductive age are associated with prolonged TTP in natural conception.
Wider implications of the findings: We did not find a correlation between AMH and TTP in SC ongoing pregnancies, possibly because AMH reflects oocyte quantity rather than quality in younger women of reproductive age. Prospective studies including women of a broader age range, ensuring pre-pregnant measurement of AMH and sperm concentration in pregnancy-planners are needed. Identifying reliable measures to advice women on their future fecundity would be an important step towards preventing unwanted childlessness.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies) – The study received funding from MSD and the European Union (EU) Interregional projects "ReproSund" and "ReproHigh". The authors have no conflict of interest.
Trial registration number: NA.
Keywords: anti-Müllerian hormone, time-to-pregnancy, natural conception, female fecundability, fertility prediction

O-067 Predicting live birth from *in vitro* fertilisation: a novel pre-treatment prediction tool

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[4]*University of Birmingham, School of Health and Population Sciences, Birmingham, United Kingdom*

Study question: The aim of this study was to build and validate a model to predict live birth for women undergoing their first fresh non-donor IVF cycle using key covariates *available before start of treatment*.
Summary answer: Our validated final model allows estimation of the probability of live birth, *before* commencing treatment, for women undergoing their first fresh non-donor cycle of IVF, while accounting for: age, body mass index (BMI), ethnicity, ovarian reserve, history of previous live birth, history of miscarriage, and cause and duration of infertility.

What is known already: The two most widely recognised prediction models for live birth following IVF were developed on data from 1991 to 2007. Given the advancements in assisted reproduction technology over time, there is a need for a more up-to-date model built on recent data. Furthermore, the existing IVF outcome prediction models do not incorporate key pre-treatment predictors, such as BMI, ethnicity and ovarian reserve, which are readily available.
Study design, size, duration: In this cohort study a model to predict live birth was derived using data collected from 9915 women who underwent IVF treatment at any CARE (*Centres for Assisted Reproduction*) clinic from 2008 to 2012. Model validation was performed on data collected from 2723 women who underwent treatment in 2013.
Participants/materials, setting, methods: Data were collected from 12 fertility clinics within the CARE consortium: Nottingham, Manchester, Northampton, Sheffield, Dublin, Bolton, Boston, Derby, Leicester, Mansfield, Northampton and Peterborough. Multivariable logistic regression was used to develop the model. Discriminatory ability and calibration were assessed using the c-statistic and calibration slope test respectively.
Main results and the role of chance: The predictors in the final model were female age, BMI, ethnicity, antral follicle count (AFC), previous live birth, previous miscarriage, cause and duration of infertility. Upon assessing predictive ability, the c-statistic for the final model and validation cohort was (0.6204; 95% CI 0.6088–0.6321) and (0.6167; 95% CI 0.5988–0.6401) respectively. The calibration slope was not statistically significant ($p = 0.28$), suggesting the model fitted the data. A 38-year-old Caucasian woman (BMI = 35; AFC = 14) with 5 years of male factor infertility and a previous miscarriage, would have a probability of live birth of 0.26. Other factors remaining the same, this probability would rise to 0.28 if her BMI was 25, and fall to 0.21 if her age was 30 and ethnicity was Black.
Limitations, reason for caution: Our model is unable to account for factors such as smoking and alcohol that can affect IVF outcome. In addition, patients and clinicians should understand this model is designed for use *before* treatment begins and does not include variables that become available (oocyte, embryo and endometrial) as treatment progresses.
Wider implications of the findings: We have developed a novel, up-to-date model, which encompasses key prognostic factors that have not previously been used, such as body mass index, ovarian reserve and ethnicity. The model can be used to build a user-friendly interface to help couples and their clinicians. It could then be possible to conduct a feasibility study of its implementation, focused on patient acceptability and impact on counselling and decision-making.
Study funding/competing interest(s): Funding by University(ies) – Dr. Dhillon is paid by the University of Birmingham, however, the study itself required no specific funding/grant.
Trial registration number: NA.
Keywords: prediction model, IVF, predictive factors, live birth

O-068 Does donor oocyte conception act as an independent risk factor for pregnancy complications?

Y. B. Jeve[1], N. Potdar[1], M. Khare[1]
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Study question: Are donor oocyte (DO) conceptions associated with increased risk of antenatal and obstetric complications compared to conceptions with autologous oocyte in vitro fertilization?

Summary answer: DO conception is associated with statistically significant increased risk of hypertensive disorders in pregnancy, intrauterine growth restriction, preterm delivery and caesarean section compared with autologous oocyte *in vitro* fertilization conceptions.
What is known already: The most common complications observed with DO conceptions are higher than expected incidence of pregnancy-induced hypertension and pre-eclampsia ranging from 16 to 40% of cases. It is suggested that immunologic intolerance between the mother and the fetus plays an important role in the pathogenesis of pre-eclampsia, however, advanced maternal age has been reported as a significant confounding factor in many studies.
Study design, size, duration: Evidence synthesis was done by performing a systematic review and meta-analysis of the existing literature. Literature search was done using Medline (1950-Oct 2014), Embase (1980-Oct 2014) and the Cochrane Library (Oct 2014) for relevant citations. In total, 16 prospective controlled studies with 82,947cycles were included in this study. Quality assessment was performed using Newcastle-Ottawa scale.
Participants/materials, setting, methods: All observational studies comparing various antenatal and obstetric outcomes in DO pregnancies with a predefined control group for comparison were included. Primary outcome measure was hypertensive disorders in pregnancy. Secondary outcome measures included gestational diabetes, intrauterine growth restriction, preterm delivery, intrauterine death and caesarean section.
Main results and the role of chance: The risk of developing hypertensive disorders in pregnancy was significantly higher in the DO conception than autologous oocyte conception (OR 3.85, 95% CI 3.06–4.85). Subgroup analysis showed increased risk in twin gestation (OR 3.77, 95% CI 2.59–5.49) and maternal age > 40 (OR 2.33, 95% CI 1.21–4.49). Similarly the risk of intrauterine growth restriction (OR 1.81, 95% CI 1.26–2.60), preterm delivery (OR 1.39, 95% CI 1.13–1.70) were significantly higher in DO conception. Risk for caesarean delivery was significantly higher (OR 2.71, 95% CI 2.23–3.30) for singleton DO pregnancies. The risk of intrauterine death and development of gestational diabetes were not statistically significant.
Limitations, reason for caution: The meta-analysis is performed on prospective observational studies since there were no randomised trials performed. Some heterogeneity between studies was observed and accordingly Fixed and Random effect models were used. We included. only published data in the review.
Wider implications of the findings: DO conception should be treated as an independent risk factor for various pregnancy complications including hypertensive disorders of pregnancy. Couples should be counselled carefully about the risks before undergoing fertility treatment. There may be a role for managing uch pregnancies be managed in dedicated Obstetric clinics with appropriate surveillance strategies to improve outcomes for mother and babies. Although hypertensive disorders of pregnancy are attributed to immunological origin by few authors, further research is required to explain the pathogenesis involved in donor egg pregnancies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: donor oocyte, pregnancy complications, IVF

O-069 The effect of small uterine dimensions on pregnancy and live birth rates after embryo transfer

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Study question: Do small uterine dimensions affect the likelihood of pregnancy and live birth after single embryo transfer?

Summary answer: Shorter uterine cavity length and uterine width are associated with decreased chances of pregnancy after *in vitro* fertilization (IVF) after controlling for confounding effects, while a shorter baseline cervical length is associated with preterm delivery.
What is known already: Women with uteri that are enlarged due to pathology such as leiomyomata are prone to subfertility. The impact of smaller uterine dimensions on embryo transfer outcomes is not as clear. A study evaluating endometrial cavity length by sounding found lower pregnancy rates among women with shorter cavities, and consequently suggested a subsequent study using ultrasonographic measurements.
Study design, size, duration: This retrospective study includes 274 nulliparous and 37 multiparous who underwent their first single blastocyst transfer from 2010 to 2014 at a university IVF center. Uterine dimensions were divided into quartiles, and data was analyzed using stepwise logistic regression, controlling for age, ovarian reserve, smoking and body mass index.
Participants/materials, setting, methods: Baseline uterine dimensions were measured by transvaginal ultrasound using a General Electric Voluson E8 machine. The endometrial cavity length and thickness; uterine body length, depth, and width; and cervical length, were evaluated between cycle day 2 and 5. Women were excluded if they had any uterine anomalies.
Main results and the role of chance: For nulliparous women, there was a lower likelihood of pregnancy among those in the lowest quartile for endometrial length (EL) ($p = 0.05$) and uterine width (UW) ($p = 0.02$). Pregnancy rate was not affected by baseline endometrial thickness (ET) ($p = 0.34$), uterine length (UL) ($p = 0.12$), uterine depth (UD) ($p = 0.30$) or cervical length (CL) ($p = 0.95$). None of the following measurements predicted progression to a clinical pregnancy with a heart beat: EL ($p = 0.06$), ET ($p = 0.14$), UL ($p = 0.79$), UD ($p = 0.06$), UW ($p = 0.73$), or CL ($p = 0.10$). Progression to a live birth was less likely in the lowest quartile of cervical length ($p = 0.02$), but none of the other measurements were predictive of a term live birth. In multiparous women,

none of the measurements predicted the likelihood of pregnancy, clinical pregnancy or live birth.
Limitations, reason for caution: This is a retrospective study with its inherent limitations and bias. However, it is the first study in the literature to evaluate this question.
Wider implications of the findings: Since shorter endometrial length and uterine width were associated with lower pregnancy rates, this could be valuable prognostic information for those undergoing IVF. Uterine width may be a useful surrogate for endometrial cavity width. Women with shorter baseline cervical lengths could be identified for closer surveillance during pregnancy, as this seems to favour pre-viable preterm delivery. Future prospective studies would be useful in further examining the relationship between small uterine dimensions and pregnancy outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None
Trial registration number: None.
Keywords: uterine size, pregnancy, embryo transfer, endometrial length, IVF

SELECTED ORAL COMMUNICATIONS

SESSION 19: ADVANCES IN DIAGNOSIS AND PREVENTION OF MISCARRIAGE

Monday 15 June 2015 **15:15–16:30**

O-070 Is there an increased risk of Down syndrome in ART as often shown in the first trimester prenatal screening: results of a large multicentre study

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Study question: The purpose of the study was to verify if the increased risk of Down syndrome (DS) calculated by the screening procedures, shown in ART, was related to a real increase of this syndrome or to a simple artefact of the equation, thus needing its adaptation for ART.
Summary answer: The equation for risk estimation at screening procedure showed an increased estimated risk of DS in ART compared to natural pregnancies. However, the total number of confirmed DS (abortions + births) was not significantly increased
What is known already: In first trimester screening for Down syndrome, risk calculation combines maternal age, nuchal translucency and 2 biochemical markers: pregnancy-associated plasma protein A (PAPP-A) and free b-hCG. Several authors reported decreased levels of PAPP-A in pregnancy after IVF/ICSI and, consequently, an increased of DS in ART pregnancies. However, which is questionable, is the origin of PAPPA-decrease, ART pregnancy itself or artefact due to treatments.
Study design, size, duration: This study is a retrospective cohort of tests performed from January 2010 to December 2013 in 2 centres performing first trimester screening of Down syndrome. In total, 28920 tests performed at gestational age 10–13 weeks were included in the analysis (19053 and 9867 in centres 1 and 2, respectively).
Participants/materials, setting, methods: The sample comprised 1510 IVF pregnancies, 436 ICSI, 351 frozen embryo transfers (FET), 719 egg donations (ED), 351 intrauterine inseminations (IIU), and 25430 natural conceptions. The risk of DS and the total percentage of DS (abortions + births) were compared. Main confounders were considered with analysis of variance-covariance and logistic regression.
Main results and the role of chance: Pregnancies with high DS risk (>1/250) were significantly more frequent in IVF (6.9%), ICSI (7.6%), FET (5.7%), IIU (6.3%), compared to natural conceptions (3.9%). In the multiple logistic model (including women's age, previous history, centre), IVF was associated to a higher risk (OR = 1.65, 95% confidence interval = 1.31–2.07), as ICSI (2.26, 1.54–3.32) and IIU (1.65, 1.11–2.46). FET and ED were not significant (1.17, 0.78–2.05 and 0.61, 0.33–1.11, respectively). Finally, PAPPA was decreased in ART, while βHCG and nuchal translucency was increased. In total, 52 DS were confirmed (1,80 *p* 1000). Their frequency was not higher with ART (2.58 vs. 1.69 *p* 1000, $p = 0.25$). In the multilogistic model, the risk was not significant higher for ART (OR = 1.57; 0.74–3.33, $p = 0.24$) and there was no difference according to ART type.
Limitations, reason for caution: This study is one of the largest performed on this topic. It relies on a retrospective cohort and on multivariate analysis. However, The number of cases with Down syndrome is still relatively low which is a limitation to analyse individual ART techniques.
Wider implications of the findings: This study clearly shows, on a large sample, that the current calculations performed to evaluate the risk of Down syndrome in the first trimester screening results in a doubling of risk in ART. On the other hand, the percentage of really diagnosed DS is not significantly increased. This still needs to be confirmed, but is also a strong argument to work on an adaptation of the equation in case of ART, to obtain a more reliable risk estimation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Laboratoire Eylau Unilabs, Paris.
Trial registration number: NA.
Keywords: Down syndrome, first trimester screening, ART, pregnancy

O-071 Correlation between ultrasound findings and abnormal karyotypes in the embryos from early pregnancy loss after *in vitro* fertilization-embryo transfer

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Study question: To evaluate the correlation between ultrasound findings and karyotypes in the embryos from patients with early pregnancy loss (EPL) after *in vitro* fertilization-embryo transfer (IVF-ET).
Summary answer: ultrasound findings are associated with abnormal karyotypes in embryos from EPL after IVF-ET.
What is known already: Approximately 10–15% of natural pregnancies end with miscarriage in the first trimester, and about 50% of spontaneous miscarriages result from chromosomal abnormalities. Whether the ultrasound findings of embryos from EPL are related to specific chromosomal abnormalities remains unclear. Few studies have evaluated the correlation between ultrasound findings and karyotypes in embryos from EPL after IVF-ET, but existing results are inconsistent.
Study design, size, duration: The embryos data from 793 IVF patients who experienced EPL between July 2005 and December 2011 were retrospectively analyzed. The samples of chorionic villi from all miscarriage tissues were collected.
Participants/materials, setting, methods: This analysis included 793 subjects with EPL after IVF-ET. All subjected were examined by transvaginal ultrasonography before diagnosis EPL. Karyotyping of miscarriage tissues was performed using cytogenetic analysis by comparative genomic hybridization (CGH) analysis plus fluorescence in situ hybridization technology(FISH) technology. The correlation between ultrasound findings and karyotypes was evaluated.
Main results and the role of chance: In the 793 embryos from EPL, the abnormal karyotype rate was 44.77% (355/793). According to different ultrasound findings, the abnormal karyotype rates in the groups of small gestational sac, early symmetrical arrested growth, small embryonic pole, normal ultrasound, only yolk sac, and empty gestational sac were 58.33% (14/24), 56.17% (91/162), 50.49% (52/103), 44.67% (67/150), 44.51% (77/173), and 29.83% (54/181). Compared to the study population, the prevalence of chromosomal abnormalities was significantly higher in the early symmetrical arrested growth group, but markedly lower in empty sac group ($P < 0.05$). Trisomy 16 was the most frequent chromosomal abnormality in the only yolk sac, early symmetrical arrested growth and small embryonic pole groups. Monosomy X was the most common aneuploidy in the empty gestational sac and normal ultrasound groups.
Limitations, reason for caution: The abnormal karyotype rate in the small gestational sac group was 58.33% (14/24), and was not associated with a specific chromosomal abnormality. Thus, further study with a larger sample may be helpful to elucidate this inconsistency.

Wider implications of the findings: All patients in this study received IVF treatment and gestational age was accurate, which are helpful to assess the ultrasound findings. We used CGH technology to overcome the limitation of long-duration cell culture for conventional chromosome G-banding. DNA from chorionic villi was extracted for CGH analysis using conventional DNA extraction methods, and FISH detection was used to exclude polyploidy. The ultrasound findings may indicate the frequency of specific chromosomal abnormality in the embryos from EPL.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproductive and Genetic Hospital of Citic-Xiangya.
Trial registration number: None.
Keywords: ART, early pregnancy loss, karyotype, ultrasound findings

O-072 Miscarriages in female rheumatoid arthritis patients – associations with serology, disease activity and anti-rheumatic treatment

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Study question: Are miscarriages in women with rheumatoid arthritis (RA) associated with RA serology, disease activity or periconceptional use of anti-rheumatic drugs?
Summary answer: RA patients who miscarried tended to be older, to have higher disease activity, to be anti-citrullinated protein antibody (ACPA) positive and to have a past of methotrexate use. This indicates that miscarriages are more likely to occur in RA patients with a more severe disease.
What is known already: Since human embryo implantation appears to be facilitated by local inflammatory reactions of the endometrium, chronic inflammatory conditions like rheumatoid arthritis (RA) may increase the risk for miscarriage. Several reports confirm an increased risk of miscarriage in women with RA. However, the association of miscarriage with RA disease activity or the use of anti-rheumatic medication is unclear, mainly due to lack of prospective studies.
Study design, size, duration: within a nationwide prospective cohort study on pregnancy in RA (PARA study), 213 patients were enrolled preconceptionally in 2002-2008 and followed until 6 months after delivery or miscarriage.
Participants/materials, setting, methods: Variables of interest were: maternal age, presence of rheumatoid factor, presence of ACPA, disease activity, use of non-steroidal anti-inflammatory drugs, use of sulfasalazine, past use of methotrexate, and total number of disease modifying anti-rheumatic drugs. Variables with a p-value <0.20 in the univariate analysis, were further analysed using logistic regression.
Main results and the role of chance: Amongst 162 pregnancies 28 miscarriages occurred [17% (95% CI = 12.2–24.0%)]. Women who miscarried were older than women with an ongoing pregnancy (33.9 ± 3.9 vs 32.0 ± 3.8 years, p = 0.022), tended to be more often ACPA positive (82 vs 60%, p = 0.058), to have higher disease activity scores (DAS 28 of 3.92 ± 0.94 vs 3.59 ± 1.17, p = 0.166) and more often to have used methotrexate in the past (82 vs 68%, p = 0.174).

Logistic regression showed a tendency towards a higher odds ratio to miscarry for increasing age [OR 1.11 (0.99–1.25) per year increase, p = 0.076], presence of ACPA [OR 2.49 (0.86–7.20), p = 0.092], and past methotrexate use [OR 2.52 (0.86 – 7.08), p = 0.091]. The association with disease activity was less significant in the multivariate analysis [OR 1.27 (0.86–1.88), p = 0.227].
Limitations, reason for caution: Although the PARA study is the world's largest prospective cohort on pregnancy in RA, the frequency of miscarriages was relatively low, limiting the possibilities for multivariate analysis. The relatively low number of miscarriages may be due to a known healthy cohort effect in the PARA study.
Wider implications of the findings: Since both ACPA positivity as well as previous methotrexate treatment, both markers for a more active disease, showed an association with the occurrence of miscarriage, it is likely that disease severity increases the risk for miscarriage. Chronic inflammation and the use of anti-inflammatory drugs may cause a disturbance in embryo implantation and decidualisation. This may also be true for other auto-immune diseases or for women using anti-inflammatory drugs periconceptionally for other conditions.
Study funding/competing interest(s): Funding by national/international organization(s) – Dutch Arthritis Foundation (Reumafonds).
Trial registration number: NA.
Keywords: miscarriage, rheumatoid arthritis, inflammation

O-073 Decidualization of human endometrial stromal cells regulates cellular and extracellular retinoic acid levels at implantation

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Study question: To investigate the role of retinoids in decidualization of human endometrial stromal cells (HESCs).
Summary answer: Decidualization decreases cellular levels of retinoic acid (RA) and retinal. Conversely, treatment of HESCs with RA inhibited decidualization, further emphasizing the importance of the retinol pathway in endometrial preparation. Decidualizing HESCs may regulate extracellular levels of retinoids, critical for post-implantation embryogenesis, through induction of metabolic enzymes and the transport protein.
What is known already: Optimal decidual transformation of HESCs is essential for embryo implantation and placental formation. Retinoids are key regulators of cellular differentiation/apoptosis and important for maternal tolerance of the embryonic semi-allograft. Excessive levels, however, are embryotoxic. Previous microarray data studies indicated that genes involved in retinol signaling and metabolism are highly regulated in the endometrium during the implantation window and upon decidualization.
Study design, size, duration: This study was approved by the Local Ethics Committee of Juntendo University, Faculty of Medicine (No.14–103). Timed endometrial biopsies (LH + 7–11) were processed for primary HESC cultures.
Participants/materials, setting, methods: Primary HESCs were decidualized with 8-bromo-cAMP, progesterone and cortisone in the presence or absence of RA or retinal. Various molecular techniques, including chromatography, RTQ-PCR, and Western blot analysis, were employed to define the role and mechanisms of retinoid metabolism signaling.
Main results and the role of chance: Compared to undifferentiated HESCs, cellular concentrations of RA and retinal were decreased in decidualizing cells. Treatment of primary cultures with RA suppressed the expression of decidual markers (PRL, IGFBP1, and 11bHSD1) in a dose-dependent manner. Decidualization also decreased the expression of the RA-binding proteins CRABP2 and FABP5 in HESCs. Expression of the RA receptors, RARalpha (associated with apoptosis induction) and PPARdelta (associated with cell differentiation), were inhibited and induced upon decidualization, respectively. Treatment with RA or retinal selectively upregulated the expression of CRABP2 and RARalpha, but not FABP5 or PPARdelta. In addition, decidualization was associated with induction of the enzymes, which convert retinal to retinol, and the retinol transport protein RBP4, and these expression was further enhanced in response to treatment with retinal.
Limitations, reason for caution: The present results were obtained by *in vitro* analysis of HESCs. These *in vitro* findings do not necessarily reflect the complex *in vivo* situations.
Wider implications of the findings: Apart from promoting differentiation of the endometrium, the wholesale reprogramming of the retinol signaling, transport, and metabolism pathways in decidualizing HESCs may be critical in limiting exposure of the implanting conceptus to RA in early pregnancy.
Study funding/competing interest(s): Funding by University(ies) – Funding by JSPS KAKENHI Grant Number 25861508, the Uehara Memorial Foundation, and Juntendo University Young Investigator Award 2013.
Trial registration number: None.
Keywords: decidualization, retinoid metabolism, endometrium, all-trans-retinoic acid, 11β-hydroxysteroid dehydrogenase type 1

O-074 The effect of bone marrow mesenchymal stem cells (MSCs) on the recurrent spontaneous abortion (RSA)

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Study question: The aim is to study the effect of bone marrow mesenchymal stem cells (MSCs) on the recurrent spontaneous abortion (RSA).
Summary answer: Injection of MSC through uterine horn make the CD4 $^{+}$T cells and macrophages at the maternal-fetal interface shifted to the immune tolerance phenotypes.
What is known already: It is reported that MSCs have the effect on the innate immune sysytem and adaptive immune system, including neutrophils, DCs, monocytes, T cells and so on.
Study design, size, duration: The abortion-prone (CBA/J × DBA/2) mice were used.
Participants/materials, setting, methods: We utilized the abortion-prone (CBA/J × DBA/2) H-2$^{\delta}$ × H-2^{k} mice. The CBA/J mice were treated with RFP labeled mouse MSCs (MSC-RFP), and they were divided into the control group, the tail vein MSC-RFP group (MSC-vein), the uterine horn PBS group and the uterine horn MSC-RFP group (MSC-horn), and then they were mated 14 days after the treatment. Subsequently, the mice were killed at the Day 12.5 of gestation, and the embryo resorption rates were observed. The placentas and uteruses were collected, and the distribution of MSCs at the maternal- fetal interface was detected by immnohistochemistry. In addition, we collected the spleens and uteruses in each group and cultured the primary cells. Next, we analyzed whether there were differences in the phenotypes of CD4 $^{+}$T cells and macrophages, IL-4, IL-10, TNF-a and IFN-g among these groups by flow cytometry.
Main results and the role of chance: Compared with other groups, the embryo resorption rate of MSC-horn group dramatically decreased ($P < 0.05$). The immunohistochemical results showed that MSCs–RFP were observed at the maternal-fetal interface in the MSC-horn group. No matter which treatment was given, there were no significant differences in IL-4, IL-10, TNF-a and IFN-γ in CD4 $^{+}$T cells in spleens of each group ($P > 0.05$). There were no significant differences in IL-10 and IL-12 in monocytes in spleens of each group ($P > 0.05$). In contrast to the other groups, the levels of IL-4 and IL-10 in CD4 $^{+}$T cells at the maternal-fetal interface in MSC-horn group strikingly increased, and the TNF-a notably decreased ($P < 0.05$). IL-10 in macrophages was obviously higher than other groups ($P < 0.05$), and IL-12 in macrophages was significantly lower than other groups ($P < 0.05$).
Limitations, reason for caution: It need to be investigated further.
Wider implications of the findings: It may be used to treat the recurrent spontaneous abortion in the future.
Study funding/competing interest(s): Funding by national/international organization(s) – Postdoctoral Science Foundation of China.
Trial registration number: No. 2014M550573.
Keywords: MSC, recurrent spontaneous abortion, the maternal-fetal interface

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SELECTED ORAL COMMUNICATIONS

SESSION 20: REPRODUCTIVE SURGERY

Monday 15 June 2015 **15:15–16:30**

O-075 Reduced clinical pregnancy rates by ART in women with a history of unilateral oophorectomy. Results of a large multi-center cohort study

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Study question: Is there a reduced pregnancy rate by ART treatment in women with unilateral oophorectomy (UO)?
Summary answer: Significantly reduced pregnancy rates after IVF/ICSI were found in women with previous UO when compared to women with both ovaries, both crude and after adjustment for age, and regardless if the transfer of embryos was performed in the fresh cycle or using frozen-thawed embryos.
What is known already: Previous case-control studies and small cohort studies have reported similar pregnancy rates by ART in women with UO when compared to control women. Consistently, women with UO had needed higher doses of gonadotropins and fewer oocytes were retrieved at ovum pick up (OPU). In all previous studies multiple embryos were replaced during the treatments.
Study design, size, duration: Multicentre cohort study including at three ART centres in Sweden. All women underwent IVF/ICSI treatments between 2003 and 2014. Single embryo transfer (SET) was performed in 77% of the treatments. The dataset included all fresh and frozen consecutive treatments.
Participants/materials, setting, methods: The exposed cohort included 76 women with UO who underwent 139 IVF/ICSI cycles and the unexposed cohort 12879 control women who underwent 22477 IVF/ICSI cycles. Primary outcome was clinical pregnancy rate. Clinical pregnancies were ultrasound-verified at week 8–9 and analysed per OPU and per embryo transfer.
Main results and the role of chance: The two groups were comparable and did not differ significantly with regard to age, BMI or performance of IVF or ICSI. Clinical pregnancy rate/OPU differed significantly between women with UO when compared to controls, both after fresh embryo replacement (24.5% vs. 32.4%) and cumulative (32.4% vs. 42.9%), and both crude ($p = 0.030$ and $p = 0.043$, respectively) and after adjustment for the women's age ($p = 0.021$ and $p = 0.031$, respectively). The cumulative pregnancy/OPU odds ratio was about 30% significantly lower in the women with UO; OR = 0.66 (CI 0.45–0.96, $p = 0.031$). The age-adjusted OR for live birth/OPU was also lower in women with UO compared to controls 0.71 (CI 0.47–1.05, $p = 0.090$).
Limitations, reason for caution: Although a significant reduction in clinical pregnancy rates was observed in women with UO, the reduced likelihood of achieving a live birth was not significant. The small sample size of exposed women might lack sufficient power for the investigation of differences in live births between the groups.
Wider implications of the findings: The present large cohort study is the first indicating reduced chance to pregnancy by ART, predominantly performed with SET, in women with previous UO. The findings contradict the earlier notion that fertility treatment outcome is not affected by a previous UO. As live birth events appear more seldom than pregnancy, our study might not have had enough power to detect a difference in live births.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – The local Research, Education and Development Council, Department of Obstetrics and Gynecology, Södersjukhuset. Clinical research grants from Stockholm County Council.
Trial registration number: NA.
Keywords: unilateral oophorectomy, pregnancy, infertility, assisted reproductive techniques, ovarian surgery

O-076 Core-pulling Salpingectomy: A Novel Surgical for Hydrosalpinx before IVF-ET

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Study question: We investigated the effects of a new surgical procedure to treat hydrosalpinx prior to IVF-ET.
Summary answer: Patients who underwent core-pulling salpingectomy received IVF-ET and achieved increased clinical conception rate compared to the conventional salpingectomy, but the difference was not statistically significant.
What is known already: The fluids secreted by epithelial cells in dilated fallopian lumens are toxic to embryos. They interfere with embryo development, embryonic implantation, conception, and increase the rate of abortion.
Study design, size, duration: *In vitro* fertilization and embryo transfer (IVF-ET) is the primary treatment option for infertile patients with hydrosalpinx. However, if the hydrosalpinx is not treated first, outcomes of IVF-ET are compromised.
Participants/materials, setting, methods: Infertile females receiving treatment for hydrosalpinx ($n = 633$) were divided into one exposed group and two comparison groups. The exposed group was patients receiving pretreatment

with core-pulling salpingectomy (n = 105). The first comparison group were patients who had conventional salpingectomy prior to IVF-ET (n = 104), and the second comparison group were patients receiving IVF-ET without a history of previous hydrosalpinx (n = 424). Outcome in the exposed group, ovarian reserve, ovarian responsiveness and conception rates were compared to the two comparison groups.

Main results and the role of chance: After core-pulling salpingectomy, antral follicle number, endocrine profile, total dosage of ovulation-inducing agents required for ovarian stimulation, E2 level on the day of HCG administration and the number of oocytes retrieved following core-pulling salpingectomy were significantly higher compared to conventional salpingectomy, but not different from women without hydrosalpinx. Patients who underwent core-pulling salpingectomy received IVF-ET and achieved increased clinical conception rate compared to the conventional salpingectomy, but the difference was not statistically significant.

Limitations, reason for caution: All participants were women from 23 to 44 years old. They were included if they met the following criteria: (i) both ovaries present; (ii) FSH < 12 IU/l, estradiol <80 pg/ml and prolactin in the normal range before ovarian stimulation; (iii) normal uterine cavity; (iv) normal thyroid-stimulating hormone concentration or euthyroid as determined by the investigator; and (v) no current or past diseases affecting the administration of gonadotrophins. Couples with male factor and tubal tuberculosis were excluded.

Wider implications of the findings: Laparoscopic core-pulling salpingectomy should be recommended for patients with hydrosalpinx before receiving IVF-ET. This procedure did not interfere with ovarian reserve or responsiveness, and improved the conception rate.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Science and technology development plan of Yantai City.

Trial registration number: NA.

Keywords: infertility, hydrosalpinx, salpingectomy

O-077 Imaging of organ viability during uterine transplantation surgery

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Study question: Is there a role for biomedical photonics, and in particular, two novel, experimental techniques – a liquid crystal tuneable filter based multispectral imaging and laser speckle contrast analysis – in assessing uterine perfusion and viability pre-, intra- and post-transplantation surgery?

Summary answer: When assessing the overall picture, multispectral imaging may be an efficient tool to assess uterine tissue re-oxygenation in both time and space in the period immediately following the re-anastomosis, as well as in the post-operative period overall. Laser speckle contrast analysis has demonstrated potential with both qualitative and quantitative application.

What is known already: Uterine transplantation surgery has been proposed as a treatment for permanent absolute uterine factor infertility in the case of loss of the uterus. Due to the complexity of the vasculature correct re-anastomosis of the blood supply during transplantation surgery is a crucial step to ensure reperfusion and viability of the organ. While techniques such as fluorescent dye imaging have been proposed to visualise perfusion there is no gold standard for intraoperative visualisation of tissue oxygenation.

Study design, size, duration: This was a longitudinal study involving a small- and large-animal model (nine rabbit cross-transplants and five sheep auto-transplants). The study was performed at the Royal Veterinary College and Imperial College London, United Kingdom between June 2012 and June 2013.

Participants/materials, setting, methods: The above mentioned techniques were used to monitor uterine oxygen saturation and blood flow before and after transplantation. An absorption spectrum was calculated at each spatial pixel location using reflectance data from a reference standard, and the relative contributions from oxy- and deoxyhaemoglobin were calculated using a least-squares regression algorithm.

Main results and the role of chance: Results acquired during animal surgeries show that cornual oxygenation changes are consistent with those observed in point measurements taken using a pulse oximeter, showing reduced *SaO2* following re-anastomosis. Values obtained using the multi-spectral imaging laparoscope were lower than those taken with the pulse oximeter, which may be due to the latter's use of the pulsatile arterial blood acquired.

The preliminary results show that LASCA has the potential to determine blood flow, as well heart rate, respiratory rate and oxygen saturation.

As a first attempt to trial two experimental technique in a previously untested area of medicine, the experience may be considered as useful. With further trials in the future in both the animal and human models, a more definitive conclusion may be arrived at.

Limitations, reason for caution: Multispectral imaging and laser speckle contrast analysis remain a prototype. For the former, a major disadvantage is motion artefacts. They are introduced by breathing, peristalsis and relaxation of tissue during image acquisition, with cornual peristalsis an issue. For the latter, the acquired data was limited and difficult to conclude from.

Wider implications of the findings: The use of multispectral imaging and laser-speckle contrast analysis here is the first such case with respect to fertility surgery and has demonstrated promise of possible future use in a human model. Accurate, real-time, non-contact imaging modalities, which use a mathematical model to process the final results, are an improvement on current tools. In addition, other parameters can be derived: heart rate, respiratory rate and blood flow.

Study funding/competing interest(s): Funding by national/international organization(s) – Research was funded by Womb Transplantation UK (registered charity).

Trial registration number: NA.

Keywords: uterine transplantation, multispectral imaging, laser speckle contrast analysis, pulse oximetry, uterine perfusion

O-078 Effect of hemostatic method on ovarian reserve following laparoscopic endometrioma excision; comparison of suture, hemostatic sealant and bipolar cauterisation; a systematic review and meta-analysis

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Study question: Is there a difference between various hemostatic methods used in laparoscopic endometrioma excision with regard to preservation of ovarian reserve measured by serum anti-Müllerian hormone (AMH) levels?

Summary answer: Bipolar cauterization (BC) use for hemostasis after laparoscopic endometrioma excision is associated with a greater loss of ovarian reserve than suturing the cyst bed or applying hemostatic sealent (HS).

What is known already: Excisional surgery for endometrioma has a negative effect on ovarian reserve. Hemostatic methods may contribute further to this effect. Several studies have compared BC with suturing or HS. No systematic review has been undertaken previously.

Study design, size, duration: Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE and OVID MEDLINE(R) In-Process and Other Non-Indexed Citations databases were screened until August 2014. Abstracts of the annual meetings of the American Society of Reproductive Medicine, the European Society of Human Reproduction and Embryology, and the American Association of Gynecological Laparoscopists were searched.

Participants/materials, setting, methods: Seven-hundred-twelve articles were identified and 18 were assessed in detail. Of these, 6 studies were included in the qualitative analysis. Four studies evaluating 213 women in total were included for meta-analysis. The main outcome measure was the rate of change in serum anti-Müllerian hormone (AMH) 3 months after surgery, expressed as a percentage of the preoperative AMH level. Subgroup analyses were done for studies comparing BC with HS or sutures. The Cochrane risk of bias tool was used for risk assessment. Grade system was used to evaluate quality of evidence. Study-to-study variation was assessed by using the Chi^2 statistic. The results were combined for meta-analysis using the Mantel-Haenszel model. All results were combined for meta-analysis with Revman Software.

Main results and the role of chance: This meta-analysis showed that alternative hemostatic methods were associated with a significantly lesser decline in ovarian reserve than BC. The mean decline in serum AMH levels was 6.95% less with alternative hemostatic methods than BC (95% CI –13.0% to –0.9%, p = 0.02) 3 months after surgery. When subgroup analysis of the studies comparing BC with HS or with sutures was performed, neither hemostatic method appeared to protect ovarian reserve significantly better than BC. Qualitative analysis concluded that 5 out of 6 studies reported greater decline in serum AMH levels with BC compared to alternative hemostatic methods. While HS was suggested to protect ovarian reserve better than BC, it was not clear whether suturing was more protective than BC.

Limitations, reason for caution: Limited number of studies were published on the subject, and some authors of original trials did not provide the required information. Blinding was not possible in any trial. The quality of evidence is low to moderate.
Wider implications of the findings: Available evidence suggests that alternative hemostatic methods preserve the ovarian reserve better than BC. However, taking the cost and possible adverse effects of HS in to consideration, choosing HS over BC may not be justified. Suturing may be preferred by experienced surgeons and more high quality studies comparing BC with sutures are needed.
Study funding/competing interest(s): Funding by University(ies) – Koc University School of Medicine.
Trial registration number: PROSPERO CRD 42014013848.
Keywords: endometrioma, laparoscopy, ovarian reserve, excision

O-079 Vascularity after laparoscopic myomectomy depends on suturing methods

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Study question: Does vascularity of uterus after laparoscopic myomectomy vary depending on operative procedure?
Summary answer: Simple interrupted suturing is superior to continuous suturing in terms of vascularity evaluated using contrast enhanced magnetic resonance imaging (CE-MRI).
What is known already: Uterine rupture during pregnancy is a rare but serious complication associated with myomectomy. Uterine scar repair after myomectomy has been evaluated with ultrasound examination, but there has been no clear evidence regarding appropriate operative procedure or contraception period to minimize the risk of uterine rupture.
Study design, size, duration: A prospective cohort study was conducted in a single institution. In total, 21 patients with symptomatic intramural uterine fibroids underwent laparoscopic myomectomy (LM) between June 2013 and April 2014.
Participants/materials, setting, methods: In 12 patients, continuous suturing of uterine wound in 2 or 3 layers was performed using braided absorbable suture thread (group A). In 9 patients, simple interrupted suturing was performed (group B). Three-months after surgery, uterine wound vascularity was evaluated using CE-MRI.
Main results and the role of chance: The ratio of avascular area to cross-sectional area of fibroids before surgery was measured in each patient. In group B, there was a statistically significant decrease of avascular area compared to that in group A (1.3% vs. 5.6%, $p < 0.01$).
Limitations, reason for caution: This is a prospective cohort study and not a randomized trial.
Wider implications of the findings: Simple interrupted suturing of the uterine wound might be associated with prompt uterine healing, shortening of contraception period or reduction of uterine rupture after LM.
Study funding/competing interest(s): Funding by hospital/clinic(s) – There is no funding to support the present study. We also have no conflict of interest.(We cannot check the option of "no Funding").
Trial registration number: NA.
Keywords: uterine fibroid, laparoscopic myomectomy, suturing

SELECTED ORAL COMMUNICATIONS

SESSION 21: STRESS IN INFERTILITY AND ENDOMETRIOSIS

Monday 15 June 2015 **15:15–16:30**

O-080 Men living with endometriosis: perceptions and experiences of male partners of women with the condition

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Study question: How do male partners of women with endometriosis understand the condition and what role do they play in supporting women in managing their symptoms and access medical treatment?
Summary answer: Endometriosis and its management significantly disrupts the lives of both women and men in many domains. Men experienced a disruption to their own quality of life and a corresponding impact on the couple relationship. Male partners played an important role in women's decision-making around and subsequent management of endometriosis.
What is known already: Endometriosis is a chronic gynaecological condition affecting women of reproductive age with an estimated incidence of 5–15%. Symptoms include dysmenorrhoea, chronic pelvic pain, fatigue, heavy menstrual bleeding and dyspareunia. It is associated with 40% of attendances at infertility clinics. Studies report strain on social and marital relationships, as well as identifying partners as an important source of support. Such studies only capture women's experiences with scant evidence about the specific impact on the male partner.
Study design, size, duration: The UK-based 'Endopart' study is a qualitative, cross-sectional interview study. Inclusion criteria for couples were a laparoscopic diagnosis of endometriosis and that couples were living together at the time of interview. Interview data were collected between April 2012 and December 2012.
Participants/materials, setting, methods: In-depth, face-to-face interviews with 22 heterosexual UK couples were conducted. Women and their partners were interviewed separately ($n = 44$). This paper draws on the interviews with the male partners. Data were analysed *in vivo* using a systematic, thematic method, informed by an interpretivist relational approach.
Main results and the role of chance: The practical and emotional impact on men of living with endometriosis was found to be substantial. Whilst all chronic conditions will affect the "well" partner to some extent, endometriosis can be particularly problematic for couples because of its potential impact on fertility and sexual relationships. Involvement in treatment-seeking and associated decision-making was one way in which men provided support to their female partners. In particular, our data demonstrate men's involvement in interactions with healthcare providers; decisions about treatment; and supporting their partners through treatment regimes.
Principles of systematic sampling were employed to ensure sample diversity regarding age, ethnicity, illness trajectory, and recruitment route. Interim outcomes from the study were discussed at an expert stakeholder workshop in order to enhance interpretive validity.
Limitations, reason for caution: The cross sectional design makes it difficult to capture the dynamic nature of the impact of endometriosis across the life course. The sample contained a significant proportion of well educated individuals. It would be helpful to confirm findings from the rich and detailed interview data with a larger quantitative study.
Wider implications of the findings: An estimated 176 million women are affected by endometriosis worldwide. There is no known cure, and treatment has variable impact on symptoms. Finding effective ways to manage the condition is crucial. Discussions of management are focused on the woman, largely ignoring the impact on men/couples. These findings have implications for the development of couple-centred management and counselling, adding unique data to the knowledge base of clinicians and others providing support to people living with endometriosis.
Study funding/competing interest(s): Funding by national/international organization(s)
The study was funded by the UK Economic and Social Research Council, grant reference: ES/J003662/1. The authors have no competing interests to report.
Trial registration number: NA.
Keywords: endometriosis, men, couples, gender

O-081 E-therapy to reduce symptoms of anxiety and/or depression in women after unsuccessful artificial reproduction technology (ART): a randomised controlled trial

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Study question: Does an e-therapy program for women during fertility treatment reduce the chance of having clinically relevant symptoms of anxiety and/or depression after an unsuccessful artificial reproduction technology (ART) treatment?
Summary answer: This study did not prove that this personalised e-therapy program reduces the chance of having clinically relevant symptoms of anxiety and/or depression shortly after a first unsuccessful ART treatment.
What is known already: Internet-based interventions are promising in reducing psychological distress, especially when treatment is personalised to specific risk profiles of patients. However in fertility care, the beneficial effects of personalised therapy on psychological distress still have to be evaluated.
Study design, size, duration: A prospective, two-arm, parallel group, single blind randomised controlled trial with a 1:1 allocation was conducted. To reduce the percentage of women suffering from emotional distress from 48% to 20%, 72 non-pregnant women were needed. In total, 120 women were randomised between February 1st, 2011 and June 1st, 2013.
Participants/materials, setting, methods: Women at risk for emotional maladjustment were included. We used symptoms of anxiety and depression measured by the Hospital Anxiety and Depression Scale (HADS) as outcome measure. The control group received psychological support on request (usual care), whereas the intervention group also received access to a personalised e-therapy program.
Main results and the role of chance: We were able to analyse 38 women in the intervention group versus 42 in the control group. There was no significant reduction in the percentage of women scoring above the cut-off level for clinically relevant symptoms of anxiety and/or depression 2 weeks after the first unsuccessful ART treatment; intervention group 30% (95% CI: 17%–42%) vs. control group 41% (95% CI: 23%–58%), risk difference 11%, $p = 0.30$. However, exploratory analysis showed that the e-therapy might have a positive effect 3 months after ART, reflected by the risk difference of 19% (intervention group 22% versus control group 40%, $p = 0.06$).
Limitations, reason for caution: Of all women screened as at risk, 151 (55.8%) declined participation. Moreover, exclusion of women with HADS >13 could have reduced the possible benefit of the e-therapy program. Finally, the e-therapy program only focused on women, whereas in fertility care it is important to involve the partner as well.
Wider implications of the findings: Internet interventions for treating mood and anxiety disorders have produced encouraging results, although the effect sizes vary. In fertility care, this is the first randomised controlled trial that evaluates a personalised e-therapy program to reduce symptoms of anxiety and depression during ART treatment. Future research should focus on a detailed process evaluation and the timing of offering personalised psychosocial care.
Study funding/competing interest(s): Funding by national/international organization(s) – NutsOhra (Study Number 0702-94) funded this study. There were no competing interests.
Trial registration number: ClinicalTrials.gov NCT 01283607.
Keywords: ART, E-therapy, psychology, anxiety, depression

O-082 Three Natural cycle IVF treatment imposes less psychological stress than one conventional IVF treatment cycle

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Study question: Is the psychological stress reduced during and following three natural cycle *in vitro* fertilization treatment cycles (NC-IVF) compared to one conventional IVF treatment cycle (cIVF; classical *in vitro* fertilisation with gonadotropins)?
Summary answer: Women undergoing three NC-IVF treatment cycles showed significantly less depressive symptoms and were more satisfied with the treatment compared to women undergoing one cIVF treatment cycle. The psychological distress decreased and the quality of life increased during the course of three NC-IVF treatments.
What is known already: IVF is the one of the most stressful infertility treatments, leading to high levels of psychological stress. NC-IVF has been shown to be less or equally expensive per achieved pregnancy than cIVF and imposes less discomfort and risks. The pregnancy rate following three modified NC-IVF treatment cycles is similar than one cIVF treatment cycle. Until know it has never been systematically evaluated if NC-IVF is less psychological stressful for infertile patients than cIVF.
Study design, size, duration: A prospective study was performed with NC-IVF (without and with 25 mg clomifen citrate per day) and cIVF patients May 2013 until December 2014. The level of mental distress was analysed by validated psychological questionnaires filled in online before, during (NC-IVF) and after completed treatment cycle(s) at home.
Participants/materials, setting, methods: We analysed psychological distress (BSI), depression (CES-D), infertility specific distress (IBS), influence of fertility problems on daily life (FertiQoL) and quality of life (WHOQOL-Bref). The outcome measures were assessed before starting the treatment (T1), before the first (T2), second (T3), third NC-IVF treatment (T4) and after the pregnancy test (T5).
Main results and the role of chance: Data of 32 NC-IVF and 26 cIVF patients who completed the T1 and T5 questionnaires were evaluated. At T1 were no differences in psychological variables between the two groups. At T5 the pregnancy rate was equal in the two groups. At T5 NC-IVF patients had a significant lower level of depression (CES-D; $z = -2.156$, $P < 0.02$) and a higher satisfaction with the treatment (FertiQoL Treatment; $z = -1.727$, $P < 0.04$) than cIVF patients. During the course of NC-IVF treatment there was a reduction of infertility specific distress (IBS; $t(40) = 2.2$, $P < 0.03$), an increase of quality of life (WHOQOL-Bref; $t(39) = 2.5$, $P < 0.02$) and lower influence of fertility problems (FertiQol; $t(23) = 2.3$, $P < 0.03$).
Limitations, reason for caution: Even though all patients were offered both treatment options, some bias concerning a more positive expectation concerning the NC-IVF treatment cannot be excluded. It cannot be excluded that the dropout rate has some impact on the study results.
Wider implications of the findings: NC-IVF treatment seems to be less stressful for infertile patients. Furthermore, previous studies have shown that modified NC-IVF treatments can be equally effective and less risky compared to cIVF. Therefore this kind of treatment should be considered as an alternative treatment option in certain cases, especially in psychologically distressed women.
Study funding/competing interest(s): Funding by University(ies), Funding by commercial/corporate company(ies) – MSD Merck Sharp & Dohme GmbH. No competing interests.
Trial registration number: NA.
Keywords: natural cycle IVF, psychological distress, depression

O-083 The risk of clinically diagnosed unipolar depression among men in ART treatment – a national register-based cohort study of 37,913 men

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Study question: Are men who initiated assisted reproductive technology (ART) treatment, due to male factor infertility, at a higher risk of a clinically diagnosed unipolar depression compared to men in ART treatment due to female factor or unexplained infertility?
Summary answer: Men who initiate ART treatment due to male factor infertility are not at a higher risk of developing a clinically diagnosed unipolar depression compared to men in ART treatment due to female factor or unexplained infertility.
What is known already: Studies have shown that men in fertility treatment for male factor are more distressed and experience more negative emotions in relation to feelings of stigma, loss and self-esteem compared to men in fertility treatment due to other causes. Furthermore, low semen quality may be associated with depression. Previous studies on these topics are both qualitative and quantitative and limited in sample size. Only one previous study has explored clinically diagnosed depression in men ART treatment.

Study design, size, duration: A national, register-based cohort study; 37,913 cohabitant male partners to women in ART treatment recorded in the Danish IVF register were identified via the Central Personal Register. Data were linked to the ICD-8 and ICD-10 codes for clinically diagnosed unipolar depression from the Danish Psychiatric Central Research Register (1969–2009).
Participants/materials, setting, methods: For the initial analysis the full cohort of 37,913 male partners were used. A Cox regression analysis was used to investigate the association between male factor infertility and unipolar depression subsequent to initiating ART treatment in a sub-population of 34,817 men without clinically diagnosed unipolar depression prior to ART treatment.
Main results and the role of chance: Of the 37,913 men cohabiting with a female partner who initiated ART treatment, 446 (1.2%) had a clinically diagnosed unipolar depression either before or after initiating ART treatment. Among the men with a unipolar depression diagnosis, 32.7% were diagnosed prior to initiating ART treatment, while the remaining 67.3% were diagnosed with unipolar depression after initiating ART treatment. In the sub-population (n = 34,817), only 266 men had a clinically diagnosed unipolar depression. The full sub-population was included in the further analysis. Men with male factor infertility did not have an increased risk of unipolar depression compared with men in ART treatment due female factor or unexplained infertility (adjusted Hazard Ratio = 1.04 (0.79–1.36), p = 0.804).
Limitations, reason for caution: Only men with a clinically diagnosed unipolar depression recorded at a psychiatric hospital are included in the national registers; i.e., only severe cases of depression. Men who were treated with antidepressive medication and/or in counselling outside the hospital setting were not recorded in the Central Psychiatric Register.
Wider implications of the findings: Though previous studies have shown that male factor infertility is a potential severe stressor for men in fertility treatment, it is very reassuring to see that in this large national cohort study, male factor infertility was not a risk factor for developing a clinically diagnosed unipolar depression subsequent to treatment when compared to men in couples with different infertility diagnoses. Counselling men in fertility treatment should include this very important information.
Study funding/competing interest(s): Funding by national/international organization(s) – Research grants are funded by the Danish Health Insurance Foundation and Merck Sharp & Dohme. The funders had no influence on the data collection, analyses or conclusions of the study. No conflicts of interests to declare.
Trial registration number: NA.
Keywords: men, ART treatment, depression, infertility diagnosis

O-084 Infertility coping strategies and their effect in infertility stress: is the relationship equivalent across cultures?

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Study question: Is the relationship between infertility-related coping (namely, active-avoidance and meaning-based strategies) and infertility-related stress (personal, marital, and social domains) in women significantly invariant across cultures?
Summary answer: Culture can affect the way infertility-related coping strategies influence infertility stress. The effects of active-avoidance coping on personal, marital, and social stress depend on the country of origin. While meaning-based coping had similar impact on stress domains across all countries, the effect on social stress was only significant in Sweden.
What is known already: The effect that coping strategies have on the way women deal with the infertility stressor is widely known. A large number of studies have shown the negative influence of avoidant coping strategies on infertility stress, and some have reported a mitigating effect of seeking a positive meaning in infertility. However, there are no studies comparing these relationships between countries.
Study design, size, duration: This study emerges from multinational collaborations with the Copenhagen Multi-centre Psychosocial Infertility (COMPI) research programme. Data was collected administrating the same measures across countries between 2000 and 2013, with samples differing only in culture. Among the nine collaborating countries, three samples were eligible to this study: Denmark, Sweden and Greece.
Participants/materials, setting, methods: Participants were 1609 women seeking fertility treatment in Denmark, Greece, and Sweden. The *COMPI Coping Strategies Scales* assessed the use of active avoidance and meaning-based coping strategies. The *COMPI Fertility Problem Stress Scales* assessed personal, marital and social stress. Multi-group analyses were conducted and critical ratios ($|Z|$) were used to compare parameters.
Main results and the role of chance: Positive associations between active-avoidance coping strategies and personal, marital, and social stress were found in all countries. The deleterious effect of active-avoidance coping on personal and social stress was significantly higher for Danish women ($|Z| > Z_{0.975}$). Stronger effects were also found on personal and marital stress in Greek women when compared to Swedish ($|Z| > Z_{0.975}$). Meaning-based coping was found to have a decreasing effect on all stress domains, but the association with social stress was significant only in the Swedish sample. Meaning-based coping had similar impact on stress domains across all countries ($|Z| < Z_{0.975}$).
Limitations, reason for caution: Although the design of this study is based on solid theoretically driven hypotheses and replicates other longitudinal designs based on a single culture, claims of directional influence cannot be made due to its cross-sectional nature. Because samples were derived from European countries, results cannot be generalizable to other continents.
Wider implications of the findings: Understand the impact of culture when coping with infertility has became especially relevant with cross-border reproductive care. This research represents a first step towards increasing practitioners' knowledge on cultural specificities. Results suggest that active-avoidance strategies can be particularly harmful to Danish and Greek women.. Meaning-based coping strategies are important for clinicians to train, as they can be protective of personal and marital stress independently of the patient's country of origin.
Study funding/competing interest(s): Funding by national/international organization(s)
This work is supported by European Union Funds (FEDER/COMPETE – Operational Competitiveness Programme) and by national funds (FCT – Portuguese Foundation for Science and Technology) under the projects PTDC/MHC-PSC/4195/2012 and SFRH/BPD/85789/2012.
Trial registration number: NA.
Keywords: infertility coping strategies, infertility stress

INVITED SESSION

SESSION 22: RISKS AND BENEFITS OF BEING MALE

Monday 15 June 2015 **17:00–18:00**

O-085 Sex-specific aspects of meiotic failure in relation to meiotic checkpoints and genome integrity of germ cells

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During meiotic prophase, all chromosomes have to stably associate (synapse) with their partner chromosome, and exchange genetic information, in order to ensure correct segregation and ploidy reduction. These processes both depend on the formation and repair of around 200 programmed DNA double-strand breaks (DSBs) at hotspots that are located throughout the genome. In male meiotic prophase, the presence of the largely heterologous X and Y provides a challenge to the system. An intricate regulatory system ensures that an obligate crossover is formed in the small homologous region that they share, the so-called pseudoautosomal region. The heterologous regions remain unsynapsed, and display persistence of DSBs. This triggers global transcriptional inactivation of both the X and Y chromosome, culminating in formation of the XY body in the periphery of the spermatocyte nucleus. When meiotic DSB repair, or chromosome pairing, or both, are somehow impaired for multiple or all chromosomes, overall DSB repair stalls, and XY body formation fails, and this

triggers a very robust checkpoint in male mice, leading to a complete arrest in meiotic prophase. This prevents further meiotic and postmeiotic progression of cells with a damaged genome. In addition to this prophase checkpoint, a very efficient meiotic metaphase checkpoint can be activated by even a single chromosome misalignment in male mice. It is not clear to what extend similar checkpoints are activated in men that display a maturation arrest phenotype in testicular biopsies, based on morphological criteria. We are using a fluorescent immunostaining approach, to investigate whether similar checkpoints are activated in human males with meiotic arrest. We are analysing remnant material of paraffin embedded testis biopsies of azoospermic patients with Johnson score 3–6 (JS3–6; $n = 21$) and Johnson score 9–10 (control; $n = 15$), using specific antibodies to asses meiotic entry, XY body formation, meiotic metaphases, and the presence or absence of postmeiotic spermatids. Complete failure of XY body formation was observed in only 10% of the JS3–6 patients. Analyses of activation of other checkpoints and of the efficiency of meiotic DSB repair in relation to checkpoint activation are currently underway. The results from this type of approach may help to estimate the risk of obtaining elongated or condensed spermatids with a damaged or aneuploid genome that might have escaped checkpoint activation mechanisms in testis biopsies of azoospermic men.

Keywords: meiosis, sex chromosomes, spermatogenesis, meiotic checkpoint, DNA repair

O-086 Why are males dying younger? Evolutionary and functional aspects of maternal mitochondrial inheritance and mitochondria in ageing

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Mitochondria are central to eukaryotic life. They retain their own genome (mtDNA) and this molecule has important roles in energenesis and a growing list of other processes. mtDNA is almost universally inherited solely through the female lineage, which provides opportunity for selection to operate asymmetrically between males and females. A growing body of work suggests that mtDNA mutations may have stronger effects on phenotype in males than females – an idea I termed 'Mother's Curse". In particular, I pointed out that male specific traits, such as sperm function, would be profoundly affected by this asymmetry in selection. Recent work also implicates this selection asymmetry in the strong sexual dimorphism in ageing observed across a diversity of species. In this talk I will review some of the background around the development of the Mother's Curse hypothesis, particularly around the effects predicted on male reproductive functions, evidence that supports the hypothesis, and then consider areas in biology where this theory might have impact.

Keywords: mitochondria, fertility, sperm, fitness

INVITED SESSION

SESSION 23: HOW TO ORGANIZE EARLY PREGNANCY CARE IN YOUR DEPARTMENT

Monday 15 June 2015 **17:00–18:00**

O-087 Early pregnancy units: Are they worth it?

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The emergence of early pregnancy units (EPU) began in the late 1980's following triage audit of emergency gynaecology patient admissions which demonstrated unnecessary admissions to hospitals and bed blockage. The seminal paper of Bigrigg and Read (1991) heralded the cost-effective analysis of setting up an out-patient, ultrasound- based assessment of early pregnancy symptoms and complications which remain the commonest gynaecology emergency. This acted as a spring well for the local development and delivery of emergency assessment of early pregnancy leading to the appearance of a national network across the UK (earlypregnancy.org.uk) under the aegis of the Association of Early Pregnancy Units.

The bewildering array of terms used to describe the plethora of early pregnancy events and complications was rationalised in 2005 by the Early Pregnancy specialist interest group of the European Society of Human Reproduction and Embryology (ESHRE) and soon after by the Royal College of Obstetricians and Gynaecologists (Farquharson et al, 2005: RCOG guideline 2006). The basis for this initiative was to emphasise the ultrasound scan assessment as a more patient-orientated understanding and to make the distressing diagnosis of early pregnancy loss (EPL) involve a fully trained team who are empathic and caring when dealing with breaking bad news.

The sheer scale of national demand for EPU capacity is reflected, certainly within the UK, with provision of over 250,000 scans each year which correlates with the estimated number of miscarriage (200,000) and ectopic pregnancy (15,000) diagnoses. These impressive figures are seen against the backdrop of 700.000 births many of whom self-refer for reassurance scan in early pregnancy prior to antenatal booking. It is of utmost importance to provide adequate educational support for all EPU health care professionals. As a result, the level of competence at scanning remains the biggest variable in care provision across the European Union (EU).

The adoption of high resolution transvaginal ultrasound allied to serial measurement of serum HCG levels has helped discriminate cases of life-threatening ectopic pregnancy amenable to both medical and/or surgical management. The NICE guideline (2012) on ectopic pregnancy and miscarriage has attempted to assess evidence based practice in a highly diverse area of practice. Early pregnancy has been the subject of increasing research interest with international collaborative RCT's planned, in recruitment phase or completed.

In terms of training and education, early pregnancy has 'come of age' and is a popular choice for trainees in areas of specialisation for healthcare professionals.

Keywords: early pregnancy, the value of early pregnancy units

O-088 Recurrent pregnancy loss programs: optimizing care and research opportunities

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Recurrent pregnancy loss (RPL) affects as many as one in 20 couples seeking parenthood. Despite extensive research worldwide, causes of recurrent pregnancy loss remain elusive. Genetic testing of miscarriage tissue offers the promise to identify pregnancy loss due to numeric chromosome errors and submicroscopic additions or deletions to the genome, which may be responsible for lethal embryonic developmental defects. Without answers, patients are left to make decisions without information, and prolonged unresolved grief.

Recurrent pregnancy loss evaluation is complex, requiring an interdisciplinary rather than traditional specialty-based approach. Therefore, this requires changes in the standard organizational model of health care delivery to optimize clinical care and research, and teaching across disciplines. Proximity to an IVF Program allows convenient access to assisted reproductive technologies, if required. Following evaluation and development of a management plan, close monitoring and supportive care in early pregnancy is associated with high live birth rates.

A robust RPL database is essential for clinical research. Informed consent at time of consultation allows prospective collection of demographics, evaluations, diagnosis and management to be entered into a database, along with subsequent pregnancy outcomes. A data dictionary is essential to optimize data entry consistency. Data entry and management personnel can optimize data entry and storage.

Concurrent storage of blood (from both partners), urine, endometrial and miscarriage tissue, and placental tissue and cord blood with ongoing pregnancies, facilitates RPL clinical and basic science research, rather than selectively collecting for a specific study. Research banking is essential to advancing the field in our lifetime. International collaboration is essential for clinical trials since the sample size is usually not attainable with a few RPL centers.

Keywords: recurrent pregnancy loss, recurrent miscarriage, patient care, research

INVITED SESSION

SESSION 24: LONG-TERM CONSEQUENCES OF TREATED CHILDHOOD CANCER

Monday 15 June 2015 **17:00–18:00**

O-089 Ovarian function in long-term survivors of childhood cancer

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Childhood cancer has become a curable disease. Currently, two-third of all children with cancer reach long-term survival. Overall survival of childhood cancer has increased dramatically over the past decades, which has urged clinicians to pay attention to short- and long-term adverse effects of cancer treatment (Anderson, 2013).

Th emost frequent long-term side effect of cancer treatment is gonadal dysfunction, often resulting in impaired fertility (Lie Fong et al., 2008, 2009). Gonadal dysfunction does occur especially after treatment with alkylating agents or pelvic radiotherapy. Fertility can also be impaired as a result of cranial irradiation by disruption to the hypothalamic-pituitary-gonadal axis. The magnitude of this impairment depends on the treatment modality, the total cumulative dosages, as well as on the genetic susceptibility of a cancer survivor (Anderson 2013; Van Dorp et al., 2013a). Besides infertility in women cancer treatment might also cause a subsequent loss of bone mass due to the depletion of estrogens.

Ovarian function is nowadays assessed by measuring the serum levels of anti-Müllerian hormone (AMH) (Visser et al, 2012). In adult women it has been shown that AMH levels are already reduced before the start of chemotherapy (Lawrenz et al., 2012). This indicates that not only therapy but also the disease- and general health status may influence ovarian function at the time of diagnosis. Similarly, in adolescent and young adult women diagnosed with cancer significant damage to gametes has been observed before the start of treatment (Fabbri et al., 2011). We have recently shown that AMH levels in pre-pubertal girls with newly diagnosed cancer are already compromised before treatment starts, suggesting that the disease itself affect ovarian function (van Dorp et al., 2014).

The occurrence and the magnitude of gonadal dysfunction after cancer treatment varies according to chemotherapy regime and dosages, pelvic radiotherapy used as well as age at diagnosis. Moreover, genetic variation, which determines ovarian reserve in normal healthy women, may modify ovarian reserve in female childhood cancer survivors as well (van Dorp et al., 2013b).

Ovarian dysfunction after chemotherapy has been well described; premature follicular depletion will result in early menopause. Byrne and colleagues followed up women treated for cancer before the age of 20 years and recorded that nearly half of those treated with radiotherapy and chemotherapy had reached menopause by the age of 31 years, compared with 5% of controls (Byrne et al.,1992).

Potential ovarian mechanisms behind increased infertility include a loss of ovarian reserve and genotoxic effects on oocytes. One study (Barton et al., 2013) has prospectively analyzed reduced ovarian reserve in relation to fecundity in young women. They and others concluded that there was no delay in conception in women with low concentrations of AMH (Hagen et al., 2012). The genotoxic effects on germ cells of both radiotherapy and alkylating agent chemotherapy have long been a cause for concern, although the risk of genetic disease does not seems to be increased in the children of cancer survivors (Wallace et al., 2012; Anderson 2013).

Women who have had radiotherapy treatment to a field that included the uterus are at increased risk of adverse outcome in pregnancy, including late miscarriage, premature delivery, low birth weight and postpartum hemorrhage. Preconception counseling may be appropriate and these women should be advised that pregnancy needs to be supervised in a high-risk obstetric unit (Lie Fong et al., 2012; Wallace et al., 2013).

Last but not least one should consider cryopreservation of ovarian tissue in girls at high risk of premature ovarian insufficiency. Cryopreservation of ovarian tissue in pre-pubertal girls remains experimental. In post-pubertal girls, oocyte cryopreservation may be an option (Wallace et al., 2013).

Keywords: ovarian function, childhood cancer, survivor, management, fertility

O-090 Testicular function in long-term survivors of childhood cancer

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Testis has been shown to be highly susceptible to the toxic effects of cancer therapy at all stages of life, even in childhood despite the relative quiescence of the hypothalamic-pituitary (HP)-gonadal axis. Future fertility relies on the presence of spermatogonial stem cells (SSCs) to produce a constant supply of spermatozoa from puberty onwards. It is generally accepted that cancer treatment which results in a complete loss of SSC will lead to permanent infertility.

Chemotherapy: Acute leukemias, the most common cancer type in children, involve treatment with antimetabolites and vinca-alkaloids which inhibit DNA and RNA synthesis and mitosis. Long-term follow-up of leukemia survivors indicates that treatment with these chemotherapeutic drugs does not completely deplete the SSCs. Treatment with a high cumulative dose of alkylating agents is one of the major factors decreasing the probability of fertility. Many studies have described threshold doses for long term subfertility. However, a recent large study of non-irradiated childhood cancer survivors failed to identify any threshold dose for alkylating agent exposure that predicted impaired spermatogenesis after long-term follow-up. There may be other factors, in addition to absolute doses and regimen, such as genetic variation in drug metabolizing pathways that modulate the impact of alkylating agent exposure on spermatogenesis or its recovery.

Radiotherapy: Even low scattered irradiation doses can damage spermatogonia: permanent azoospermia can result when the testes are exposed to doses higher than 6–10 Gy. Leydig cells are more resistant to radiation damage. Leydig cell dysfunction is associated at doses ≥25 Gy. Radiotherapy exposing the HP region ≥25 Gy contributes to risk of androgen deficiency.

Hematopoietic stem cell transplantation: Germ cell failure with raised serum levels of FSH and decreased testicular growth in puberty are observed among most of the male patients after hematopoietic stem cell transplantation (HSCT) irrespectively to conditioning therapy. However, the probability for recovery of spermatogenesis post-treatment, is associated with the type of conditioning therapy, age of the patient, time interval since transplantation and absence of chronic graft-versus-host-disease. One third of adult HSCT patients receiving high dose cyclophosphamide had sperm in the ejaculate after a recovery period of 1 year. But after total-body-irradiation (TBI), recovery of spermatogenesis never occurred before the 4th year after transplantation. Similar findings have recently been reported in the pediatric population. HSCT conditioning with busulfan or cyclophosphamide was associated with better pubertal growth with larger adult testicular volumes, lower serum levels of FSH and the more frequent presence of spermatozoa than TBI conditioning.

Assessment of gonadal dysfunction: Semen analysis is a gold standard as the primary surveillance modality for evaluation of spermatogenesis. Monitoring of growth and pubertal development and progression is recommended for pre- and peri-pubertal survivors treated with radiotherapy exposure of the testes and HP region. Referral to a reproductive medicine clinic is recommended for survivors treated with potentially gonadotoxic therapy. Counselling regarding the risk of androgen deficiency is recommended for survivors treated with radiotherapy exposure of the testes or HP region. Survivors treated with one or more potentially gonadotoxic treatment should be aware of the risk of physiological sexual dysfunction including erectile and ejaculatory dysfunction.

Male fertility preservation: The gonads should be shielded from irradiation when possible. Sperm cryopreservation should be offered to pubertal and post-pubertal cancer-diseased boys. In adolescents, measurements of testicular volume are helpful in predicting the chance for successful retrieval of spermatozoa and semen production. The chances of improving fertility after sterilizing cancer therapy remain poor. Rapid progress in the development of novel experimental strategies to generate fertile gametes from cryopreserved testicular tissue may provide new methods for fertility preservation among pre-pubertal patients in the future.

Keywords: infertility, androgen deficiency, childhood cancer therapy, recovery

SELECTED ORAL COMMUNICATIONS

SESSION 25: PARAMEDICAL SELECTED ORAL SESSION – LABORATORY

Monday 15 June 2015 **17:00–18:00**

O-091 Organizing the lab: the effect of time to injection in intracytoplasmic sperm injection (ICSI) on ongoing pregnancy

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Study question: The purpose of this study is to investigate whether the timing of the start of ICSI after ovum pick-up (OPU) influences the probability to become pregnant. As, specifically, extreme poor semen quality requires a more time consuming ICSI procedure, the time that ICSI is started is inevitable subject to variation.
Summary answer: Differences of ICSI injection interval times between 1.0 h up to 6.0 h since ovum pick up does not influence the ongoing pregnancy rate (OPR).
What is known already: It is argued that 4 h are needed to complete nuclear and cytoplasm maturation of oocytes before injection, despite an omission of confirmative large studies. A fixed time-interval after OPU of the injection for all patients is not feasible, because of the variation of the laboratory workload (number of ICSIs per day) and semen quality. However, it is challenging to organize the lab procedures without causing detrimental effect on fertilization, embryo quality or pregnancy outcome.
Study design, size, duration: This retrospective study includes all first cycles of patients who visited our clinic between February 2002 up to April 2014. The ICSI injection interval is defined as the time interval between OPU and injection. The ICSI injection interval was categorized and evaluated in relation to: pregnancy, ongoing pregnancy and abortion.
Participants/materials, setting, methods: The first ICSI cycles of 3032 women were included in this study. The treatments were related to type of sperm used: either ejaculated, non-ejaculated, fresh/frozen. The median female age was 32.0 years (range: 19.8–43.8 years) and the median injection time interval was 2.3 h (range: 0.6–7.3).
Main results and the role of chance: The percentage of ongoing pregnancies was 39% (95% confidence interval (CI): 37 – 41%) and this was not statistically significant different between the ICSI injection time intervals and even so after adjustment for basic female characteristics: age, FSH doses, number of oocytes and of fertilized oocytes. In addition, on average the start of ICSI with fresh sperm was 36 min (95% CI: 30–43) earlier compared to frozen sperm, while the mean start of ICSI was similar with respect to ejaculated or non-ejaculated sperm. After OPU, injection can be started as soon as 1.0 h after OPU without reducing the chance to pregnancy.
Limitations, reason for caution: The groups with very early (<1.0 h) and the very late (>6.0 h) injection times are small, and may be related to lower pregnancy rates. The primary outcome in this study is OPR of the first cycle; we did not consider the cumulative outcome of the cryopreserved embryo's.
Wider implications of the findings: The work load in an IVF-lab is not constant. The limitations of personnel and ICSI- microscopes together with the difficulty of some ICSI's, implicate decision making of when to start an ICSI procedure. Although the ongoing pregnancy rate may be lower before 1.0 h and after 6.0 h, pregnancy rates are still good; extreme long or short periods between OPU and injection is not desirable, but in extreme conditions it could be acceptable.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Radboud University Medical Center, Nijmegen, The Netherlands.
Trial registration number: NA.
Keywords: ICSI, timing, pregnancy, injection, ongoing pregnancy

O-092 Reliable High Resolution SNP-Array Analysis of Human Embryos for Genomic's Aberration Screening and Karyomapping

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Study question: Can High-Resolution Single Nucleotide Polymorphism Array (SNP-A) analysis be reliably used to screen for genomic aberrations on human embryos from patients undergoing *in vitro* fertilization (IVF) treatment?
Summary answer: Empirical evidence demonstrates that High-Resolution SNP-A can be reliably used to detect chromosomal aberrations on embryos that have undergone trophectoderm (TE) biopsy. Previously characterized samples with known genetic profiles were analyzed by SNP-A in a blinded fashion to determine the ability of SNP-A to identify previously known and novel aberrations.
What is known already: FISH-PGD and BAC-arrays have been used in embryo screening but the resolution (size of the smallest aberration detected) and mosaic sensitivity are limited. SNP arrays can detect both chromosome segmental imbalances and aneuploidy, and may overcome the limitations of FISH and BAC arrays in embryo screening and PGD.
Study design, size, duration: This was a retrospective study of 84 samples with and without detectable chromosomal aberrations by alternative technologies (FISH, NGS, aCGH), The 84 samples included TE biopsies (N = 16), leukocytes from health individuals (N = 44).... The study was run and analyzed between June 2014 and January 2015.
Participants/materials, setting, methods: Samples were either TE biopsies comprising 2–5 cells or manually picked leukocytes from peripheral blood.
All samples were amplified using whole-genome amplification followed by CytoScanÒ Cytogenetics Suite Protocol. A reference file (for normalization purposes) was generated using the 44 normal leucocytes samples (from similar amount of starting cells) using Chromosome Analysis Suite Software.
Main results and the role of chance: The SNP-A methodology was robust; all analyzed samples (100%) yielded results. Aberrant/non-aberrant status was concordant with those obtained using a predicate methodology in all cases (100%). Further evaluation on the aberrations and genotypes is being conducted.
Limitations, reason for caution: The method evaluated provides a higher potential of detecting smaller aberrations when compared with BAC-array, aCGH or NGS. However, a bigger study to evaluate its clinical efficacy and utility is advisable.
Wider implications of the findings: This is the first study reporting analytical assessment of high resolution SNP-A for chromosomal aberrations screening in embryos obtained for IVF. High resolution SNP-A have the potential to discover smaller chromosomal aberrations that other methods (BAC arrays, acGH, FISH, NGS), with the additional value of the genotypes that could serve for karyomapping and forensic evidence of the embryos when compared to samples obtained by amniocentesis or after birth.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies) – National Science Foundation of China (No. 81222007). Affymetrix INC. RD.
Trial registration number: NA.
Keywords: single nucleotide polymorphism array, preimplantation genetic diagnosis, chromosomal aberrations

O-093 Frozen-thawed *in vitro* matured oocytes collected at the time of ovarian tissue processing, for the purpose of fertility preservation for transsexual persons, show normal spindle formations

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Study question: Does long term androgen treatment in female to male transsexual persons (FTMs) have an effect on the spindle structure and chromosome alignment of *in vitro* matured cryopreserved oocytes derived from cumulus enclosed oocytes (CEOs) isolated at the time of ovarian tissue (OT) processing and cryopreservation, before or after vitrification.
Summary answer: Spindle structure analysis and chromosomal alignment is normal in *in vitro* matured CEOs that were identified at the time of OT

processing. The metaphase II (MII) oocytes do not seem to be morphologically affected by long-term androgen treatment.
What is known already: It has been shown, for oncological patients, that after OT processing and cortex freezing, CEOs can be found in the remaining solution. These CEOs can be the result of tissue processing where cortical antral follicles are mechanically disrupted. Others have stated that the CEOs are derived from surplus medulla tissue. These CEOs can be *in vitro* matured until they reach metaphase II stage. This has not yet been described for fertility preservation programs for FTMs.
Study design, size, duration: *In-vitro* matured CEOs reaching MII stage were collected and split into 2 groups; group 1 was immediately fixed for spindle staining and group 2 was first vitrified and warmed and then analyzed for spindle structure and alignment of the chromosomes. Statistical analysis was performed by Fisher's exact-test.
Participants/materials, setting, methods: 16 FTMs were included with a mean age of 24.1 ± 6.2 years, following a period of testosterone treatment (53.6 ± 21 weeks). A total of 680 CEOs were collected, IVM culture was performed using supplemented M199-medium, open vitrification using the cryotop system, spindle structure analysis was assessed by immunostaining and confocal imaging.
Main results and the role of chance: After 48 h *in vitro* maturation, 38.1% CEOs were at MII-stage (259/680). Those MII-oocytes were split over 2 groups: (1) 126 MII-oocytes in the non-vitrification group, and (2) 133 MII-oocytes, which underwent vitrification. Immediately after warming, the survival rate was 73.7% (98/133); after 1 h of culture 68.4% (91/133) and after 2 h of culture 67.7% (90/133). These 90 survived MII-oocytes were fixed and stained for spindle/chromosome alignment analysis. Both the non-vitrified and the vitrified group, showed comparable results concerning normal spindle structure and chromosomes alignment, 85.7% (108/124) versus 92.2% (83/90) ($P = 0.269$).
Limitations, reason for caution: The results are based on transsexual persons whose ovaries were processed at the time of transition surgery, after long term androgen hormonal treatment. Although the *in vitro* matured frozen warmed oocytes seem morphologically normal, the biological competence of these oocytes like fertilisation capacity and implantation potential was not analysed.
Wider implications of the findings: CEOs can be collected during OT cryopreservation for FTMs. The MIIs from the FTMs show a normal spindle structure and chromosome alignment before and after vitrification. From a morphological point of view, the *in vitro* matured oocytes do not seem to be affected by the androgen treatment. This finding can maximise fertility preservation options for FTMs.
Study funding/competing interest(s): Funding by University(ies) – This study has not been supported by any grants and the authors declare no competing interests.
Trial registration number: This research is conducted with the approval of the local ethics committee (2012/780).
Keywords: human ovarian medulla tissue, metaphase-II oocytes, vitrification, spindle, *in vitro* maturation

O-094 Laser assisted hatching of blasotcysts prior to ET may improve outcome

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Study question: Does laser assisted hatching (LAH) and subsequent blastocyst collapse prior to Embryo Transfer affect pregnancy and implantation rates in fresh IVF cycles.
Summary answer: Fresh embryo transfer of a single blastocyst following laser assisted hatching may improve implantation
What is known already: While LAH studies have been very well documented, there is very little published regarding LAH of fresh blastocysts, a recent presentation at ASRM meeting 2015 (Henderson et al) concluded that LAH of fresh blastocysts may benefit patients over 40 years.
Study design, size, duration: · This was a prospective cohort study of 235 patients undergoing fresh blastocyst transfer between August 2013 and December 2014 at City Fertility Centre.
Participants/materials, setting, methods: · All patients undergoing fresh stimulated IVF cycles with either standard IVF or ICSI insemination were included; donor oocyte, CGH cycles and patients having double embryo transfer (DET) were excluded from analysis. Embryos were assessed on the morning of embryo transfer and LAH performed 5–15 min prior to embryo transfer. The results were compared using Fishers Exact test where $p < 0.05$.
Main results and the role of chance: A total of 272 cycles were included; 136 cycles with LAH with blastocyst collapse and 136 controls. The biochemical and clinical pregnancy rate for the LAH group was 52.2% and 45.6% and in the control group 38.8% and 31.3% respectively. These results were significant when compared using Fishers Exact test ($p < 0.5$).
Limitations, reason for caution: This was not a RCT
Wider implications of the findings: With greater numbers the aeitology of the patients could be reviewed to determine if this practice is more beneficial to a particular sub group of patients**.**
Study funding/competing interest(s): Funding by hospital/clinic(s) – CFC
Trial registration number: NA.
Keywords: blastocyst, hatching

INVITED SESSION

SESSION 26: TREATING MITOCHONDRIAL DISEASE THROUGH ASSISTED REPRODUCTIVE TECHNOLOGIES – MHR SESSION

Tuesday 16 June 2015 **08:30–09:30**

O-095 Preventing the transmission of mitochondrial DNA disease

H. J. M. Smeets[1], S. C. E. SalleveltH.[2], J. C. F. DreesenM.[3], C. E. M. De Die[3], A. B. C. Otten[3], D. M. E. HellebrekersI.[4], D. C. Samuels[5], I. F. M. De Coo[6]
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Mitochondrial disorders are among the most common inborn errors of metabolism, in at least 15% caused by mitochondrial DNA (mtDNA) mutations, which can occur *de novo* or are maternally inherited. Considering the clinical severity and lack of treatment, preventing transmission to subsequent offspring is often being requested, but reproductive options are not always evident. Recurrence risks vary considerably, ranging from high and unpredictable for many familial mtDNA mutations to low for often *de novo*, large single mtDNA deletions. In the latter case prenatal diagnosis (PND) is offered for reassurance. We studied if the same applied to *de novo* mtDNA point mutations. We performed PND in 4 likely *de novo* mtDNA disease cases, based on absence of the point mutation in multiple maternal tissues. The mtDNA mutation was absent in all 4 prenatal samples. In literature 107 cases with *de novo* mtDNA point mutations were reported, but PND in a subsequent pregnancy was only performed in 6 cases. None showed the mutation. The absence of the mtDNA mutation in 64 siblings of individuals with a presumed *de novo* mtDNA mutation further adds to the low recurrence risk of *de novo* point mutations. However, for ethical reasons most asymptomatic minor siblings of an index patient were not tested for the mutation. Only few reports from family studies describe recurrence, but the data is not unambiguous. So, for *de novo* mtDNA point mutations and deletions the recurrence risk seems to be low and PND can be offered for reassurance. PND is also the most suitable option for female carriers with a low mutation load demonstrating skewing to the extremes. Preimplantation Genetic Diagnosis (PGD) is currently the best reproductive option for most maternally transmitted heteroplasmic mtDNA point mutations. Embryos with mutant load below a mutation-specific or, if

not possible, general expression threshold of 18% are transferred. A total of 19 PGD cycles were performed in our lab for 4 different mtDNA mutations (m.3243A > G, m.8993T > G/C, m.8344A > G, m.14487T > C). Of the 100 embryos diagnosed, 32 were transferable and 14 were transferred, leading to 4 pregnancies. Two have resulted in the birth of a healthy child. All carriers produced oocytes below the threshold. In general blastomere mutation load was representative for the whole embryo, but rare outliers occurred, which could lead to a wrong conclusion. Therefore, we always analyse 2 blastomeres of an embryo. Another patient carried both a *de novo* m.3243A > G mutation and was compound heterozygote for mutations in the polymerase gamma (POLG1) gene, the latter most likely explaining the clinical symptoms. PGD was performed for the mother on both the m.3243A > G and POLG1 mutations in separate blastomeres of the same embryo and a healthy embryo was transferred. None of the embryos carried the m.3243A > G mutation. Data on the oocytes and embryos of a carrier during multiple cycles generates further insight in the size of the individual genetic bottleneck and distribution of mutant load during oogenesis. Although the data is still limited, based on the m.3243A > G and m.8993T > G PGD cases only, and assuming the absence of selection, the size of the bottleneck ranges from about 10 tot 100 mtDNA molecules, which is less than the 200 reported in literature. The distribution pattern of the mutation loads varies, ranging from random (m.324A > G), derived from 2 populations (m.3243A > G) and skewed (m.3899T > G). These data can be used to resolve the mechanisms of the bottleneck further and to predict the chance of having offspring below the threshold of expression in individual carriers. Finally, nuclear genome transfer techniques have recently been approved in the UK and will offer additional reproductive options especially for carriers with high heteroplasmy levels or homoplasmic mtDNA mutations.

Keywords: mtDNA disease, genetic bottleneck, prenatal diagnosis, preimplantation genetic diagnosis, de novo disease

O-096 Towards therapeutic application of IVF-based techniques to prevent transmission of mtDNA disease

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The fertilised egg contains >200,000 copies of mitochondrial DNA (mtDNA). Unlike nuclear DNA, which we inherit from both parents, mtDNA is exclusively maternally inherited. Pathogenic mutations in mtDNA cause a range of debilitating and life-threatening diseases. MtDNA mutations may affect all, or only some, copies of mtDNA. In the latter case, known as heteroplasmy, the severity of disease is proportional to the ratio of mutated to non-mutated mtDNA. Because of random segregation and sampling effects during early embryonic development, women who are heteroplasmic for a given mtDNA mutation produce oocytes with varying mutation loads. It is therefore very difficult to predict the risk of serious disease in the children of affected women. While preimplantation genetic diagnosis (PGD) may be used to select embryos with the lowest mutation load, it is not useful for women who consistently produce oocytes with mutation loads above the threshold for mtDNA disease. An alternative approach, currently being developed, is to uncouple the inheritance of nuclear DNA from mtDNA by transplanting the nuclear DNA from the egg of an affected woman to that of an unaffected donor. This would enable women with mutated mtDNA to have a genetically related child with a greatly reduced risk of transmitting mtDNA disease. Following > 5 years of public debate and scrutiny, the UK Parliament has recently voted in support of a change to legislation to enable the UK Regulator to consider applications from clinics to offer the new techniques in clinical treatment to prevent transmission of mtDNA disease. We are currently engaged in preclinical studies to investigate the likely safety and efficacy of the new techniques. An update of progress towards clinical treatment will be provided.

INVITED SESSION

SESSION 27: ENDING TREATMENT: NOT OUR BUSINESS?

Tuesday 16 June 2015 **08:30–09:30**

O-097 Complicated Decisions: How individuals and couples end treatment

J. C. Daniluk[1]
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The number of patients who elect to end treatment prior to achieving a viable pregnancy is estimated to range from 20% to 65%. Concerns have been raised about these high "drop out" rates, particularly in cases when the prognosis is good and patients do not have to bear the full financial costs of treatment. In response to these concerns, considerable attention has recently been paid in the literature to the importance of patient-centered care, early identification of psychosocial vulnerabilities, and the availability of adequate, stage-specific counselling and tailored interventions. Efforts to reduce treatment burden, improve the quality of medical care, and support patients through the physical, emotional, and psychological challenges of fertility investigations and treatments should be vigorously pursued. However, these efforts may not be sufficient to fully address "premature" treatment termination. The decision to end fertility treatment prior to achieving a viable pregnancy is indeed complicated. The literature indicates that these decisions are based not just on prognosis or the financial costs of treatment, but on a range of psychosocial factors specific to the individual, their significant relationships, their values and cultural beliefs, as well as their perceptions of the risks and personal costs associated with continued treatment. This presentation will include a brief overview of the research literature addressing the primary reasons that patients end treatment prior to achieving a viable pregnancy. The literature on the short- and long-term outcomes for individuals and couples who end treatment before they are able to realize their parenting dreams and goals will also be discussed. Emphasis will be placed on the importance of patient empowerment through patient-centered care, shared decision-making, language, and empathic communication. The need to expand our definition of successful treatment outcomes will also be underscored. While we may not always be able to ensure *happy* endings, this presentation will focus on what we can do to contribute to *helpful* endings.

Keywords: ending treatment, decision-making, helpful endings

O-098 Women's long-term trajectories of psychological adjustment during and after IVF treatment

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[4]*Radboud University Nijmegen Medical Centre, Department of Medical Psychology, Nijmegen, The Netherlands*

Research on psychosocial adjustment to infertility and fertility treatment has mostly focused on group-based responses to treatment (means), but neglected to investigate how each individual adjusts across time. The goal of the present study was to describe the most common longitudinal adjustment trajectories (anxiety, depression) of women during and after In-Vitro-Fertilization (IVF) treatment and to explore demographic, diagnosis, treatment and psychosocial predictors of these trajectories. Finally we investigated differences in mental-health 11–17 years after treatment according to adjustment trajectories. Women attending the Radboud University Nijmegen Medical Center in The Netherlands (1998–2000, STRESSIVF study) were consecutively recruited. Before the start of the first IVF cycle (T1) data was collected on demographic, diagnosis and psychological (marital relationship, social support, helplessness and acceptance infertility cognitions, avoidant and problem-focused coping) variables. Anxiety (STAI-S) and depression (BDI) were assessed before start, 1 month after their 1st cycle (T2), 6 months

(T3) and 2.5 years after their last cycle (T4). Treatment outcome data after the first and last cycle and compliance behaviour (compliers, discontinuers, persisters) was collected. Mental-health data was collected from OMEGA project (n = 180). Latent class growth mixed modelling with maximum-likelihood-estimation was implemented to identify subgroups of patients with distinct longitudinal trajectories (T1–T4). Predictors of trajectory membership were investigated with multinominal-logistic regressions. Response rate was 84%. 36 women (9.4%) were excluded because they lacked data on more than two time-points. 348 were retained. Average age was 32. 23% had children before treatment. 26% achieved pregnancy after the 1st cycle and 51% after the last. 67% complied with treatment, 23% discontinued prematurely and 10% did more than 3 cycles. Four good-fitting anxiety trajectories were identified (BIC = 7579.71, AIC = 7506.51, posterior classifications ≥.79). Resilient trajectories (n = 232,66.7%) were characterized by stable positive functioning and Recovery trajectories (n = 85,24.43%) by an initial pathological response with return to normal functioning. Chronic (n = 15,4.31%) trajectories presented consistent low levels of psychological functioning and Delayed (n = 16,4.6%) trajectories were characterised by a late pathological response. Three good-fitting depression trajectories were identified (BIC = 4114.32, AIC = 4041.13, posterior classifications ≥.90). Resilient (n = 299,85.92%), Recovery (n = 32,9.2%) and Delayed (n = 17,4.89%). For anxiety, compared with resilient participants, chronic participants reported longer infertility duration (OR = 2.367), higher marital dissatisfaction (OR = 1.176) and were more likely to discontinue treatment prematurely (OR = 7.904). Compared with resilient participants, delayed participants were less likely to have children (OR = 0.55), reported higher marital dissatisfaction (OR = 1.172) and lower social support (OR = .722). Compared with resilient participants, recovery participants reported higher helplessness infertility cognitions (OR = 1.154). For depression, compared with resilient participants, delayed participants were less likely to achieve a pregnancy after the last cycle of treatment (OR = 1.154), reported higher marital dissatisfaction (OR = 1.098), lower social support (OR = .669) and lower infertility acceptance cognitions (OR = .671). Compared with resilient participants, recovery participants were less likely to achieve a pregnancy in their 1st cycle (OR = .161), reported higher marital dissatisfaction (OR = 1.069) and higher helpless infertility cognitions (OR = 1.272). Participants with a resilient anxiety trajectory presented better mental-health than participants with a delayed trajectory (p = .003). Participants with a recovery (p = .002) and resilient (p < .001) depression trajectory presented better mental-health than participants with a delayed trajectory. Most individuals adjust well. Only a very small percentage of patients will never adjust across treatment but 14–33% will present adjustment difficulties at some point during or after treatment. Patients with less satisfaction in the partnership, with less social support and a stronger focus on the child-wish are less likely to be resilient. Recovery trajectories seem to be associated with achieving pregnancy after the first cycle. Discontinuation from treatment is associated with a chronic anxiety trajectory. Overall results suggest that adjustment is multi-determined by demographic, psychosocial, behavioural (i.e., compliance) and treatment factors, and not only a function of treatment outcome.

Keywords: psychosocial adjustment, individual adjustment trajectories, risk factors, mental-health, latent class growth mixed modelling

INVITED SESSION

SESSION 28: ASRM EXCHANGE SESSION – IMPACT OF ENVIRONMENTAL TOXINS ON REPRODUCTIVE HEALTH

Tuesday 16 June 2015 **08:30–09:30**

O-099 Impact of environmental toxins on female reproduction

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Reproductive health and ultimately our reproductive capacity are under strain globally. Indicators of reproductive adversity include increased rates of infertility/longer time to pregnancy, premature pubertal onset, increased miscarriage rates, poor birth outcomes, developmental disorders, and reproductive disorders including male and female factor infertility, and endometriosis, polycystic ovarian syndrome (PCOS) and uterine fibroids. Since these changes have occurred in a relatively short timeframe, genetics alone is unlikely, and environmental factors are now considered part of reproductive risk. In developed and developing countries, air pollution, stress, nutrition, and chemicals in agricultural areas, personal care and household cleaning products, and in industrial waste, pesticides, and nearly ubiquitous plastics are of concern. This lecture will focus on representative examples of environmentally-based reproductive compromise and epidemiologic and experimental evidence for epigenetic and trans-generational persistence of some modifications and phenotypes with an eye to how to minimize risk and maximize reproductive success, with a focus on female reproduction.

Keywords: environment, reproductive risk, environmental toxicants, endocrine disrupters

O-100 Impact of environmental toxins on male reproduction

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Occupational and environmental risks to the reproductive system are a public health concern. Population exposure to toxic environmental chemicals is global and ubiquitous. Reports of occupational exposure to chemicals harmful to the male reproductive system date back to Ancient Rome. More recently, epidemiologic evidence supports an association between environmental toxic exposures and reproductive outcomes in the male. A toxicant, whether a chemical, physical, or biologic agent acts by interrupting biologic processes either by a direct chemical action or indirectly when metabolic products alter physiologic control systems in the reproductive system. Potential sites of disruption include the hypothalamic-pituitary-testicular axis, spermatogenesis, sperm function and transport, fertilization and embryo development, and alteration of the epigenetic code. Animal *in vivo* studies suggest that adverse reproductive outcomes are dependent on age of exposure, dose and duration of exposure, and may be trans-generational. The relationship between exposure and outcomes in the human are primarily derived from observational studies that analyze the relationship between the chemical of interest and the change in incidence or prevalence of the reproductive disorder of interest. The most extensively studied toxicants can be categorized into industrial chemicals and agricultural chemicals. A unifying hypothesis proposes that select toxicants are endocrine disrupting chemicals or EDCs, which alter functions of the reproductive endocrine system, primarily via hormone receptors, and consequently cause adverse outcomes. Potential adverse effects have been documented through out the reproductive life cycle in laboratory animals and in some wildlife species. The evidence that human reproductive health has been adversely affected by exposure to chemicals is less definitive; however, a group of studies report a relationship between exposure to EDCs and decreases in sperm production and/or function; and an increase in GU abnormalities and testicular cancer. Current efforts are focused on better methods of laboratory and clinical evaluation; and on careful systematic review of the current scientific evidence to assess the quality and strength of both the animal data and the epidemiologic data, to determine, in as objective an approach as possible, the strength of the evidence of toxicity.

Keywords: toxicity, sperm, infertility, environment

INVITED SESSION

SESSION 29: PARAMEDICAL INVITED SESSION: NURSING

Tuesday 16 June 2015 **08:30–09:30**

O-101 Setting up a networking platform for single women using donor semen

M. Salomon[1], K. K. Schønning[1], H. S. Krogh[1], T. M. Loving[1]
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Study question: Evaluate the use of a patient-network for single women undergoing fertility treatment with donor semen in terms of attendance,

benefits for mother and child and the duration and the value of the contacts they established.
Summary answer: Setting-up this patient-network requires minimal resources and the women evaluated it as beneficial, both during the fertility treatment and after their child was born. Women who stopped having contact with the Solo-mother network or those who didn't get pregnant recommend a similar network to other Solo-mothers.
What is known already: The decision to become Solo-mother using donor semen in Denmark is still controversial. For some women, the decision can be associated with many considerations and difficult choices. The Fertility Clinic, Rigshospitalet, Copenhagen have since 2010 create Solo-mothers networks that allows women to support each other, and allows a long-term opportunity to form relationships with other mothers and children with the same background.
Study design, size, duration: This study was conducted using online questionnaire (www.enalyzer.com). In total 135 women (16 networks) were invited. The survey was anonymous. Inclusion criteria: women who were active in one of 16 Solo-mother networks for at least 4 months. The 16 networks were initiated at least 9 months earlier. Six were excluded.
Participants/materials, setting, methods: There were 129 participants providing 78 responses (61%). All women participated in two introductory meetings facilitated by nurses, after which the Solo-mother networks were left alone. All 16 Solo-mother networks established each, their own group on Facebook for communication. The online questionnaire was launched using these groups on Facebook.
Main results and the role of chance: Responses (n = 78). Totally 79% (n = 62) were still active in the networks, whereas 21% (n = 16) were not active any more. In total 17% (n = 13) had a partner. Overall 69% (n = 54) delivered a child and 9% (n = 7) achieved the child with a partner. On a 5-point-Linkert-scale from very-important to not-important stated 90% (n = 70) it as very-important or important that a nurse facilitated the first two meetings, and they totally-agree or agree that the network has led to: 72% (n = 56) having a better network; 74% (n = 58) got new friends, and 77% (60) feeling less alone. When asking only women who had delivered a child (n = 54) they totally-agreed or agree the Solo-mothers network helped them: 61% (n = 33) during fertility treatment, and 65% (n = 35) after the child was born. They stated that 67% (n = 36) of their children have contact with each other.
Limitations, reasons for caution: The participants were invited through Facebook. If they were no longer active on Facebook they wouldn't receive the invitation. Some women did not attend the Solo-mother network because they worried that it might be stressful, if they did not get pregnant.
Wider implications of the findings: In Denmark number of single women treated with donor semen has increased since 2007, even though having a child with donor semen is not their preferred choice to parenthood (Salomon et al., in press, Obstetricia et Gynecologica Scandinavica 2015). We believe that the opportunity to join a Solo-mother network will contribute to the well-being of these women and their children. Setting-up a patient-network like this could easily be used to the benefit of other patients groups.
Study funding: The evaluation of the solo-mother networks was partly funded by a grant from Nordic Cryobank.
Trial registration number: NA.
Keywords: single women, solo mothers, donor semen, fertility treatment, network

O-102 Needs and determinants that have an influence on the intention to use preconception care among Flemish women

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Study question: *The study question is two-folded (1)* what are the preconception-related needs of reproductive-aged Flemish women, and *(2)* which determinants and associated factors have an influence on their intention to use preconception care (PCC) in the future?
What is known already: Despite the increased use of prenatal care services, reproductive outcomes remain stable over the last two decades. Many of the adverse reproductive outcomes (such as preterm birth, low birth weight, congenital malformations …) are associated with maternal and paternal risk factors that can be addressed before conception through PCC. Although PCC has been recommended by internationally recognized bodies (such as WHO), most women do not request it. Little is known about women's reasons for not using PCC.
Study design: Cross-sectional study.
Participants/materials, setting, methods: *Study part 1:* 242 reproductive-aged women with a desire to have (additional) children were recruited online through social media and discussion forums, and in the Women's Clinic of Ghent University Hospital, Flanders, Belgium; *study part 2:* the study is ongoing and preliminary results will be presented at the conference.
Main results and the role of chance: *Study part 1:* reproductive-aged women are interested in PCC and prefer to receive this care directly from a professional caregiver. The gynecologist (93%) was the most preferred PCC supplier, followed by the midwife (73%) and the GP (63%). Preconception-related information needs were high. Most women wanted information about nutrition (82%), environmental exposures (76%), work conditions (80%), and medical conditions (54%-74%). Information needs were higher among women with certain medical conditions, such as a (history of) mental illness (OR = 3.50; 95% CI 1.08–11.36), a (history) of eye- and otolaryngological diseases (OR = 2.22; 95% CI 0.95–5.21), and being overweighed (OR = 2.22; 95% CI 1.01–4.93). Few women indicated that they need preconception-related support. Women with overweight had a higher need for lifestyle-related support (p = 0.001); *study part 2:* the study is ongoing.
Limitations, reasons for caution: *Study part 1:* women were recruited on a voluntary basis, which increases the risk of selection bias. Our sample tends to over-represent women with a higher socioeconomic profile. The actual needs and attitude towards preconception care can be different than our findings suggest; *study part 2:* the study is ongoing.
Wider implications of the findings: *Study part 1:* our study results indicate that PCC should be offered to women of reproductive age as they are interested in PCC and have high information needs. Midwives can have an important role in providing preconception care as more women prefer to receive PCC from a midwife than a GP; *study part 2:* the study is ongoing.
Study funding/competing interest(s): This study was funded by the Research Foundation – Flanders (FWO). The authors declare that they have no competing interests.
Trial registration number: (1) B670201420381, (2) B670201422053.
Keywords: preconception care, needs assessment, determinants

SELECTED ORAL COMMUNICATIONS

SESSION 30: INNOVATIVE ASPECTS OF BLASTOCYST DEVELOPMENT

Tuesday 16 June 2015 **10:00–11:30**

O-103 Qualitative and quantitative grading of human blastocysts and its association with live-birth rate and neonatal outcome

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[5]*Karl Franzens University, Department for Mathematics and Scientific Computing, Graz, Austria*

Study question: Does qualitative and quantitative blastocyst assessment on day 5 allow for prediction of neonatal outcome (placental and birth weight, live birth, malformation)?
Summary answer: After adjustment for gestational age vitrified blastocysts resulted in a higher birth weight as compared to fresh ones. Trophectoderm (TE) but not inner cell mass (ICM) or blastocyst expansion was associated with the sex of the embryo as well as the rates of implantation, pregnancy, miscarriage, and live birth.
What is known already: The practice of culturing embryos until blastocyst stage for transfer has become more common in the field of ART with the development of optimized culture media. However, it should also be noted that

several drawbacks have been reported for blastocyst culture, such as monozygotic twinning, preterm delivery, congenital malformations or increased birth weight. With respect to the latter it seems that in humans intrauterine growth is impaired as early as in the second trimester of pregnancy.
Study design, size, duration: This prospective analysis comprises all fresh and vitrified/warmed single blastocyst transfers meeting the inclusion criteria during an 18-month period.
Participants/materials, setting, methods: A total of 254 blastocysts (162 fresh and 92 vitrified/warmed) were included which showed at least full stage guaranteeing the presence of an extensive blastocoel and distinct cell lineages. Qualitative scoring of blastocysts was done according to the classical criteria expansion, inner cell mass as well as trophectoderm appearance. In parallel, all three parameters were quantified semi-automatically. Placental and birth weights and malformations, respectively, were provided by the maternity hospitals.
Main results and the role of chance: Vitrified blastocyst transfers led to a significantly higher birth ($P = 0.012$) but not placental weight ($P = 0.106$) as compared to the fresh counterparts. Degree of expansion was not related to outcome nor was ICM grade and area. However, hatching blastocysts had significantly smaller ICMs as compared to full ($P > 0.01$) and expanded blastocysts ($P < 0.05$). Trophectoderm quality and cell number were the only parameters that were significantly related to rates of implantation, pregnancy, and live birth. Pregnancies that went to live birth could be distinguished from those pregnancies that aborted (biochemical pregnancies, missed abortion with and without heart activity) on the basis of trophectoderm grade ($P < 0.05$) and cell number ($P < 0.001$). Male blastocysts had a 2.53 higher chance to show TE of quality A as compared to female ones ($P = 0.04$).
Limitations, reason for caution: Since the routine performance of the laboratory was to transfer and vitrify blastocysts with the largest ICM, number of study blastocysts that showed very small ICMs of class C was limited. In general, it should be kept in mind that two-dimensional measurement may not accurately reflect 3-dimensional structure of the cell-lineages.
Wider implications of the findings: The presented correlation of TE with outcome indicates that sooner or later trophectoderm scoring will replace inner cell mass scoring in terms of priority. This would automatically require a rethinking process in terms of blastocyst selection and cryopreservation strategy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Landes-Frauen- und Kinderklinik Linz, Austria.
Trial registration number: NA.
Keywords: blastocyst expansion, inner cell mass, placental and birth weight, trophectoderm, live-birth

O-104 For patients with repeated implantation failures, a morphologically poor blastocyst could affect the implantation rate of a morphologically good blastocyst during a double blastocyst transfer

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Study question: Increasing the number of transferred embryos or blastocysts is sometimes selected as the strategy for patients with repeated implantation failure (RIF). Does this strategy actually contribute to an increase in the implantation rate for the patients with RIF?
Summary answer: Although double blastocyst transfer (BT) with two morphologically good blastocysts (MGBs) increases the chances for a multiple pregnancy, it does not increase the pregnancy rate compared with a single BT with a morphologically poor blastocyst (MPB) among patients with RIF.
What is known already: An increase in the number of transferred blastocysts is believed to also increase the pregnancy rate. Therefore, a double BT is sometimes selected as one of the strategies for patients with RIF. However, a double BT also increases the risk for multiple pregnancies. We previously demonstrated that the implantation rate for a double BT with morphologically good and poor blastocysts was significantly lower than that for a single good blastocyst.
Study design, size, duration: This retrospective study was performed between April 2009 and September 2014 and included 634 cycles for 354 patients who had experienced more than two failures with a single BT. Patients received either a vitrified-warmed single or a double BT in either the natural ovulatory cycle or the HRC.
Participants/materials, setting, methods: A MGB consists of an ICM and TE of grade A or B. The remainders are MPB. We compared pregnancy rates among the groups that received a single BT with a MGB, a double BT with two MGBs, or a double BT with both a MGB and a MPB.
Main results and the role of chance: The pregnancy rate in the group that received double BTs with two MGBs was 45.6%, which was comparable to the single BT group with a MGB (37.8%) and to the double BT group with both a MGB and a MPG (39.5%). The multiple pregnancy rate for the double BT group with two MGB was 14.6%, but there was no multiple pregnancy in the single BT group.

Table

	Single BT		Double BT	
	MGB	MPB	MGB + MGB	MGB + MPB
BT cycle, *n*	468	3	90	76
Transferred MGB, *n*	468	0	180	76
Transferred MPB, *n*	0	3	0	76
Pregnancy rate; %	37.8	0	45.6	39.5
Implantation rate; %	37.8	0	26.1*	22.4*
Multiple pregnancy rate; %	0	0	14.6	13.3

*$p < 0.001$ vs single BT

Limitations, reason for caution: In this study, patients with more than two unsuccessful IVF cycles were selected, because the definition of RIF in the literature usually ranged between 2 and 6 unsuccessful IVF cycles. Therefore, the patients in this study would have been categorized as falling somewhere between severe and mild RIF cases.
Wider implications of the findings: The implantation rates of a double BT with two MGBs or one each of a MGB and a MPB were significantly lower than that of a single BT with a MGB. A double BT didn't decrease the pregnancy rate, but it did increased multiple pregnancies. Therefore, even for treatment of RIF patients, we should avoid double BTs when two or more MGBs might be acquired.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The authors have received no funding for this study, and they have no financial interests in any companies. Also, there are no competing interests.
Trial registration number: This study is not an RCT study, so no trial registration number was assigned.
Keywords: morphologically good blastocyst, morphologically poor blastocyst, mplantation rate, repeated implantation failure

O-105 Blastocyst development in single-step versus sequential culture media of the same brand: analysis of 386 sibling oocytes

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Study question: To compare embryo development between sibling oocytes cultured in single-step and sequential media produced by the same manufacturer (SAGE).
Summary answer: Embryo culture in single-step medium (One-Step) is associated with significantly higher blastocyst formation rates (BFR) and overall blastocyst quality compared to culture in sequential media (Cleavage-Blastocyst).
What is known already: Embryo culture is a complex task, and culture media play a key role for embryo development *in vitro*. Two different approaches are commercially available: single-step, a medium formulated for the entire preimplantation period, based on the principle of 'letting the embryo choose', and sequential media, which mimic the environment of the female reproductive tract, requiring medium renewal at least every 48 h. The question of which system performs better remains highly contentious.
Study design, size, duration: Prospective randomized study of 386 sibling oocytes from 50 women (aged ≤ 39 years) undergoing oocyte retrieval procedure for intracytoplasmic sperm injection (ICSI) at Ferticlinic, Villa Margherita, from September to December 2014.
Participants/materials, setting, methods: A total of 386 injected oocytes were randomly allocated in two different dishes containing single-step (group A) and sequential medium (group B) respectively. Culture was performed up to blastocyst stage in multi-gas incubators (Sanyo) at 37°C, 5% 02, 5.5% CO2. Medium change was performed only for sequential medium on Day-3.
Main results and the role of chance: No significant difference was detected in the fertilization rate between group A and B, respectively 84.76% (178/210) vs.

79.55% (140/176). On day-3, the percentage of grade-A, (77.53% vs. 64.29%), grade-B (7.87% vs. 12.86%), grade-C (14.61% vs. 22.86%) and grade-D embryos (0% vs. 0%) did not differ as well. On day-5, culture in one-step yielded a significant higher BFR [144/178 (80.9%) vs. 94/140 (67.14%) $P = 0.04$] and more top-quality blastocyst rate [76/144 (52.78%) vs. 24/94 (25.53%), $P = 0.003$].

Limitations, reason for caution: This was a prospective randomized study with sibling oocytes. To confirm the result, it is necessary to evaluate clinical data in a prospective RCT with randomization of patients. Furthermore, studies evaluating epigenetic effects of the two different approach should be conducted.

Wider implications of the findings: Probably we should critically reexamine the conviction that sequential culture approach, mimicking the female reproductive tract environment, is the ideal strategy. The additional handling required for medium renewal and the lost of accumulated endogenous growth factors changing culture dish is likely to provide an environmental stress to embryos, making this choice not worthwhile and preferring the one which leaves embryos undisturbed.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Ferticlinic, Villa Margherita, Rome, Italy.

Trial registration number: NA.

Keywords: embryo culture, single medium, sequential media

O-106 Blastocyst inner cell mass and trophectoderm grading lends to euploidy predictability

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Study question: Can independent quality grades for blastocyst inner cell mass (ICM) and trophectoderm (TE) predict early embryo euploidy? Can a chart utilizing combined ICM and TE blastocyst grades be made to predict the best choice for embryo transfer?

Summary answer: ICM and TE grades show significance for euploid predictability. Blastocysts with a grade A resulted in higher euploid predictability. When combining grades to access overall blastocyst quality, TE grade is more predictive. Experience dictates that a grade A TE should be preferentially selected for transfer to optimize implantation success

What is known already: Morphology has been the most used and reliable method for embryo transfer selection. Ahlstrom and coworkers (ESHRE 2013) provided strong embryo transfer evidence that an 'A' quality TE grade versus ICM grades is more predictive of pregnancy success in fresh, non-PGS cycles. Early embryo aneuploidy is widely accepted as a major reason of implantation failures. However, to-date, there are no clear morphologic characteristics that accurately predict genetic normality.

Study design, size, duration: Retrospective analysis of ICM and TE quality grades was associated with 1,311 euploidy screened blastocysts. Patients autonomously chose to perform PGS-trophectoderm biopsy/microSecure vitrification-all cycles ($n = 288$), between January 1, 2014 and December 31, 2014. Array CGH and NextGen sequencing was used for aneuploidy determination.

Participants/materials, setting, methods: On day 5-6, all expanded blastocysts were assigned a quality grade for ICM and TE. Grades ranged from A (good) and B (fair) to C (poor). All blastocysts with ICM or TE quality grades of A or B were used for analysis. Statistical significance was determined by Fishers exact test.

Main results and the role of chance: Independent quality grade analyses indicate that grade 'A' blastocysts resulted in statistically higher euploidy predictability. Euploidy occurrence for ICM revealed: grade A-57%, grade B-43%; and TE: grade A-62%, grade B-37%. Combined blastocyst grade euploidy analysis indicated: AA-62%, BA-62%, AB-41%, and BB-33%. A comparative chart was made to determine which combined blastocyst grade had the best predictive ability for euploidy. The chart indicated that when TE was accessed as grade A, euploidy predictability was highest and when TE was graded B euploidy predictability was lowest. ICM grades were predictive with emphasis on grade A, but poor quality graded ICM embryos with top quality grade TE showed significance for similar or higher euploidy predictability. Overall, high levels of implantation (85%) and ongoing pregnancies (79%) were achieved.

Limitations, reason for caution: Morphology assessments are a non-diagnostic procedure, which is subject to technician and program variation. Although blastocyst grades are predictive of potential euploidy status, the morphological quality assessment is not absolute. Therefore, some poor morphological graded blastocysts may be euploid, while other high quality blastocysts are in fact aneuploid.

Wider implications of the findings: Euploidy determination in our clinic has facilitated routine single embryo transfer with increased success, while drastically decreasing twinning rates. Although this study highlights that morphology can be predictive, it clearly is not diagnostic. Many embryos could be incorrectly selected or de-selected for transfer, thus effecting overall take home baby rates. Preimplantation screening is the best option for embryo selection to eliminate the inherent variability associated with morphological assessments, with or without developmental time lapse imaging.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Southern California Center for Reproductive Medicine.

Trial registration number: NA.

Keywords: blastocyst, embryo grading, euploidy

O-107 Identifying chromosomes with significant roles in blastocyst development

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Study question: Aneuploidy screening helps to identify embryos that will not become healthy babies; however, little is known about the influence of individual chromosome aneuploidies (ICAs) on embryonic development. Knowledge of which ICAs affect early embryonic development may help us to understand where critical genetic elements controlling early development can be found.

Summary answer: Trisomies were transmitted preferentially to blastocysts for chromosomes 1, 6, 9, 11, 17 and 20 whereas monosomy was transmitted preferentially for chromosome 18. This suggests that preferentially transmitted trisomic chromosomes carrying excess genetic material enable blastocyst formation whereas preferentially transmitted monosomic chromosomes carrying genetic material preclude blastocyst formation.

What is known already: Most aneuploid embryos are incapable of developing into living babies. We routinely identify aneuploid embryos and deselect them for transfer since their prospects for live birth are slim. However, we know that some aneuploid embryos are capable of blastocyst formation, implantation and development to advanced fetal stages since they may be found in products of conception and in live births.

Study design, size, duration: We compared the incidences of ICAs in embryos biopsied on day 3 with embryos biopsied as blastocysts. Knowledge of ICAs for which the incidence varies between day 3 and the blastocyst stage may help us to learn more about where genetic elements that control embryonic development are found.

Participants/materials, setting, methods: Array Comparative Genomic Hybridization (aCGH) Data for 560 day 3 embryos and 1014 blastocysts were assembled. Incidences of aneuploidy for each chromosome (ICAs) were determined and compared. Transmittance of each ICA from day 3 to blastocysts was calculated as the ratio of incidences for each ICA (blast/day 3).

Main results and the role of chance: Incidences of euploidy (day 3:28.8%; blastocyst: 35.8%) were significantly different. ICAs were found for every chromosome (1-22, X, Y) both on day 3 and in blastocysts. The mean incidences of aneuploid chromosome/aneuploid embryo (day 3: 2.06 ± 1.64; max = 23; blastocyst 3.23 ± 3.35 max = 13) differed significantly. Incidences of autosome ICAs (per aneuploid embryo) averaged 0.069 (range: 0.033 [trisomy 11] to 0.125 [monosomy 16]) for day 3; 0.046 (range: 0.013 [monosomy 3] to 0.096 [monosomy 16]) for blastocysts. Transmittances averaged 0.655 (range: 0.23 [monosomy 1] to 1.36 [trisomy 11]). Comparing transmittances of trisomy:monosomy for each chromosome, we estimated the effect of two copies of the chromosome. Trisomies 1, 6, 9, 11, 17 and 20 and monosomy 18 were transmitted preferentially to blastocysts.

Limitations, reason for caution: ICAs were assessed in two different groups of patients (day 3 versus blastocyst biopsy) rather than investigating the progression from day 3 to blastocyst in embryos with known ICAs. While our method avoids the issue of harm from day 3 biopsy, it lacks the statistical sensitivity expected from a paired approach.

Wider implications of the findings: ICAs occur with unequal incidences in both day 3 embryos and blastocysts. Lower incidence of multiple aneuploidies in blastocysts suggests that embryos with multiple aneuploidies progress poorly to the blastocyst stage. Accentuated inhomogeneity of incidences for ICAs in blastocysts suggests preferential transmittance. The significantly different transmittance of trisomies 1, 6, 9, 11, 17 and 20 and monosomy 18 suggest that genetic elements enabling progression to the blastocyst stage lie on these chromosomes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – NYU Fertility Center.
Trial registration number: NA.
Keywords: blastocyst formation, aneuploid chromosomes, genetic control of development, PGS

O-108 Number of trophoectoderm cells removed for biopsy is correlated with first trimester miscarriage

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Study question: Trophectoderm biopsy provides the possibility of removing several cells, which allows more accurate genetic diagnosis. Although it is recommended not to remove more than 10 cells, in many cases, the technique itself makes it difficult. This could affect embryo implantation and miscarriage in early stages, despite being euploid embryos.
Summary answer: The removal of more than 10 cells during trophectoderm biopsy increases the rate of miscarriage in the first trimester in euploid embryos after comprehensive chromosome screening (CCS).
What is known already: Day 3 biopsy is considered to be harmful to the embryo. The risk of misdiagnosis due to mosaicism and the aggression for the embryo decreasing dramatically the implantation rate making it unacceptable. In contrast, trophectoderm biopsy offers the possibility of obtaining several cells without apparent embryo damage. However, we have not enough information to ascertain which is the number of cells that can be drawn without affect clinical outcomes.
Study design, size, duration: Prospective study. We include the known clinical results of 161 euploid embryos transferred coming from 98 women that underwent CCS treatments from January to September 2014.
Participants/materials, setting, methods: At least one euploid embryo was transferred to 98 patients. Assisted hatching was performed on day 3 using laser pulses (Saturn Active, Research Instruments). On day 5 of development, conventional trophoectoderm biopsy was done. Images were recorded using Cronus software, and trophoectoderm cells were counted. Clinical outcomes were evaluated.
Main results and the role of chance: We removed ≤ 10 cells in 105 blastocysts (group I), and more than 10 in 56 (group II) according to the hatching of the embryos. These embryos were chromosomally analyzed by array-CGH. Euploidies embryos were transferred on day 6. The global clinical pregnancy rate (sac visualization after 6 weeks of gestation) was 51.8%. There was no statistically significant difference in the clinical pregnancy rate between groups I and II. However, an increase in the first trimester miscarriage was strongly associated with the biopsy of more than 10 cells (6.3 % in group I vs 25% in group II, with an odds ratio 6.45, 95% confidence interval 1.26-32.90).
Limitations, reason for caution: Study currently under development to increase the number of cases and test this assertion and to ascertain which is the ideal number of trophoectoderm cells for biopsy.
Wider implications of the findings: The results obtained in this preliminary study confirm that the cells that give rise to the placenta and extraembryonic tissues play a crucial role in the maintenance of early stages of embryo development. The biopsy of more than 10 cells in the blastocyst stage may be detrimental, perhaps not for the embryo implantation, but for the later development. According to our results we should remove less than 10 cells.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu.
Trial registration number: NA.
Keywords: CCS, biopsy procedure, miscarriage, ART

INVITED SESSION

SESSION 31: LIVE SURGERY SESSION: OVARIAN PATHOLOGY AND SURGERY

SELECTED ORAL COMMUNICATIONS

SESSION 32: WHAT IS NEW IN MANAGEMENT OF POOR OVARIAN RESPONSE

Tuesday 16 June 2015 **10:00–11:30**

O-109 Efficacy of Dehydroepiandrosterone (DHEA) to overcome the effect of ovarian ageing (DITTO): a double blinded randomized placebo controlled trial

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Study question: To evaluate the effect of pre-treatment DHEA supplementation on the outcome of In-Vitro Fertilisation (IVF) treatment in women predicted to have poor Ovarian Reserve (OR).
Summary answer: Pre-treatment DHEA supplementation doesn't seem to improve the ovarian response as measured by the number of oocytes retrieved or clinical pregnancy rates during IVF treatment in women predicted to have poor OR.
What is known already: Ovarian ageing is a cause of subfertility and is associated with poor outcomes of IVF treatment. A few clinical studies have shown that DHEA can improve ovarian response and increase the chances of pregnancy after IVF treatment in women with a poor OR suggesting DHEA may help to overcome the effect of ovarian ageing.
Study design, size, duration: A single centre, double blinded, placebo controlled, randomized trial was performed over two years with 60 women undergoing IVF. Subjects were randomized, based on a computer-generated pseudo-random code using random permuted blocks of randomly varying sizes, to receive either DHEA or placebo with both capsules having similar colour, size and appearance.
Participants/materials, setting, methods: 60 with poor OR based on antral follicle count (≤ 10) or anti-Mullerian hormone (< 5 pmol/L) undergoing IVF were recruited. They were randomised to receive DHEA 75 mg/day or placebo for at-least 12 weeks before starting ovarian stimulation. They had long protocol using hMG 300 IU/day. Data analysed by "intention to treat".
Main results and the role of chance: The recruitment rate was 39% (60/154). A total of 53 participants (28 vs 25 in the study and the placebo groups respectively) were included in the final analysis after excluding seven, who did not commence the trial medication due to various reasons. The number (median; range) of oocytes retrieved (4; 0-15 vs. 4; 0-18 respectively; $P = 0.69$) and clinical pregnancy rates (7/28, 25% vs 9/25, 36% respectively; $P = 0.38$) were similar between the study and control groups. While the mean (± standard deviation) basal DHEA levels were similar at recruitment (9.1 ± 5.1 vs 7.5 ± 2.4 ng/ml respectively; $P = 0.3$), the DHEA levels at pre-stimulation stage were higher in the study group than in the control group (16.3 ± 5.9 vs 11.2 ± 4.5 ng/ml respectively; $P < 0.01$). No serious adverse events noted in both groups.
Limitations, reason for caution: The study was planned as both a proof of principle trial with the ovarian response as a surrogate for clinically important outcomes, and as pilot to evaluate the feasibility of conducting a late phase trial to test the effect of DHEA on live births. The successful recruitment suggests such a definitive trial would be feasible, but the lack of effect on ovarian response suggests it is a low priority.

Wider implications of the findings: The data from the study do not support the idea of using DHEA as an adjunct to IVF for improving treatment outcome in women predicted to have poor OR. While there has been a great deal of attention in the use of pre-treatment DHEA in predicted poor responders recently, this practice should be restricted to as part of large RCTs.
Study funding/competing interest(s): Funding by University(ies) – Early Career Research and Knowledge Transfer scheme, University of Nottingham. Nottingham University Hospital (NUH) Charity.
Trial registration number: EudraCT number: 2011-002425-21; http://www.clinicaltrials.gov; NCT01572025; CTA reference: 03057/0053/001-0002.
Keywords: DHEA, IVF, poor ovarian reserve, antral follicle count, anti-mullerian hormone

O-110 Effects of recombinant-LH supplementation on the proteomic profile of follicular fluid from poor responder patients: focus on follicular growth factors and oocyte maturity markers

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Study question: May recombinant-LH supplementation during IVF cycles in poor responder patients influence the pathways involved in follicular growth and oocyte maturity? Which pathways of follicular signaling are influenced by recombinant-LH supplementation? Can these factors explain the clinical advantages observed in older poor responder patients after recombinant-LH supplementation?
Summary answer: In poor-responder patients, recombinant-LH supplementation during IVF influences the pathways involved in follicular growth and oocyte maturity. The treatment significantly increases follicular levels of EGF, ERK-1/2 and AKT-1 and particularly the availability of the phosphorylated forms (active forms). These evidences explain the improvements in qualitative and quantitative ovarian response.
What is known already: Poor/fragmentary data was available regarding the effects of recombinant-LH in in-vitro granulosa and theca cells of human origin. No data was available from in-vivo studies regarding the effects of recombinant-LH supplementation on SCF, EGF, ERK-1/2 and AKT-1 pathways in the follicular fluid of older-poor-responder women undergoing IVF cycle. Evidence from in-vitro and animal studies seems to confirm that recombinant-LH, in addition increasing cAMP levels (involved in steroidogenesis), activates the ERK-1/2 (proliferation) and AKT-1 (anti-apoptotic) pathways.
Study design, size, duration: Observational longitudinal crossover study on 28 poor-responder patients > 42 years older. All patients underwent COS using rFSH alone in the first cycle (s-COH group) and using rFSH with rLH supplementation (ex-COH group) in the second cycle. We compared follicular concentrations of SCF, EGF, Erk 1-2, p-Erk 1-2, Akt-1 and p-Akt-1 between the two groups.
Participants/materials, setting, methods: After the achievement of hypotalamic suppression (long-agonist protocol), stimulation was performed using rFSH-300IU/day (plus rLH150IU/day in ex-COH) for 5-days and subsequently adjusted according to biochemical/sonographical features (s-COH). Follicular levels of SCF, EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were detected using appropriate ELISA-Kit and reported in pg/mL, ng/mL or Unit/mL, according to the manufacturer's indications.
Main results and the role of chance: Follicular levels (56 samples) of EGF, Erk 1-2, p-Erk-1/2, Akt-1, p-Akt-1 were significantly different between s-COH versus ex-COH, with the exception of SCF. In detail, mean value of EGF was 9,40 ± 2,92 vs 11,75 ± 3,95 pg/mL [$p < 0.05$], ERK 1-2 184,82 ± 50,15 vs 332,14 ± 111,35 pg/mL [$p < 0.001$], p-ERK-1/2 20,89 ± 3,41 vs 40,18 ± 10,37 U/mL [$p < 0.001$], AKT-1 5,35 ± 2,45 vs 10,42 ± 2,08 ng/mL [$p < 0.001$], p-AKT-1 28,07 ± 8,98 vs 42,36 ± 10,06 U/mL [$p < 0.001$], SCF 830,25 ± 364,09 vs 735,43 ± 300,39 pg/mL [p:n.s.]. The increasing intra-follicular levels of proteins (particularly in their active conformation) involved in cellular proliferation and anti-apoptotic pathways may explain the better clinical outcome observed after rLH supplementation. The absence of significant variations in SCF levels confirmed both that this pathway is activated only by FSH stimulation and that differences collected in other pathways is generated by rLH signaling.
Limitations, reason for caution: The potential bias linked to the type of the study (pilot study with low sample size), the peculiar features of the patients (older poor responder), the collection and assay of total follicular fluid for any treatments and not for single follicle require caution in the interpretation of data.
Wider implications of the findings: The type of study and its results strongly suggests that the differences in terms of follicular growth factors and oocyte maturity markers are linked to LH-signaling. The lower levels of SCF in the ex-COH group, though not statistically significant, suggest that LH can partially replace/potentiate the effects of FSH on follicular growth (SCF is exclusively controlled by FSH-signaling). This is the first in-vivo study reporting these evidences, partially demonstrated by in-vitro/experimental studies on animals.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This research was granted by Merck Serono Group. Authors declare no competing of interest.
Trial registration number: PMA-2012-13-rLH-PMA.
Keywords: ART, poor responder, follicular growth factors, oocyte maturity markers, recombinant LH

O-111 Beneficial effects of melatonin on oocytes and embryo quality in aged IVF patients

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Study question: The aim of the present study was to evaluate the role of melatonin supplementation on the main IVF outcomes in aged patients underwent IVF
Summary answer: Data reported in the manuscript clearly demonstrate that melatonin administration during ovarian stimulation in IVF patients increases intrafollicular melatonin concentrations and improve oocytes and embryos quality in over 40 patients
What is known already: IVF patients treated with melatonin had a greater number of mature oocytes and a lower number of immature oocytes compared not treated patients. Indeed, it is very likely that melatonin promotes the elimination of oxygen free radicals. Furthermore we demonstrated that exogenous administrated melatonin is able to accumulate in the follicles.
Our suggestion is to give a melatonin supplementation to aged patients underwent IVF.
Study design, size, duration: From July 2009 to December 2013 358 patients were assessed for elegibility in this prospective double blind randomized controlled trial.
Participants/materials, setting, methods: 358 infertile women aged over 40 underwent a shortdown-regulation protocol with a gonadotropin-releasing hormone (GnRH) analogue and a combined stimulation protocol with urinary and recombinant FSH. The patients were randomized into two groups: Group A, 178 patients who received melatonin (5 mg); group B, with 180 patients who did not received melatonin.
Main results and the role of chance: There were significant statistical differences comparing group A with group B in terms of mature oocytes (48.2% vs 35.0% $p = 0.008$); oxidative stress (CARR U 190 ± 41 vs 388 ± 64 in group A and B respectively), antioxidative capacity (AOCs) (1,76 ± 0,4 vs 0,89 ± 0,2 in group A and B respectively), progesteron concentration in follicular fluid (10,4 ± 1.1 vs 4,3 ± 0,8 in group A and B) and grade I embryos (45,7% vs 30,4% $p = 0.0045$). Melatonin intrafollicular concentrations were significantly increased after melatonin treatment (213 ± 51 pg/mL versus 69 ± 23 pg/mL, $P = 0.0013$).
Limitations, reason for caution: Statistical power calculation was based on a level of 0.05 with 80% power to detect a 20% difference with 50 evaluable patients per group. Sample size needed was 214 (Confidence Interval 4; Confidence level 95%). The difference between treatments was evaluated using a two-sided, 95% confidence interval.
Wider implications of the findings: Melatonin supplementation during IVF protocols in aged patients improve oocyte and embryo quality increasing progesterone production and scavenging free radicals. Furthermore, melatonin is efficiently accumulated in the follicular fluid.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Praxi DS, Praxi Provita.

Trial registration number: NCT01540747.
Keywords: melatonin, IVF aged patients, oxidative stress, antioxidative capacity

O-112 Supplementation of medroxyprogesterone acetate in modified natural cycles to prevent premature ovulation for IVF/ICSI patients with diminished ovarian reserve

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Study question: Is it possible to prevent premature ovulation using orally progestin (medroxyprogesterone acetate, MPA) in modified natural cycles based on freeze-all policy for patients with diminished ovarian reserve (DOR)?
Summary answer: Supplementation of MPA in modified natural cycles is an effective treatment to prevent premature ovulation and improve cycle programme for patients with DOR.DOR patients would have more chance to obtain oocytes/embryos for subsequently frozen-thawed embryo transfer (FET). The viable embryo rate per oocyte retrieved in modified natural cycle with MPA was not significantly increased.
What is known already: Premature ovulation is a current challenge for patients with DOR, even with using of GnRH anaglue. Our previous study indicated that progesterone can prevent premature LH surge in controlled ovarian stimulation in normal ovulatory women, both in the follicular-phase ovarian stimulation and luteal-phase ovarian stimulation. We analysed the clinical and embryological outcomes of using MPA in modified natural cycle in patients with DOR.
Study design, size, duration: A prospective controlled open-label cohort study was conducted between 2014 Jan and November at Shanghai Nineth People's Hospital, Shanghai, China. 204 infertile women with spontaneous cycle, higher basal FSH (10-30 IU/l) or no more than 5 antral follicles were included and allocated into two groups (MPA group and natural cycle) in alternatively order.
Participants/materials, setting, methods: In MPA group, MPA 10 mg/d was administrated from menstrual cycle day 3. After 5-7 days, follicular monitoring was conducted every 2–4 days, low dose of hMG was added if necessary. When one dominant follicle reached mature, triptorelin 100 μg and hCG 1000 IU were used for trigger. Oocyte retrieval was performed 35-36 h later. Natural cycle was as controls. All viable embryos were cryopreserved for subsequent embryo transfer. The primary endpoint was the number of oocytes retrieved.
Main results and the role of chance: In MPA group, the premature ovulation rate was significantly decreased into 2.0% compared with 10.8% in natural cycle ($P < 0.05$). The mean number of oocyte retrieved and viable embryos were significantly increased in MPA group (1.1 ± 0.6 vs 0.8 ± 0.6, $P < 0.05$; 0.6 ± 0.6 vs 0.4 ± 0.5, $P < 0.05$). The fertilization rate and cleavage rate were similar between the two groups. 14.71% (15/102) patients performed emergency advanced oocyte retrieval in natural cycle while no one advanced oocyte retrieval in MPA groups. The mean interval from trigger to oocyte retrieval in MPA group was significantly longer than those who were triggered in natural controls (35.3 ± 0.6 h vs 31.5 ± 5.8 h, $P < 0.01$). The cycle cancel rate of no viable embryos was slightly lower in MPA group but not reach significance (50.0% vs 61.8%, $P = 0.091$). The viable embryo rate per oocytes retrieved was similar between the two groups (51.3% vs 51.9%, $P > 0.05$).
Limitations, reason for caution: DOR patients have to accumulate enough viable embryos for subsequent FET in IVF clinic. No pregnancy outcome was reported due to the policy of transfer two embryos in our clinic.
Wider implications of the findings: Supplementation of MPA in modified natural cycle provides a novel sight to overcome premature ovulation for patients with DOR. DOR patients have more chance to accumulate embryos for subsequent FET. Progestin priming with using MPA in the follicular phase may permit folliculogenesis but delay or inhibit ovulation mechanism. The MPA treatment improved the oocyte quantity rather than the quality in modified natural cycle in patients with DOR.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The Natural Science Foundation of Shanghai (grant number 14411934600).
Trial registration number: ChiCTR-OCH-14004176.
Keywords: natural cycle, progesterone, in vitro fertilization

O-113 The comparison of mild stimulation vs. controlled ovarian hyperstimulation protocol in poor ovarian responders: a prospective randomized study

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Study question: Is there any difference in clinical outcomes between mild stimulation and controlled ovarian hyperstimulation (COH) in-vitro fertilization (IVF) cycles in poor ovarian response (POR) patients?
Summary answer: Comparing with COH protocol, mild stimulation was associated with fewer gonadotropin (Gn) consumption and lower number of oocyte retrieved, while, no significant difference was observed in the number of good quality embryos, clinical pregnancy rate (CPR) per embryo transfer (ET), CPR per oocyte pick-up (OPU) and CPR per cycle.
What is known already: POR is associated with lower number of oocyte retrieved and poorer clinical outcomes, and represents a significant therapeutic challenge in assisted reproductive technologies, with no single effective stimulation protocol has been established to increase ovarian response. Mild ovarian stimulation, characterized by lower dose of exogenous gonadotropin consumption and shorter stimulation length has been utilized in poor responders, however, there is no consistent conclusion concerning the comparison between mild stimulation and conventional COH.
Study design, size, duration: This is a prospective randomized non-inferiority study, from Jan to Nov. 2014, POR patients were randomized to mild stimulation (letrozole + small dose of Gn) or COH group (GnRH-a long 'stop' protocol). The randomization is achieved by computer-generated list. Neither patients nor physicians are blinded to the treatment assigned.
Participants/materials, setting, methods: POR patients who met 'Bologna criteria' were recruited in this study, 50 patients were assigned to mild stimulation, while 55 to COH group. The two groups were matched in basic characteristics, such as, age, basal FSH, AFC and AMH etc.
Main results and the role of chance: No significant difference in basic characteristics, such as age (36.2 ± 4.8 vs. 36.2 ± 5.4), AFC (4.5 ± 1.8 vs. 4.9 ± 1.2), basal FSH (9.4 ± 4.1 vs. 9.4 ± 3.8), E_2(39.2 ± 18.2 vs. 42.9 ± 30.5) and AMH (0.8 ± 0.6 vs. 0.9 ± 0.7) levels was observed between mild stimulation and COH group (All $P > 0.05$). The cycle characteristics were listed in following table.

Table

	mild stimulation	COH group	*P*
Gn consumption (IU)	934.90 ± 298.75	3016.36 ± 711.92	0.000
E_2 level on Day_{hCG} (pg/ml)	615.46 ± 534.87	1335.48 ± 667.51	0.000
LH level on Day_{hCG} (IU/L)	7.33 ± 6.16	2.37 ± 1.72	0.000
Endometrial thickness	9.06 ± 2.38	11.79 ± 2.43	0.000
No. of mature follicles	3.10 ± 1.83	4.33 ± 2.68	0.007
No. of oocytes retrieved	2.67 ± 1.98	4.02 ± 3.12	0.013
No. of transferrable embryos	1.24 ± 1.15	1.90 ± 1.73	0.027
No. of good-quality embryos	1.06 ± 1.11	1.29 ± 1.32	0.352
No. of embryos transferred	1.59 ± 0.50	1.67 ± 0.48	0.561
CPR/ET	48.1% (13/27)	46.7% (14/30)	0.911
CPR/OPU	26.5% (13/49)	26.9% (14/52)	0.964
CPR/cycle initiated	26.0% (13/50)	25.5% (14/55)	0.954

Limitations, reason for caution: This study was part of an ongoing RCT research on the comparison of mild stimulation and COH in POR patients. After statistical calculation, the sample size should be 250 per arm to make sure enough patients recruited and analyzed. Therefore our conclusion needed to be interpreted with cautiousness.
Wider implications of the findings: Based on our results regarding less doses of exogenous Gn consumed and an equivalent CPR, mild ovarian stimulation protocol should be recommended to POR patients. Considering the higher number of transferrable embryos associated with COH group, the cumulative pregnancy rate should be used as the primary outcome in future study. At last, large-scale multi-center RCT is needed to verify our results.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The reproductive medicine center of sixth affiliated hospital of SYSU.
Trial registration number: ChiCTR-TRC-13003454.

Keywords: mild stimulation, controlled ovarian hyperstimulation, poor ovarian responder, clinical outcomes, IVF

O-114 Serum stem-cell-factor assays in poor responder patients undergoing IVF: a new tool to establish sense or no sense in performing follicle aspiration

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Study question: In poor responder patients undergoing IVF, could the follicular stem cell factor concentration (f-SCF) be associated with the quantitative and qualitative characteristics of ovarian response? Is f-SCF correlated with serum concentration at ovulation induction (s-SCF)?May s-SCF be considered a new tool to establish whether to perform follicle aspiration or not?
Summary answer: The f-SCF correlates strongly with quantitative and qualitative ovarian response, oocyte fertilization-rate and number of viable embryos. s-SCF is strongly correlated with the f-SCF. s-SCF demonstrated good accuracy in predicting both cycles in which no oocytes are collected and cycles in which at least one or three oocytes are collected.
What is known already: Granulosa cells surround the developing oocyte, providing a critical microenvironment for follicular growth while in the developing follicles they produce SCF, which can act on theca cells, stromal cells and oocytes via c-kit receptor. The expression patterns of SCF and c-kit in the ovary, as well as the effects of SCF on oocytes and theca cells, suggest that SCF may be important for many stages of follicular development.
Study design, size, duration: Observational-longitudinal study on 28 poor-responder. All patients underwent Controlled-Ovarian-Hyperstimulation (COH) using rFSH in first cycle (s-COH group) while rFSH plus rLH supplementation (ex-COH group) in the second cycle. We compared f-SCF concentration between the two groups, f-SCF with s-SCF concentration and s-SCF value with qualitative and quantitative ovarian response
Participants/materials, setting, methods: Long-agonist-protocol was performed using rFSH-300 IU/day (plus rLH150 IU/day in ex-COH) for 5-days subsequently adjusted according to biochemical/sonographic features. f-SCF and s-SCF levels (pg/mL) were detected using appropriate ELISA-Kit. We considered as quantitative ovarian response number of follicles > 14 mm and MII oocytes while as qualitative oocytes fertilization-rate and number of viable embryos.
Main results and the role of chance: We collected and analyzed 112 samples (56-follicular fluid and 56-serum). The comparison of s-COH and ex-COH showed no significant differences in term of both f-SCF (830,25 ± 364,09 vs 735,43 ± 300,39 pg/mL) and s-SCF (884,98 ± 387,01 vs 783,18 ± 325,55 pg/mL). Considering all treatments, the correlation between f-SCF and s-SCF values showed a strong association ($R^2 = 0.982$, $p < 0.001$). The s-SCF value > 1400 was associated with 60% probability of collecting at least 3 oocytes, 85% of fertilization-rate and 75% probability of having at least 2 viable embryos. Patients with > 1000/s-SCF/ < 1400 showed a 27% probability of collecting at least 3 oocytes (58% of at least 1 oocyte), 65% fertilization rate, 46% probability of having at least 1 viable embryo. s-SCF < 1000 and < 400 showed respectively 21% and 0% probability of collecting at least 1 oocyte.
Limitations, reason for caution: The potential bias linked to the peculiar features of the patients (older poor responder), the exclusive use of recombinant gonadotropins, the collection and assay of total follicular fluid per cycle and not for single follicle requires caution in the interpretation of data.
Wider implications of the findings: If this pioneeristic data will be confirmed, s-SCF assay may be introduced in clinical practice when approaching older-poor-responder-patients. Particularly in a public health setting, in which usually the number of treatments offered is limited and linked to number of oocyte retrievals, the availability of a real-time/non-invasive/less-expensive serum-test able to predict with good accuracy the number of oocytes, fertilization-rate, and number of viable embryos may improve the cost-effectiveness of treatment offered to this cohort of patients.
Study funding/competing interest(s): Funding by University(ies) – Authors declare no funding. Authors declare no competing of interest.
Trial registration number: PMA-2012-13-rLH-PMA.
Keywords: ART, poor responder, stem cell factor, predictive model, growth factors

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SELECTED ORAL COMMUNICATIONS

SESSION 33: WHAT'S NEW IN PGS?

Tuesday 16 June 2015 **10:00–11:30**

O-115 Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study

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Study question: Can next-generation sequencing (NGS) be used efficiently for preimplantation genetic screening (PGS) of embryos from IVF patients with clinical indications of recurrent pregnancy loss (RPL), recurrent implantation failure (RIF) and previous aneuploid conceptions (PAC) in a clinical setting?
Summary answer: This is the first randomized clinical study on the efficiency of NGS for preimplantation genetic screening in comparison to array comparative genomic hybridization (aCGH). Our data clearly demonstrate that NGS screening results in similarly high ongoing pregnancy and implantation rates for PGS patients compared to aCGH screening.
What is known already: Previous randomized clinical trials with FISH screening of a limited numbers (5-12) of chromosomes resulted in disappointing pregnancy outcomes. Recent studies with aCGH screening of 24 chromosomes have resulted in a significant increase in ongoing pregnancy and implantation rates for PGS patients. More recent advances in next-generation sequencing have provided new methods for screening embryos from IVF cycles. However, there is still limited information about clinical application of NGS in IVF and PGS treatments.
Study design, size, duration: IVF patients ($n = 172$) at mean age 35.2 ± 3.5 years were enrolled in this prospective randomized, single-blind, pilot interventional study in our multiple IVF clinics from July 2013 to June 2014. The cohort patients requested PGS treatments with the clinical indications of RPL ($n = 72$), RIF ($n = 63$) and PAC ($n = 37$).
Participants/materials, setting, methods: The enrolled patients were randomized into NGS (Group A, $n = 86$) and aCGH (Group B, $n = 86$). Blastocysts were vitrified after biopsy on day 5. One to two euploid blastocyst were transferred to individual patients based on the PGS results. Clinical pregnancy and implantation outcomes were compared between the two groups.
Main results and the role of chance: Comparative data analysis revealed that the demographic parameters of the enrolled patients in Group A and Group B were similar ($p > 0.05$). The fertilization and blastocyst rates were also comparable between the two groups ($p > 0.05$). NGS detected all types of aneuploidies including trisomy, monosomy, dual and complex chromosomal abnormalities accurately compared to aCGH. Moreover, NGS screening identified euploid blastocysts for transfer and resulted in similarly high ongoing pregnancy rates for PGS patients compared to aCGH screening (74.7% vs. 69.2%, respectively, $p > 0.05$). The observed implantation rates were also comparable between Group A and Group B (70.5% vs. 66.2%, respectively, $p > 0.05$). Additionally, there was no significant difference in miscarriage rate between Group A and Group B (1.3% vs. 2.6%, respectively, $p > 0.05$).

Limitations, reason for caution: Although NGS brings distinct clinical benefits for many IVF patients, the approach is not for all infertile patients, especially those with diminished ovarian reserve and with balanced translocations. Further randomized studies with a larger sample are required to define the role of NGS in assisted reproductive treatments.
Wider implications of the findings: With the observed high accuracy of aneuploidy screening across all 24 chromosomes and the resulting high clinical pregnancy and implantation rates after transfer of the screened embryos, NGS has demonstrated an efficient, robust high-throughput technology for PGS in IVF clinics. With the enhanced capability of detecting segmental imbalances, NGS platforms, at a high read depth, may detect aneuploidy and imbalance translocations at the same time.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study is supported by internal funding. CEM is a full time scientists at Illumina, which provided NGS chips and reagents for this study. All the other authors have no conflicting interests to declare.
Trial registration number: NA.
Keywords: NGS, aCGH, PGS, ongoing pregnancy, implantation

O-116 Comparison of comprehensive chromosome screening (CCS) results obtained from in vitro fertilization (IVF) cycles treated with low stimulation protocols and standard stimulation protocols

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Study question: Is there any difference in aneuploidy rates and preimplantation genetic diagnosis (PGD) cycle outcome between blastocyst-stage embryos produced after low stimulation IVF cycles and standard stimulation cycles and if yes, which is the most advantageous?
Summary answer: No difference was seen in regards to aneuploidy rates between embryos produced using the two different treatments. Consequently, standard stimulation cycles which often result in higher number of embryos were found to have twice as much the chances of producing a euploid embryo for transfer and establishment of pregnancy.
What is known already: Stimulation procedures vary amongst different centers but are in general similar to methods considered standard. Usage of a low stimulation procedure could result in a better cycle outcome while, it is expected to be less stressful to the patient. Such protocols will be particularly useful in avoiding patient over-stimulation. Furthermore, embryos produced through IVF often show high aneuploidy rates. It will be valuable to determine if such strategy results in better CCS outcomes.
Study design, size, duration: A retrospective analysis of 69 PGD cycles performed between January 2014–December 2014 was carried out. All cycles were derived from a single assisted reproduction center in the USA. Results from CCS of 246 blastocysts were collected and analyzed.
Participants/materials, setting, methods: Average maternal age between the two groups of patients was similar (37 for the low stimulation patients, 35.3 for the standard stimulation patients; $p = 0.11$). Results obtained from samples derived from patients ³ 40 years old were excluded from the study. Array comparative genomic hybridization was used for CCS of biopsied samples.
Main results and the role of chance: The amount of embryos that were found to be euploid after CCS was determined to be similar between the two patient groups ($p = 0.26$). Specifically, 45.8% (22/48) of embryos derived from low stimulation cycles were determined to be euploid compared to 55.6% (110/198) of embryos derived from standard stimulation cycles. Only approximately half (19/37) of the low stimulation cycles were found to have a euploid embryo for transfer while, all standard stimulation cycles (32/32) had at least one euploid embryo for transfer. As a consequence, patients being treated with low stimulation protocols had to undergo twice the amount of cycles than patients being treated with standard stimulation protocols in order to find a euploid embryo to transfer for initiation of pregnancy.
Limitations, reason for caution: Although this study was of considerable size, more cycles and embryos have to be assessed and follow-up data from cycles that had a transfer need to be obtained before any definitive conclusions are drawn.
Wider implications of the findings: This study provides evidence that incidence of aneuploidy remains the same regardless of the stimulation protocol administered to each patient. As shown, patients being treated with standard stimulation protocols are expected to have higher chances of having a euploid embryo available for transfer per cycle completed. However, definitive conclusions about a potential benefit to patients can only be reached after acquisition and examination of clinical outcome follow-up data.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Institutional funding.
Trial registration number: NA.
Keywords: in vitro fertilization, hormonal stimulation, comprehensive chromosome screening, aneuploidy

O-117 Preimplantation genetic screening using comprehensive chromosome screening technology improves embryo selection: a meta-analysis of randomized controlled trials

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Study question: Does preimplantation genetic screening (PGS) using comprehensive chromosome screening (CCS) technology improve embryo selection in in-vitro fertilization (IVF) cycles?
Summary answer: When used in patients with normal ovarian reserve, PGS using CCS technology increases clinical implantation rates (IR) and sustained IR (probability that an embryo would implant and progress beyond 20 weeks gestation).
What is known already: Most of the randomized controlled trials (RCTs) on PGS using fluorescence-in-situ-hybridization (FISH) technology after cleavage-embryo biopsy showed no increase in live birth rates. CCS technology, which analyses the whole chromosome complement, can be achieved using different genetic platforms, and has been extensively tested and validated in PGS cycles (PGS-CCS). Whether PGS-CCS improves embryo selection in IVF remains unclear.
Study design, size, duration: A meta-analysis of RCTs on PGS-CCS published before January 2015 was performed. The clinical outcomes of interest included in the meta-analysis are clinical IR and sustained IR. 267 embryos transferred after PGS-CCS were compared to 383 embryos transferred after selection based on standard morphology criteria alone.
Participants/materials, setting, methods: We searched Medline, Cochrane Central, EMBASE, Scopus, Web of Science, Google Scholar, as well as related articles from relevant authors on the subject. RCTs were eligible if they compared PGS-CCS to other methods of embryo selection. The three authors independently screened for eligibility, and assessed the quality of the RCTs.
Main results and the role of chance: Out of 750 citations identified, sixteen articles met initial eligibility criteria and were further analyzed. Of these, only three RCTs met full inclusion criteria, allowing direct comparison of PGS-CCS to routine IVF care based on embryo morphology selection. In one study, the CCS technology used was array comparative genomic hybridization (aCGH), and in the two remaining others, quantitative polymerase chain reaction (qPCR) was used. In these studies, all embryo biopsies were performed on day 5-6 of embryo development. PGS-CCS is associated with significantly higher clinical IR, with a relative risk [RR] of 1.29 [95% CI: 1.15–1.45], and also a significantly higher sustained IR, with a RR of 1.39 [95% CI: 1.21–1.60].
Limitations, reason for caution: Randomization was performed in the presence of blastocysts available for biopsy from patients with normal ovarian reserve, which may introduce selection bias and overestimate success rates for PGS-CCS. Extrapolation of these results to a different population (e.g. poor responders) and/or embryo stage biopsy remains uncertain for the time being.
Wider implications of the findings: By increasing IR in IVF cycles, PGS-CCS might be helpful when used in the setting of elective single embryo transfer practice. Therefore, it represents an ideal tool for embryo selection in order to overcome the marked differences in outcome of single- versus double-embryo transfer. Results from ongoing RCTs conducted on different patient populations

(e.g. decreased ovarian reserve), and different embryo stage biopsy (e.g. polar body, day-3) may further clarify the role of this technology.
Study funding/competing interest(s): Funding by hospital/clinic(s) – ART-PGD Center, CHU Sainte-Justine, Montreal, Canada.
Trial registration number: NA.
Keywords: preimplantation genetic screening, omprehensive chromosome screening, embryo selection, elective single embryo transfer

O-118 Segmental abnormalities can be detected in the DNA of the blastocyst cavity extracted by blastocentesis

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Study question: Can the array-Comparative Genomic Hybridization (aCGH) analysis of DNA extracted from the blastocoelic fluid predict the chromosomal status of the blastocyst, including segmental abnormalities?
Summary answer: The DNA from the blastocoelic fluid (BF) reflects the chromosomal status of the blastocyst trophectoderm (TE) cells. Also segmental abnormalities can be detected by aCGH analysis after blastocentesis
What is known already: Unbalanced segmental abnormalities can be a reason for embryo demise; or, if compatible with life, they can cause congenital abnormalities and mental retardation in the offspring. Identified in approximately 10% of clinical pregnancies, they are especially frequent in carriers of reciprocal translocations, but they can also originate as de novo rearrangements. Data from the analysis of BF suggest that it can be a reliable source of DNA to assess the embryo chromosomal status.
Study design, size, duration: Study: Prospective blinded study for the chromosomal analysis of 44 blastocysts on which aCGH had already been performed on Polar bodies (PBs) or blastomeres in a Preimplantation Genetic Testing program. Of them, 18 blastocysts from 6 couples, generated at least one embryo with a segmental abnormality. Duration: January-December 2014.
Participants/materials, setting, methods: Of the 6 couples with embryos having segmental abnormalities, 2 were carriers of a balanced translocation, 4 had a normal karyotype. Both BF and TE cells were retrieved from each blastocyst, and the DNA was blindly analyzed by aCGH. Results were compared with those previously obtained from PBs and blastomeres.
Main results and the role of chance: DNA was found in 43/44 tested BFs (98%). In the subgroup of 18 blastocysts, in 14 cases the aCGH analysis revealed full correspondence between BF and the results from PBs (n = 8) or blastomeres (n = 6). In the remaining 4 cases, aneuploidies predicted by blastomeres were found in the BF with the addition of new abnormalities. In 8 of the 18 studied blastocysts (44%), segmental abnormalities had been detected by PBs (n = 2) or blastomeres (n = 6). Three were found in embryos from translocation carriers, 5 in embryos from couples with a normal karyotype. In all 8 cases, DNA from the corresponding BF presented the same segmental abnormality predicted by the analysis at previous stage also for small alterations (33 Mb). All results were confirmed in corresponding TE cells.
Limitations, reason for caution: Small size of samples analyzed.
Wider implications of the findings: The present data confirm that blastocentesis can be an alternative source of DNA for preimplantation genetic testing, since the DNA from BFs reflects the chromosomal status of the corresponding embryo. Furthermore, these preliminary results suggest that this approach is reliable to the point to permit the detection of segmental aneuploidies, both in couples carrier of reciprocal translocation and in couples with normal karyotype.
Study funding/competing interest(s): Funding by hospital/clinic(s) – S.I.S.Me.R.
Trial registration number: NA.
Keywords: blastocele, segmental aneuploidies, blastomere, polar bodies, preimplantation genetic screening

O-119 Blastocyst chromosome distributions persist into pregnancy

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Study question: Is there a pattern to chromosome copy number errors in a large, single centre cohort of blastocysts and does the testing platform influence the outcome?
Summary answer: Blastocysts display defined patterns in the type of aneuploidies observed and the chromosomes involved, with both reflecting the patterns seen in pregnancy loss, neither of which are influenced by the testing platform used.
What is known already: Limited studies have investigated the specific nature of the aneuploidies observed in blastocysts, with various rates of simple vs complex aneuploidies and equal prevalence of monosomies and trisomies being reported. Preliminary data obtained from a smaller, single platform data set in our laboratory, indicated the trend toward fewer complex aneuploidies, fewer monosomies and a distinct chromosomal pattern in blastocysts, prompting extended analysis of a large data set over multiple platforms.
Study design, size, duration: Retrospective analysis of the chromosomal complement of 1029 blastocysts biopsied for preimplantation genetic screening (PGS) between June 2012 and December 2014.
Participants/materials, setting, methods: All patients undertaking PGS with blastocyst biopsy in a single clinic were analysed. PGS was performed using either 24Sure array comparative genomic hybridization (CGH) or single nucleotide polymorphism arrays with parental support through Natera (SNP) at the patient's discretion.
Main results and the role of chancel: Euploidy rate varied by age as expected, averaging 52.3% with no difference between platforms (p = 0.493 age corrected). Although aneuploidy rates increased with age the mean number of errors per blastocyst did not (< 34 years, 2.44 errors versus > 40 years, 2.36 errors). Overall 59.5% of blastocysts contained a single error, with a total of 79.3% simple aneuploidies (1-2 errors) and only 20.7% complex aneuploidy (> 2 errors). The proportion of monosomies was 23.8% compared with 76.2% trisomies. Chromosomes 13, 15, 16, 18, 21 and 22 are the most commonly associated with pregnancy loss and interestingly were the most prevalent chromosomes in blastocyst aneuploidies. This distribution was significantly non-random (chi-square goodness of fit, $p < 0.0001$) and consistent across both CGH and SNP (chi-square test for independence, p = 0.941).
Limitations, reason for caution: This is a retrospective analysis of patients undergoing PGS for a variety of clinical indications and is potentially influenced by this population bias. The testing platform used was not randomized in this study but rather selected by patients based on cost, reported accuracy and information provided by the respective tests.
Wider implications of the findings: This large, single centre, multi-platform data set differs significantly from previously reported findings in regard to the prevalence of monosomies and trisomies in blastocyst samples, with the current data more closely approximating the frequencies seen in prenatal testing. The confirmation of a distinct pattern of chromosomal involvement, which mimics the chromosomal errors indentified in pregnancy loss, support the use of blastocyst biopsy as the most clinically relevant time to perform pre-implantation genetic screening.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Monash IVF.
Trial registration number: NA.
Keywords: PGS, blastocyst, aneuploidy

O-120 Do culture conditions, patient factor or female age affect incidence of segmental chromosome errors in preimplantation embryos?

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Study question: Is the frequency of segmental chromosome errors related to a specific patient group? Does female age affect this frequency? Do culture conditions in different IVF clinics have an effect?
Summary answer: Cleavage stage embryos from younger patients (< 37 years) showed a significant increase in the frequency of segmental abnormalities. Moreover, differences in procedures at individual clinics appear to affect the incidence of segmental aneuploidy. This is important given that segmental imbalance can have significant mental and physical consequences for affected children.
What is known already: Chromosome abnormality affects almost half of all embryos generated through IVF and is believed to be the leading cause of implantation failure and miscarriage. The majority of these abnormalities affect entire chromosomes. However, a significant minority, accounting for 12-15%

of all errors, affect parts of chromosomes. The cause of such 'segmental' errors is unknown and their clinical impact is poorly defined.
Study design, size, duration: This is a retrospective analysis of the frequency of segmental errors in preimplantation embryos. 2794 embryos at the cleavage and blastocyst stages were studied, generated by 462 patients (average age 38.3 years, range 21-47 years) that had IVF treatment at 12 different clinics between 2008 and 2014.
Participants/materials, setting, methods: One blastomere was biopsied from cleavage stage embryos and 3-10 trophectoderm cells were biopsied from blastocyst stage embryos. Biopsy specimens underwent whole genome amplification (SurePlex), followed by labelling and hybridization to 24Sure aCGH slides (Illumina). Results were analysed using BlueFuse software, revealing aneuploidy affecting whole chromosomes and chromosome segments >5 Mb
Main results and the role of chance: In order to investigate whether specific patients produce high number of embryos with segmental errors only those that had 5 or more embryos were examined. A total of 30 patients had segmental errors affecting 50% or more of their embryos. Patients were sub-grouped according to female age into a younger (< 37 years) and an older (≥ 37 years) group. A significant increase in the frequency of segmental errors was found in cleavage stage embryos generated from the younger patient group (29% vs. 19%, $p = 0.0001$) while no significant difference was observed between the 2 groups at the blastocyst stage. The frequency of segmental errors affecting preimplantation embryos was significantly elevated in embryos derived from three of the referring IVF centres ($p = 0.001$, $p = 0.003$ and $p = 0.005$)
Limitations, reason for caution: The method used in this study only examines a part of the embryo and cannot rule out the possibility of mosaicism. Additionally, it is not possible to conclusively determine whether the segmental errors observed are meiotic or mitotic (postzygotic) in origin. Segmental imbalances <5 Mb in size cannot be accurately detected.
Wider implications of the findings: Younger patients had a higher incidence of segmental abnormalities, but this may be related to the increased proportion of male-factor infertility cases in our 'younger' patient group, rather than an effect of age. Segmental abnormalities involve chromosome breakage and could conceivably be related to sperm DNA fragmentation. Some clinics had higher rates of segmental abnormality in the embryos produced, arguing that optimisation of culture and/or simulation methods might help to avoid these abnormalities.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Reprogenetics UK.
Trial registration number: NA.
Keywords: segmental chromosome errors, female age, patient factor, culture conditions, human embryos

SELECTED ORAL COMMUNICATIONS

SESSION 34: CLINICAL ASPECTS OF ENDOMETRIOSIS

Tuesday 16 June 2015 **10:00–11:30**

O-121 Endometriosis does not alter antral follicle responsiveness to FSH administration as assessed by the Follicular Output RaTe (FORT)

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Study question: To investigate whether responsiveness of antral follicles to FSH administration, as reflected by the Follicular Output RaTe (FORT), is altered in patients with endometriosis.
Summary answer: Our results indicated that, whereas endometriosis is associated with reduced antral follicle counts (AFC) and preovulatory follicle counts (PFC), the percentage of antral follicles that successfully respond to FSH administration (FORT) remains comparable to controls, thereby supporting the hypothesis that follicle quality is not altered by endometriosis.
What is known already: Endometriosis-linked infertility has been, at least in part, attributed to disorders in the oocyte reproductive competence. Yet, quantitative ovarian defects such as reduced antral follicle endowment and poor response to controlled ovarian hyperstimulation (COH) does not constitute necessarily an evidence of altered follicle-oocyte competence. Further, responsiveness to FSH presumably is characteristic of healthy ranulose cells and has been associated to IVF-ET outcome using the FORT index, therefore, constituting an interesting clinical appraisal of follicle quality.
Study design, size, duration: Case-control study. We compared 141 infertile patients, 25 to 43 years of age, with laparoscopically-documented endometriosis and having accomplished COH for IVF-ET with other 141, age- and BMI-matched, IVF-ET candidates without endometriosis. Endometriosis was staged according to revised AFS criteria.
Participants/materials, setting, methods: Similar COH protocols and initial recFSH doses (225 IU/day) were used in endometriosis and control patients. AFC (3-8 mm) before COH and PFC (16-22 mm; day of hCG administration) were determined. The FORT was calculated by the ratio PFC x 100/AFC.
Main results and the role of chance: As expected, by design, ages and BMI were similar in both groups. Whereas AFC (14.0 ± 0.3 versus 15.9 ± 0.4 follicles, $P < 0.001$) and PFC (6.4 ± 0.2 versus 7.3 ± 0.3 follicles, $P < 0.02$) were lower in endometriosis patients than in controls, FORT remained similar in the two groups of patients (47.1 ± 1.6% versus 47.4 ± 1.9%, respectively). Incidentally, FORT results were comparable in patients with stage I-II or III-IV endometriosis and in those with or without endometrioma.
Limitations, reason for caution: FORT could not be, by design, assessed in patients having discontinued FSH treatment before hCG administration (due to a markedly insufficient follicle recruitment), since their PFC could not be established. In addition, FORT implies that 3-8 mm follicles before COH respond coordinately to FSH, which is not always the case.
Wider implications of the findings: Endometriosis is a frequent finding among infertile patients. The present study constitutes the first clinical demonstration that, although this disease is associated with a significant reduction in the absolute number of FSH-sensitive and mature follicles obtained after COH, follicular responsiveness to FSH is not affected by endometriosis. This is in keeping with hypothesis that follicle-oocyte quality is spared in women with endometriosis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: endometriosis, IVF-ET, FORT, ovarian response, follicle quality

O-122 Reproductive and pregnancy outcomes in women with endometriosis: a Scottish national record linkage study

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[4]*The University of Edinburgh, Obstetrics and Gynaecology, Edinburgh, United Kingdom*

Study question: Are women with history of endometriosis more likely to have adverse reproductive and pregnancy outcomes when compared to women without the diagnosis of endometriosis?
Summary answer: Women with endometriosis were at increased risk of miscarriage and ectopic pregnancy. Pregnancies progressing beyond the first trimester, were at higher risk of complications, including antepartum haemorrhage, preterm birth, operative delivery and postpartum haemorrhage.
What is known already: The impact of endometriosis on pregnancy and reproductive performance of women is relatively unknown. The few studies exploring the relationship between endometriosis and pregnancy outcome have been conducted in infertile women undergoing medically assisted reproduction. There is lack of large studies tilizing population based data where a definitive diagnosis of endometriosis has been established at laparoscopy.
Study design, size, duration: A retrospective population based cohort study using record linkage of routinely collected anonymised discharge data from all Scottish NHS hospitals was conducted. A total of 14,655 women were followed up over a maximum of 30 years from 1981 to 2010.
Participants/materials, setting, methods: In a nationwide Scottish study, we compared reproductive and pregnancy outcomes in 5,375 women with surgically confirmed endometriosis to 8,280 women without the diagnosis of

endometriosis (a random sample of women without a diagnosis of endometriosis and pregnant during the same time-period) using univariate and multivariate logistic regression accounting for confounding factors.
Main results and the role of chance: Pregnant women with history of endometriosis were significantly older, more likely to be nulliparous and from a more affluent social class ($p < 0.001$) when compared to those without endometriosis. On multivariate analysis, after adjusting for age, parity, socioeconomic status and year of delivery, women with endometriosis had a significantly higher risk of early pregnancy complications with adjusted OR (95% CI) of 1.76 (1.44, 2.15) and 2.70 (1.09, 6.72) for miscarriage and ectopic pregnancy, respectively. A previous diagnosis of endometriosis was associated with a significantly increased risk of adverse later pregnancy outcomes [adjusted OR (95% CI)] including placenta praevia [2.24 (1.52, 3.31)], unexplained antepartum haemorrhage [1.67 (1.39, 2.00)], postpartum haemorrhage [1.30 (1.61, 1.46)] and preterm births [1.26 (1.07, 1.49)].
Limitations, reason for caution: The study suffers from limitations inherent to an observational study with limited availability of and missing data on important co-variables such as smoking, body mass index and use of medically assisted reproduction. It is possible that there is a small proportion of women with undiagnosed endometriosis in the unexposed cohort.
Wider implications of the findings: Endometriosis predisposes women to increased risk of early pregnancy loss and later pregnancy complications. These data can be used by clinicians when counselling women with known endometriosis. Data from this Scotland wide study should be taken into account when planning health care strategies for surveillance and early identification of potential complications in pregnancy to minimize their impact on pregnancy outcome.
Study funding/competing interest(s): Funding by national/international organization(s) – Chief Scientist Office, Scotland.
Trial registration number: NA.
Keywords: endometriosis, reproductive outcomes, pregnancy outcomes

O-123 Predicting the reproductive outcome in endometriosis – a comparison between EFI and AFS scores

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Study question: To determine the better tool, scored by r-AFS and EFI scores, for predicting the reproductive outcomes in patients with surgically documented endometriosis, attempting conception
Summary answer: Correlation with EFI, is a better prognosticator of the reproductive outcome in patients with surgically documented endometriosis attempting conception due to a highly significant relationship between EFI scores and pregnancy outcomes.
What is known already: r-AFS score can only stage the levels of endometriosis into minimal, mild, moderate and severe. It cannot predict the reproductive outcome after surgery for endometriosis.
Study design, size, duration: *Study Design:* Prospective cohort study. *Sample Size:* 166. *Study Duration:* 18 months. *Lost to Follow Up:* The subjects lost to follow up were excluded from the study.
Participants/materials, setting, methods: *Materials and Setting:* EFI score was calculated post operatively (score 0 to 10) for 166 women for whom r-Afs endometriosis staging was done (minimal, mild, moderate, severe) in a private tertiary reproductive and gynaecological research centre from January 2012 to July 2013.All the participants attempted conception within six months by timed intercourse or intra uterine insemination or controlled ovarian stimulation based on a pre designed protocol. Those patients failing to conceive within six months of intrauterine insemination or those who had severe endometriosis were offered ART techniques as an alternative. *Methods*: Six groups of EFI were identified (1-3,4,5,6,7-8,9-10) and pregnancy rates were calculated for each group. Patients were excluded when they were lost to follow up, had subsequent surgery for endometriosis, or was on ovarian suppression.
Main results and the role of chance: *Main Results and Role of Chance:* EFI score and pregnancy rates showed a highly significant relation ($p < 0.05$) with patients having an EFI score of 0 with 0% pregnancy rates and those patients having a score of 6 and above with pregnancy rates unto 73%. The least function score was an important parameter in determining the EFI score. The EFI score contains historical and surgical factors, thus being a better parameter for determining the reproductive outcomes post operatively, regardless of the r-Afs scores.
Limitations, reason for caution: *Limitations*: The EFI score cannot be used to predict post operative pain. *Reason for Caution:* This is a newly devised scoring system used to predict the reproductive capacity in patients undergoing surgery for enometriosis. Larger studies are needed to determine its disadvantages. *Bias and Confounding:* Bias and confounding is eliminated by taking same samples for both criteria.
Wider implications of the findings: The study was earlier published by Adamson in a European population. This new validated endometriosis staging system is now used in an Indian population. It appears that this scoring system can be used to counsel patients about their reproductive chances after a surgery for endometriosis as well as their post operative conception options.
Study funding/competing interest(s): Funding by hospital/clinic(s) – KJK Hospital, Trivandrum, India.
Trial registration number: NA.
Keywords: reproductive outcome, endometriosis, EFI, AFS

O-124 Prognostic factors of ART outcomes in a continuous series of 359 endometriosis patients

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Study question: To evaluate the ART cumulative pregnancy rates (cPR) in a large cohort of endometriosis (OSIS) patients and to identify the determinant factors of ART outcome.
Summary answer: Globally pregnancy rates are good in endometriosis patients (44%); Yet, OSIS associated with intestinal deep infiltrating endometriosis (DIE) and past surgery for OSIS are associated with poorer ART outcome.
What is known already: ART is one of the therapeutic options offered for managing OSIS-associated infertility. Yet, published data on ART outcome in women affected by OSIS are conflicting and the determinant factors for pregnancy chances unclear.
Study design, size, duration: Observational cohort study on 359 consecutive OSIS patients, who underwent IVF-ICSI treatment at Cochin-Port-Royal Hospital between June 2005 and February 2013. Diagnosis of OSIS was histologically proven in women who had past surgery ($n = 279$ – 77.7%), or based on published imaging criteria using transvaginal sonography and magnetic resonance imaging.
Participants/materials, setting, methods: The main outcome measure was cPR per patient. We compared the characteristics of women who became pregnant and those who did not, using univariable and multivariable analysis, to identify determinant factors of fertility outcome.
Main results and the role of chance: 359 consecutive OSIS patients underwent 720 IVF-ICSI cycles. In the overall population the clinical pregnancy rate per cycle and per embryo transfer was 25.2% and 36.4% respectively. 158 (44%) patients became pregnant. Using multivariable analysis, intestinal DIE (OR = 0.31, 95% CI (0.2-0.6), $p < 0.001$), past surgery for OSIS (OR = 0.29, 95% CI (0.1-0.6), $p = 0.001$) or endometrioma (OR = 0.34, 95% CI (0.2-0.7), $p = 0.002$) were independent factors associated with lower pregnancy rates. AMH levels < 2 ng/mL and antral follicle count (AFC) < 10 were also associated with negative ART outcomes (OR = 0.49, 95% CI (0.3-0.9), $p = 0.014$ and OR = 0.41, 95% CI (0.2-0.7), $p = 0.002$ respectively).
Limitations, reason for caution: The patients in whom the diagnosis of OSIS was based on imaging rather than surgery are possibly not as accurately phenotyped as those who had surgery. This limitation is however one that affects most studies on OSIS and probably, not entirely avoidable.
Wider implications of the findings: Being a referral center for OSIS management, our OSIS population is likely to be uncommonly enriched in severe cases. A more common OSIS population is therefore likely to have even higher cPR than those reported here (44%). Our results are in line with previous studies showing that intestinal DIE is a major determinant of fertility outcomes. We also brought a new insight, concerning the negative impact of previous surgeries for OSIS on ART results.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Groupe Hospitalier Universitaire (GHU) Ouest, Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, 75679 Paris, France.
Trial registration number: NA.
Keywords: IVF, pregnancy rate, deep infiltrating endometriosis, surgery

O-125 MRI features correlate significantly with histological level of infiltration in the bowel wall in colorectal endometriosis

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Study question: Is there correlation between MRI features and histological infiltration in the bowel wall in deep colorectal endometriosis (DCE)?
Summary answer: Depth and length of the infiltration of a deep endometriosis lesion in the bowel wall based on measurements in MR images show a significant correlation and are positive predictors for the level of histological infiltration in the bowel wall in patients with DCE.
What is known already: Previous research demonstrated a relatively high sensitivity and specificity (87% and 93%, respectively) for MR imaging to diagnose the presence of endometriosis in the rectal wall and a high odds ratio (OR 39.74). However, studies that tried to define which layer of the bowel wall is infiltrated by MR imaging did not reach a definitive answer. Up to now, histopathology remains the gold standard for diagnosing deep colorectal endometriosis and level of infiltration.
Study design, size, duration: A retrospective, mono-centre study in a tertiary referral endometriosis centre between January 2001 and January 2014. Ninety-six patients with DCE, diagnosed by histopathology analysis after bowel surgery (low anterior resection or discoid resection), were enrolled in this study. Preoperative MRI was performed in all patients.
Participants/materials, setting, methods: MRIs were reassessed on level of bowel infiltration by two radiologists. They were aware that in all patients bowel resection was performed with positive histology for endometriosis. One pathologist, blinded to the MRI results, assessed infiltration of the bowel wall. MRI findings were compared with histopathology results.
Main results and the role of chance: Infiltration was scored as superficial (serosa/muscle layer) or deep (submucosa/mucosa). Lesion thickness, depth and length of lesion infiltration in the bowel wall observed by MRI correlated significantly with histological level of infiltration. Lesion thickness was significantly lower in superficial (2.54 cm) than in deep (2.92 cm)($p = 0.042$) OR 1.63 histological infiltration (reader 1). The same applied for depth of infiltration 0.74 vs. 0.96 cm ($p = 0.033$), OR 2.59 (reader 1) and 1.05 vs. 1.29 cm ($p = 0.044$), OR 2.16 (reader 2) and length 1.73 vs. 2.21 cm ($p = 0.028$), OR 1.57 (reader 1) and 2.24 vs. 2.89 cm ($p = 0.024$), OR 1.42 (reader 2). Presence of hyperintense spots, contact with bowel wall, traction of rectum, involvement of ureter, prestenotic bowel dilatation, and aspect of the infiltration did not show significant correlation.
Limitations, reason for caution: A possible limitation of the study is the long period of inclusion of patients in which quality of MRI scans conceivably have been changed and/or improved.
Wider implications of the findings: This study shows that there is a significant correlation between MRI features and the level of infiltration in the bowel wall of DCE in a large series of patients. To avoid unnecessary bowel resections the knowledge if the deep colorectal endometriosis lesion is infiltrating superficially (serosa/ muscle layer) or deep (submucosa/mucosa) could be helpful in deciding whether or not to perform bowel resection.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding.
Trial registration number: NA.
Keywords: MRI, histopathology, endometriosis, colorectal, infiltration

O-126 Pain and quality of life assessment in ART patients suffering from endometriosis (OSIS): prospective controlled study

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Study question: To evaluate the impact of ART on pain and quality of life (QOL) in women suffering from OSIS, as compared to disease-free controls.
Summary answer: During ART pain and quality of life scores are not different in OSIS and control women. No differences where observed according to the severity of endometriosis.
What is known already: The risk that ART might induce a flare of OSIS-related symptoms is not well known. Moreover the quality of life of infertile endometriosis patients undergoing ART has not been evaluated yet. This risk – theoretical but non-assessed – may dissuade gynecologists from offering ART to endometriosis patients, having them rather undergo invasive surgery.
Study design, size, duration: Prospective controlled observational cohort study conducted between January 2014 and June 2014 in a tertiary care university hospital. Diagnosis of OSIS was histologically proven in women who had past surgery (41/102; 40.2%), or based on published imaging criteria using transvaginal sonography and magnetic resonance imaging.
Participants/materials, setting, methods: 132 endometriosis patients were matched to 132 disease free women undertaking IVF immediately after each case. In all study participants, four points clinical evaluation were performed: Baseline, During synchronization, During stimulation and Post retrieval. Pain scores were evaluated using visual analogue scale. QOL was assessed using FertiQol International evaluation.
Main results and the role of chance: After excluding cancelled cycles (20 and 16, respectively) and patients lost to follow up (10 and 12 respectively), 102 OSIS and 104 controls women were retained for the study. Endometriosis women were phenotyped according to the surgical classification of endometriosis in superficial endometriosis (8; 7.8%), endometrioma (19; 18.6%) and deep endometriosis (75; 73.5%). Difference in Visual analogue scale (VAS) score between post retrieval and baseline evaluation in endometriosis reveals no significant increase of dysmenorrhea, dyspareunia, non cyclic chronic pain, gastro-intestinal symptoms during IVF compared to control group. Evaluations of quality of life with FertiQol international in endometriosis group are comparable to control group for core FertiQol (58.6 ± 15.2 vs 63.1 ± 17.1, respectively; $p = 0.131$) and treatment FertiQol (24.7 ± 6.8 vs 24.1 ± 7.0, respectively; $p = 0.670$) evaluation.
Limitations, reason for caution: Women in whom the diagnosis of endometriosis was based on imaging are possibly not as accurately phenotyped as those who had surgery. We cannot rule out that women who dropped out were those who experienced some deleterious effects nevertheless the small number of lost of follow up moderate such bias.
Wider implications of the findings: Quelling all the prevailing fears about ART in OSIS patients, our results strikingly showed no worsening of pain during ART in OSIS women, as compared to controls. During ART, pain scores followed similar pattern in OSIS and control patients. These data support the views that ART is a safe option when surgery can alter ovarian function or expose to undue surgical risks in case of extended endometriosis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Cochin institute, Gynecology and Reproductive Médicine department, Université Paris Descartes, Assistance Publique—Hôpitaux de Paris (AP-HP).
Trial registration number: NA.
Keywords: endometriosis, IVF, pelvic pain, FertiQol

SELECTED ORAL COMMUNICATIONS

SESSION 35: QUALITY AND SAFETY FOR EMBRYOS, MOTHERS AND NEONATES

Tuesday 16 June 2015 **10:00–11:30**

O-127 Obstetric and neonatal outcomes from trophoblast biopsy combined with frozen embryo transfer versus cleavage biopsy with fresh embryo transfer in PGD/PGS treatment

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Study question: To investigate whether blastocyst biopsy combined with frozen embryo transfer increase the risk of pregnancy complications and adverse neonatal outcomes compared to cleavage biopsy with fresh embryo transfer.
Summary answer: The patients after blastocyst biopsy combined with frozen embryo transfer had a better neonatal outcome but associated with increased risk of placenta-related disease, especially for pregnancy induced hypertension (PIH), when compared to cleavage biopsy with fresh embryo transfer.
What is known already: The blastocyst biopsy and vitrification strategy is becoming more popular in PGD/PGS because of potential better pregnancy outcome when compared to blastomere biopsy and fresh embryo transfer strategy. However, the obstetric and neonatal outcomes of the new strategy is still to be determined.
Study design, size, duration: We undertook a retrospective study including all children born after PGD/PGS (n = 288) with cleavage biopsy with fresh embryo transfer (PGD#1) from March 2008 to November 2012 or blastocyst biopsy combined with frozen embryo transfer (PGD#2) from January 2012 to August 2013 in Citic Xiangya Reproductive and Genetic Hospital.
Participants/materials, setting, methods: Only pregnancies with gestation > 28 weeks were included in our study. Data was collected by telephone follow-up. Obstetric and neonatal outcomes were compared in PGD#1 and PGD#2 by student-t test, chi-square test and logistic regression.
Main results and the role of chance: PGD#1 (96 singletons and 33 twins pairs)and PGD#2 (124 singletons and 35 twins pairs) were included in our study. In PGD#2, the incidence of placenta-related diseases, including PIH, placenta previa and postpartum hemorrhage, were higher than in PGD#1. But limited to the sample size, only PIH have significant difference (2.6% vs 9.0%, P = 0.032). When compared to PGD#1, PGD#2 had lower incidence of VLBW (10.6% vs 0, P = 0.025), and better gestational age [median (rang)35.57 (30.60, 38.40) vs 36.71 (31.10, 39.30) (P = 0.001)] and birthweight [median (rang)2500.00 g (1225.00 g, 3750.00 g) vs 2700.00 g (1550.00 g, 3600.00 g) (P = 0.002)] in twin pregnancies. No differences were found at other neonatal outcomes such as birth defects, neonatal hospitalization, SGA, LGA both in singletons and multiplets.
Limitations, reason for caution: Our study is limited to the retrospective design and small sample size. The measure outcomes from questionnaires by telephone not medical records were the underlying risks.
Wider implications of the findings: The higher incidence of placenta-related diseases and better outcomes of gestational age and brithweight in PGD#2 revealed that the trophoblast biopsy and vitrification of embryos may have adverse influence on placenta function. Our findings can provide advice to the mother after trophoblast biopsy and vitrification during the perinatal, which should concerned about the occurrence of placenta related diseases.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by grants from the Major State Basic Research Development Program of China (973 Program; no. 2012CB944902).
Trial registration number: NA.
Keywords: obstetric outcomes, neonatal outcomes, cleavage biopsy, blastocyst biopsy, frozen embryo

O-128 Female age, number of mature eggs and biopsied blastocysts effectively define the chance for obtaining one euploid embryo: counselling and decision-making during PGS cycles

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Study question: Is it possible to effectively predict the chance of obtaining at least one euploid embryo during blastocyst stage preimplantation genetic screening cycles based on basal and cycle parameters?
Summary answer: Female age, number of mature oocytes and biopsied blastocysts effectively predict the chance of obtaining at least one euploid embryo per started stimulation cycle, allowing for an appropriate patient counselling and optimization of oocyte/embryo banking programmes for IVF centers performing PGS in outsource.
What is known already: Most of the IVF clinics using PGS in outsource perform oocyte/embryo banking in order to optimize genetic testing costs. Main drawback of this approach is the risk of performing supplementary ovarian stimulation cycles when not required. We aimed at deliver useful data to provide a better management of PGS cycles based on decisional tree models assessing the probability of each patient of obtaining at least one euploid blastocysts (EB) based on basal and cycle parameters.
Study design, size, duration: This is an observational longitudinal cohort study performed from October-2011 and December-2014 including 734 consecutive blastocyst stage PGS cycles. Primary outcome was the obtainment of at least one euploid embryos following trophectoderm biopsy and 24-chromosome screening. We included only cycles where all oocytes were injected and resulting blastocysts analysed.
Participants/materials, setting, methods: The effect of covariates (female age, FSH, AMH, duration of infertility, previous IVF failures, previous miscarriages, infertility factor, sperm quality, stimulation protocol, MII retrieved, biopsied blastocysts) on EB achievement was assessed using forward logistic regression analysis. Recursive partitioning analysis was used to generate decisional trees.
Main results and the role of chance: From 734 egg retrieval cycles, 4992 MII were injected resulting in 1640 biopsied blastocysts. Cycles with no blastocysts were 173 (23.6%,95%CI = 20.5-26.8). 1640 blastocysts were biopsied and 656 were euploid (40.0%,95%CI = 37.6-42.4). 249 were already transferred resulting in 134 ongoing implantations (52%,95%CI = 46.2-58.9). Only female age and number of biopsied blastocysts were independently associated with the probability of having EB (OR = 0.68,95%CI 0.60-0.76 and OR = 1.83 95%CI 1.56-2.15, respectively, $p < 0.01$). A classification trees to predict EB obtainment according to significant covariates showed that female patients aged between 35-39 have a EB in 43%, 80% and 99% with 1,1-5 and > 5 blastocysts, respectively. Females aged 40-43 have a EB in 25%,58% and 78% when 1,2-3 and > 3 blastocysts were biopsied, respectively. Older ones had an overall chance of EB of 23%.
Limitations, reason for caution: These data belong from a single centre using an effective culture system and an extensively validated aneuploidy screening technology. The lack of a prospective replication cohort to validate the prediction model developed should also be acknowledged.
Wider implications of the findings: The developed decisional tree can help practitioners to decide if additional egg retrieval cycles are necessary to increase significantly the chance of euploid blastocysts obtainment and improve the cost-effectiveness of IVF/PGS cycles. These data can thus potentially improve the management of PGS cycle and the patient's counseling
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: PGS, aneuploidy screening, blastocyst chromosome screening, euploid embryo

O-129 Neonatal outcomes after the implantation of human embryos vitrified using a closed-system device

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Study question: Dose closed vitrification system (CVS) which is expected to minimize the risk of contamination poses a risk of adversely affecting embryo development?
Summary answer: There were no significant differences between the use of closed and open vitrification systems in embryo development after implantation, gestational age, birth weight, sex ratio, Apgar score, and congenital anomalies of newborns. Human embryos can be vitrified using a CVS without impairment of neonatal development.
What is known already: The freezing procedure known as an open vitrification system (OVS) maximized cooling and warming rates be by direct contact to liquid nitrogen and improved the embryo viability. However, there are some potential drawbacks of the OVS, such as the risk of cross-contamination during cooling and storage. To avoid the possible risk of contamination, several CVSs have been developed. However, new concerns such as a decrease in the cooling rate have emerged.

Study design, size, duration: Developmental competence of blastocysts vitrified by CVS after implantation, including gestational age, birth weight, sex, Apgar score, and anomalies of newborn was compared with that obtained in the case of OVS.
Participants/materials, setting, methods: The data pertaining to a total of 875 vitrified-warmed blastocysts that were single-transferred under hormone-replacement cycles between November 2011 and December 2013 were randomly divided into two groups according to the day of blastocyst vitrification (CVS, *n* = 313; OVS, *n* 562) after receiving informed consent.
Main results and the role of chance: One hundreds thirteen of 313 patients in CVS and 206 of 562 patients in OVS had deliveried. There were no significant differences between the use of CVS and OVS in embryo development after implantation, gestational age, (closed: 275.6 days, open: 274.1 days), birth weight (closed: 3127.9 g, open: 3056.8 g), proportion of Caesarian sections (closed: 36.5%, open: 40.5%), sex ratio (proportion of male babies (closed: 43.3%, open: 48.4%), Apgar score (closed: 9.3, open: 9.3), and congenital anomalies (closed: 2.9%, open: 0.5%) of newborns.
Limitations, reason for caution: It need further study will be required to assess the subsequent growth and development of the children.
Wider implications of the findings: Our study offers some insights into the safety of CVS. There were no significant differences in the developmental characteristics after implantation or in the neonatal status between the closed and open vitrification groups.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVFNamba Clinic.
Trial registration number: This study that was approved by the ethics committee of the IVFNamba Clinic (No.2012-5).
Keywords: closed vitrification system, human blastocyst

O-130 Morbidity and mortality among very and extreme preterm singletons born following assisted conception in Australia: a population study

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Study question: Do very preterm (gestational age 28 to < 32 weeks) and extreme preterm (gestational age < 28 weeks) singleton babies born to women after assisted conception have higher rates of adverse perinatal outcomes compared to spontaneously conceived very preterm and extreme preterm singleton babies.
Summary answer: This national study confirms that selected adverse perinatal outcomes including small for gestational age (SGA) and some birth defects as well as specific neonatal complications are higher among very preterm and extreme preterm singletons born following assisted conception compared to spontaneously conceived very and extreme preterm singletons.
What is known already: Preterm birth (gestational age < 37 weeks) is a global public health problem, accounting for more than one-third of neonatal deaths. Morbidity and mortality is even higher in the very preterm and extreme preterm babies. Internationally, assisted conception including assisted reproductive technology (ART) treatment (in vitro fertilization and gamete intra-fallopian transfer), artificial insemination (AI) and hyper-ovulation has been associated with increased risk of preterm birth.
Study design, size, duration: A population-based retrospective study using data from the Australian and New Zealand Neonatal Network (ANZNN) for the period 2001-2010. The network includes all neonatal intensive care units (NICUs) in Australia and New Zealand. The ANZNN data collection includes all babies born at < 32 weeks' gestation and admitted to a NICU.
Participants/materials, setting, methods: 24,069 live born singleton babies admitted to NICUs in Australia and New Zealand during 2001 to 2010. Mode of conception included spontaneous conception, hyper-ovulation, ART and AI. Outcomes included both mortality and morbidity measures, the latter categorized into birth conditions and NICU conditions. Descriptive and multivariate analyses were conducted.
Main results and the role of chance: Of the 24,069 singletons < 32weeks' gestation admitted to NICUs, 21,753 (90.4%) had information on mode of conception. Of those, 94.4% (20,530) were spontaneously conceived, 4.4% (*n* = 953) were born after ART, 1% (*n* = 216) after hyper-ovulation and 0.2% (*n* = 54) following AI. ART mothers were significantly older (mean age 34.6 versus 28.9 years), non-Aboriginal (1.0% versus 6.2%) and had a history of pregnancy-induced hypertension (20.9% versus 17.6%) ($p < 0.05$). Compared to spontaneously conceived singletons, ART singletons had significantly higher odds of birth defects (adjusted odds ratio (AOR) 1.71, 95% confidence interval (CI) 1.36-2.16), necrotizing enterocolitis (AOR 1.43, 95% CI 1.04-1.97) and major surgery (AOR 1.26, 95% CI 1.39-2.12). SGA was significantly associated hyper-ovulation (AOR 1.52, 95% CI 1.021-2.67) and AI (AOR 3.02, 95% CI 1.53-5.81) but not ART.
Limitations, reason for caution: Detailed information on ART treatment including number of embryos transferred, fertilization procedures, fresh versus thaw transfer and blastocyst versus cleavage stage embryo was not available in the ANZNN data. There was no information available on subfertile women who conceived without use of assisted conception.
Wider implications of the findings: A higher likelihood of very and extreme preterm birth remains for women giving birth after assisted conception. The differences in perinatal outcomes for singleton preterm births following different fertility treatments has implications for counselling of couples planning to access fertility treatment regarding their choice and type of treatment. More research is needed to determine whether the increased risk of adverse perinatal outcomes is due to the assisted conception itself or underlying subfertility.
Study funding/competing interest(s): Funding by University(ies) – Internal resources University of New South Wales and University of Technology Sydney.
Trial registration number: NA.
Keywords: preterm birth, very preterm, extreme preterm, assisted conception, ART

O-131 Improving the implementation of tailored expectant management in couples with unexplained infertility: results from a cluster randomized controlled trial (NTR3405)

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Study question: What is the effectiveness of a multifaceted implementation strategy compared to usual care to improve professional adherence to guideline recommendations on tailored expectant management (TEM) in couples with unexplained infertility and a good prognosis of natural conception?
Summary answer: A cluster RCT (c-RCT) showed that the multifaceted implementation strategy improved guideline adherence with eight percent compared to usual care. This is mainly the result of an increased number of professionals calculating the couple's prognosis of spontaneous conception and advising a correct TEM of at least six to twelve months.

What is known already: In couples with unexplained infertility and a good chance of natural conception (> 30%), TEM of six to twelve months is equally effective as starting medically assisted reproduction (MAR) early. TEM is therefore recommended in national fertility guidelines. However, implementation of TEM is not optimal, as MAR often starts too early, leaving room for improvement. Based on an assessment of barriers and facilitators for TEM among professionals and infertile couples, we have developed a multifaceted implementation strategy.
Study design, size, duration: We performed a c-RCT in 25 Dutch clinics, embedded in the Dutch consortium for studies in women's health. Clinics were randomized for the intervention (implementation strategy for 12 months, N = 13) or usual care (N = 12). The strategy included audit and feedback, a local protocol, an E-learning communication module and a website.
Participants/materials, setting, methods: We studied couples with unexplained infertility and > 30% chance of natural conception within 12 months. The primary outcome was adherence to TEM. We also assessed three quality indicators associated with adherence to TEM. Data collection was obtained from medical records. We performed baseline and effect measurements in all clinics.
Main results and the role of chance: At the baseline measurement we included 544 couples. In the after measurement there were 247 couples exposed to the intervention and 238 couples exposed to usual care. In the intervention group (I) adherence increased from 49% to 69%; in the control group (C) from 49% to 61%. (unadjusted OR 1.5; 95% CI 1.03-2.20). Two quality indicators supported this effect: 1. Calculation of the prognosis of spontaneous conception (I:59% to 85%, C:75% to 81%). 2. Advising a correct TEM of at least six months (I:58% to 77%, C:58% to 68%). The third quality indicator, adhering to a correct advised expectant period of at least six months, showed no difference between the intervention and control group (I:85% to 91%, C:85% to 90%).
Limitations, reason for caution: There is possible selection bias since our included couples had a higher socio-economic status than non-responders. How this might affect adherence is unclear. Furthermore, the adherence to TEM has also been influenced by the government, which tried to make TEM obligatory simultaneously to the start of the intervention.
Wider implications of the findings: An implementation strategy, based on barriers and facilitators and targeting multiple levels (patients, professional and organization), improves guideline adherence to TEM. The increased adherence leads to less couples being exposed to unnecessary fertility treatment and the associated risks, complications and burdens. To further underline the importance of improved adherence to TEM it will be necessary to investigate the effect of the strategy on other outcome measures such as experiences with care and cost-effectiveness. In order to assess which of the strategy facets had the most effect, a process evaluation will have to take place. When these facets are identified, other clinics in the Netherlands as well as other European countries that make recommendations on TEM, can use these tools to improve implementation of TEM.
Study funding/competing interest(s): Funding by national/international organization(s) – Netherlands Organisation for Health Research and Development (ZonMW).
Trial registration number: www.trialregister.nl. NTR3405.
Keywords: unexplained infertility, expectant management, prognostic models, guideline adherence, implementation strategy

O-132 Long-term risk of endometrial cancer after ovarian stimulation for in-vitro fertilization

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Study question: Since the 1980s, in-vitro fertilization (IVF) is widely used. However, late adverse health effects are still largely unknown. In view of the association of endogenous estrogen levels with endometrial cancer risk, the purpose of this study was to examine the long-term risk of endometrial cancer after ovarian stimulation for IVF.
Summary answer: After a median follow-up duration of 21.9 years after treatment, the risk of endometrial cancer was not significantly increased after ovarian stimulation for IVF treatment.
What is known already: Both endogenous and exogenous estrogens play a key role in the development of endometrial cancer. Because of their influence on endogenous estrogen levels, nulliparity, late menopause, late age at first birth, obesity, and lack of physical activity are well-established factors for endometrial cancer. Some causes of subfertility have been associated with increased risk of endometrial cancer, but results were inconsistent. Published studies have not yet examined the long-term effects of endometrial cancer after ovarian stimulation.
Study design, size, duration: This is a nationwide historical cohort study, the OMEGA study, comprising 19,158 women who started IVF and a non-IVF group of 5,950 subfertile women who underwent other fertility treatments in one of the 12 IVF centres in the Netherlands between 1980 and 1995.
Participants/materials, setting, methods: Information was collected on subfertility cause and treatment (medical records), reproductive and life-style factors (questionnaires). The Netherlands Cancer Registry provided endometrial cancer incidence (1989-2013). We calculated standardized incidence ratios (SIRs) for comparison with the general population. Multivariable Cox regression analysis was used to quantify treatment effects on endometrial cancer risk.
Main results and the role of chance: After a median follow-up duration of 21.9 years, 63 endometrial cancers were observed (SIR, 1.08; 95% confidence interval [CI], 0.83-1.38). Endometrial cancer risk was comparable after IVF (SIR, 1.16; 95% CI, 0.85-1.54) and after other fertility treatments (SIR, 0.90; 95% CI, 0.52-1.47). The SIR for nulliparous women was 1.83 (95% CI, 1.31-2.50), whereas the SIR for parous women was 0.62 (95% CI, 0.22-1.23). When comparing the IVF group with the non-IVF group, the risk after IVF was not increased (HR, 0.98; 95% CI, 0.53-1.80), adjusted for hormonal causes of subfertility and number of births. The risk did not significantly differ for 1-3, or 4 or more IVF-cycles compared with no IVF, and for different causes of subfertility, adjusted for number of births.
Limitations, reason for caution: The SIRs must be interpreted with caution, because of differences in parity and subfertility. Therefore, the direct comparison of the IVF group with the non-IVF group, while adjusting for important confounders, such as parity and subfertility cause, is very important.
Wider implications of the findings: We concluded that ovarian stimulation for IVF does not increase the risk of endometrial cancer after long follow-up. This is a reassuring finding for women who have undergone IVF treatments in the past. This reassuring result can also be used by women and their physicians in making a decision on starting or continuing with IVF treatments.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s) – This research is supported by the Dutch Cancer Society, grant nr NKI 2006-3631 and a Departmental grant from the Department of Obstetrics and Gynecology of Erasmus University Medical Center.
Trial registration number: NA.
Keywords: IVF, long-term adverse effects, endometrial cancer, cohort study, epidemiology

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INVITED SESSION

SESSION 36: EUROPEAN AND GLOBAL ART MONITORING SESSION

Tuesday 16 June 2015 **11:45–12:45**

O-133 Assisted reproductive technology (ART) in Europe 2012. Preliminary results generated from European registers by ESHRE

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Introduction: This is the sixteenth report of the European IVF-monitoring (EIM), the ESHRE register on assisted reproductive techniques (ART) organization. This report deals with the results of treatments initiated during 2012.
Methods: Data were collected from existing national registries in 33 countries (data sent in at the time of abstract deadline) and directly entered by each national coordinator into the EIM database through software developed by ESHRE. Data were analysed at ESHRE headquarters.
Results: In total, 1081 IVF clinics participated (81.2% of registered clinics in the participating countries. Next to these also 1083 IUI units reported their data. The IVF clinics reported 624 874 treatment cycles: IVF (144 914), ICSI (307 762), frozen embryo replacement (FER, 127 365), egg donation (ED, 30 437), preimplantation genetic diagnosis/screening (PGD/PGS, 8 426), in-vitro maturation (IVM, 421) and frozen oocytes replacements (FOR, 5 549). This preliminary data set shows that the number of cycles compared to 2011 increases with 2.4%. However since a number of countries still need to send in their data, the total number of ART cycles will increase even more. Data on intrauterine insemination using husband/partner's (IUI-H) and donor (IUI-D) semen were reported from 26 countries (data sent in at the time of abstract deadline). A total of 175 499 IUI-H and 43 498 IUI-D cycles were included. When interpreting the results, it is important to note that delivery rates may be underestimated due to lack of follow-up and incomplete reporting. For IVF, the clinical pregnancy rates (PR) per aspiration and per transfer were 29.4% and 33.8%, respectively and comparable to 2011. For IVF the delivery rate (DR) per aspiration was at 23.0%. For ICSI, the corresponding rates were 25.4 %, 29.5%, and 19.7%. For frozen embryos replacements, PR was 23.4% per thawing and 25.3% per transfer. The corresponding delivery rates were 16.2% and 17.5%. In oocyte donation cycles, PR and DR were 48.4% and 31.3% per transfer, respectively in the fresh cycles and 36.0% and 21.9% in the frozen cycles. For the ED cycles with FOR, the numbers are as follows: PR was 45.1% and DR was 26.2% per transfer. For PGD/PGS, in the fresh cycles the PR was 37.8% per transfer. In the frozen cycles this was 38.0%. For in vitro maturation, PR and DR were 25.5% and 22.1% per transfer respectively. Finally, 4645 replacements after oocyte freezing were reported, mainly from Italy and Spain. They resulted in 37.4% PR and 20.5% DR per thawing, respectively. Following IUI-H the pregnancy rate and delivery rate was 11.9% and 8.0%, while it was 16.6% and 11.9% respectively in IUI-D. The transfer of 1, 2, 3 and 4 or more embryos following IVF or ICSI occurred in 29.9%, 55.4%, 13.5% and 1.2% of cycles respectively. There were significant national differences in practice. The proportions of singleton, twin and triplet deliveries after IVF and ICSI showed only marginal differences compared to those in 2011, at 82.1%, 17.2%, and 0.7% respectively. The proportion of very preterm deliveries (≤ 33 weeks) increased from 9.0% for singleton pregnancies to 20.4% for twin pregnancies and 38.1% for triplet pregnancies, justifying a transfer policy aimed at decreasing the risk of multiples. IUI-H in women below 40 years of age resulted in 8.1% twin and 0.5% triplet pregnancies, thus not higher than in IVF-ICSI.
Conclusions: In comparison with previous years, 2012 will show an increase in the number of reported ART cycles in Europe. The numbers of embryos transferred remain relatively stable, as does the delivery rate. No significant change in the multiple birth rate was observed in these preliminary data.
Keywords: European IVF monitoring, national registries

O-134 ICMART world report 2011

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Study question: An update of glossary on assisted reproductive technology (ART) became necessary because of evolution of techniques and concepts since the previous version (2009) and integration into WHO guideline processes.
Summary answer: The new version clarifies some important points. It was enlarged to new categories in order to cover all aspects of ART and related fields. The number of items increased from 87 to 247.
What is known already: ART practice is expanding worldwide, and is subject to large variations in availability, practice and results across the World. Its public health importance has lead to the implementation of many registries at national and international levels, and it is the topic of many publications. Thus, the need for standard definitions is critical to allow for evaluation of studies, centers, countries, and regions results. The International Committee for Monitoring Assisted Reproductive Technologies (ICMART), an entity responsible for the collection and dissemination of worldwide data on ART, worked with WHO to publish the first glossary on ART terminology in 2006 and a revision in 2009. Such revisions are regularly needed because of the evolution of techniques and concepts.
Study design, size, duration: The revision was organized with expert panels, literature review, and stakeholder consensus meetings. The total process will last 12-15 months.
Participants/materials, setting, methods: The process was organized in 4 steps, coordinated by ICMART (F. Zegers-Hochschild) and WHO (S. Vanderpoel). First, 4 working groups of 5-8 specialists were organized (September 2014), each reviewed a subset of definitions: clinical; laboratory (subsequently divided into Andrology and Embryology); outcomes; epidemiology and public health. New definitions were also proposed for items not already covered. The second step was a consensus meeting, at WHO headquarters, with 30 attendees including the 4 groups' coordinators and representatives of the main international societies. All proposed definitions were discussed at length and a first draft was produced. In a third step, comments from WHO regional representatives, some country offices, and other external agencies were compiled. This included discussions with the external expert technical advisor group facilitated by WHO, which is compiling the 11th ICD (international classification of disease) revision. Finally, the 4th step will be a debate process during the consensus discussions in September 2015 with the integration of the glossary and official WHO guidelines for infertility.
Main results and the role of chance: The ICMART/WHO Glossary has been largely expanded, from 87 to 247 definitions. Most previous definitions have been retained but some modified, clarified or expanded. Many new definitions have been added, particularly in infertility and epidemiology/public health. All current aspects of ART are clearly defined. The role of chance is minimal, given the large coverage of specialists in infertility and other stakeholders, and the use of evidence based medicine wherever it was possible.
Limitations, reasons for caution: The glossary definitions are based on a consensus process by many stakeholders, but it was not possible to achieve universal agreement. Also, as done in the past, the glossary will be translated into the recognized UN languages.
Wider implications of the findings: It is generally accepted that measurement of human activities improves quality and outcomes. However, this is only possible if all stakeholders speak a common language. The ICMART/WHO glossary intends to provide a common terminology platform. This will allow for better analysis of international comparisons and, subsequently, a potential improvement of quality and safety. The collaboration with WHO is important to allow for an unbiased development and a broader international use of the glossary.
Study funding: World Health Organization

INVITED SESSION

SESSION 37: HUMAN DEVELOPMENT AND EVOLUTION

Tuesday 16 June 2015 **11:45–12:45**

O-135 Critical periods of human development: ART effects?

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The Developmental Origins of Health and Disease (DOHaD) hypothesis states that an adverse environment of the conceptus during early life can affect

development by inducing adaptive changes in physiology and metabolism leading to increased disease risk in adulthood. Evidence for this hypothesis can be found in the human as well as in several animal species. An increasing body of evidence from animal studies suggests that the periconception period is a very vulnerable period. Poor nutrition and sickness of the mother during this period, maternal obesity as well as in vitro culture of embryos have all been shown in animal models to increase the risk of developing chronic non-communicable diseases in adulthood. This raises concern for human IVF offspring. Although it must be stressed that the great majority of children resulting from assisted reproductive technologies (ART) treatment is healthy, adverse perinatal outcome and cardio-metabolic parameters in IVF children are reported. Patient-related factors such as type and duration of subfertility and lifestyle of the parents have been shown to be possible causal factors. However, evidence is accumulating that, similar to the animal models, also certain aspects of the ART technique itself, such as the type of culture medium used, may have an effect on the phenotype of IVF children. Whether or not these IVF technique induced effects are an indication for health-related consequences in later life is as yet unknown. In view of the fact that in the Western world, an estimated 1-3% of children born annually are conceived by IVF, more research is highly needed to investigate the effects and long-term safety of the IVF technique and culture medium on health of these children.

Keywords: birthweight, culture medium

O-136 Correcting deficient developmental trajectories: time to act?

Peter Gluckman
University of Auckland, Auckland, New Zealand

SELECTED ORAL COMMUNICATIONS

SESSION 38: MANAGING PATIENT EXPECTATIONS

Tuesday 16 June 2015 **11:45–12:45**

O-137 Infertile men's expectations and needs in relation to fertility treatment before and after their first ICSI-treatment

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Study question: What are the infertile men's expectations to and needs in relation to fertility treatment?
Summary answer: Men with low sperm quality want to be involved on equal terms as their partner in the fertility treatment. The participants wanted professionalism, empathy, and face to face information about their sperm quality, treatment process, treatment results, and the future plan.
What is known already: Infertility concerns both partners in the couple. Medical fertility treatment is mainly directed towards the women and accordingly information and support are frequently focused on the women. Male infertility is a potential severe stressor and many men with low sperm quality would like more widely to talk about it. The infertile men want a dialogue concerning fertility treatment, male infertility, the role of the male partner, and information about the psychological consequences of male infertility.
Study design, size, duration: Longitudinal, semi-structured qualitative interview study with ten men with low sperm quality undergoing fertility treatment. Participants were interviewed before and after their first ICSI-treatment attempt. The participants were selected by purposeful sampling with maximum variation. The data collection takes place between November 2014 and May 2015.
Participants/materials, setting, methods: The participants were assigned to fertility treatment at the Fertility Clinic, Hvidovre Hospital, Copenhagen, Denmark. A total of 15 men were contacted, where five did not want to participate. The interviews were audiotaped and transcribed in full. Data were analysed using qualitative content analysis following Graneheim and Lundman (2004).
Main results and the role of chance: The interviews before the participants' ICSI treatment showed that the men wanted an overall plan and an overview of the process on the time of the referral. They wished that the information about their diagnostic sperm sample was given face to face with an opportunity to ask questions. The men expected involvement in the treatment process. They desired kindness, understanding, professionalism, and empathy from the fertility clinic staff. The participants wanted detailed information about the treatment plan, the results in the process, and information about how the treatment progress. Results from follow-up interviews will be added before the congress.
Limitations, reason for caution: We do not have information about men not wanting to participate in the study. The study included only infertile men seeking ICSI-treatment and hence caution should be taken to take transfer the results to infertile men in other fertility treatment as e.g., the use of donor semen.
Wider implications of the findings: These results are in agreement with other studies concerning that the men wants the clinical staff's support and a dialog concerning male infertility. Written information, which will be given to the men together with their results from their sperm test, could be a solution to the problem of information early in the process. We also suggest that the clinics work towards more structure on information on male diagnosis and future treatment plan.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hvidovre Hospital, Copenhagen, Denmark.
Trial registration number: NA.
Keywords: male infertility, qualitative study, assisted reproductive technology, information, patient-centred care

O-138 Using the same sperm donor for siblings: what it means to parents

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Study question: How do (prospective) parents perceive and experience the importance of a genetic link between siblings born after fertility treatment with donor sperm?
Summary answer: The use of the same sperm donor for subsequent conceptions appeared quasi univocal and was accompanied by uncertainty about the same donor being available over time. Feelings of luck or relief were mentioned by parents whose children were genetic siblings.
What is known already: Several authors have noted an interesting paradox: while gamete donation allows for detachment of social parenthood from biological relatedness, it is also becoming the area in which biological notions of kinship are reaffirmed. There is an increasing demand for the right to know one's gamete donor; donor offspring are also seeking genetic half siblings through online registries. It remains fairly unchartered territory how parents negotiate the importance of a genetic link between their donor-conceived children.
Study design, size, duration: In this study, we included 35 lesbian and heterosexual couples. The in-depth semi-structured couple interviews were performed between October 2012 and October 2013. Data were analysed through step-by-step inductive thematic analysis. A continuous auditing process by the co-authors resulted in themes that were grounded in the data.
Participants/materials, setting, methods: The participants were recruited at the Department of Reproductive Medicine of a University Hospital. Twenty couples had a child conceived after a fertility treatment with anonymous donor sperm and 15 were in treatment at the time of data collection. The study was approved by the appropriate Ethics Committee.
Main results and the role of chance: We distinguished between families with siblings from the same donor, siblings from a different donor, and siblings with a different biological mother (in lesbian couples). Overall, the couples showed a clear preference to use the same sperm donor for their children. In describing the reasons for this preference, common assumptions were that the genetic link between the children generated better sibling relations, and that (visible) resemblances between the siblings would facilitate social acknowledgement

of their family construct. Uncertainty about the availability of the same donor over time seeped through in their stories. For some lesbian couples who decided that both partners should carry a child, the genetic link between mother and child was perceived as more important than a full genetic link between the siblings.
Limitations, reason for caution: This qualitative study aimed at a better understanding of the participants' experiences of and importance attached to the genetic link between their offspring and does not intend to produce generalizable results. Only recipients of anonymous donor sperm were included.
Wider implications of the findings: These results suggest that, even when the non-biological parent is acknowledged in his role within the family, there is a tendency to favor full genetic bonds where possible. Full siblings are considered 'real', 'unambiguous' kin connections. The findings also have possible implications for the clinic: the opportunity to use the same donor for subsequent conceptions seems important for (prospective) parents and should ideally be discussed before the start of the treatment.
Study funding/competing interest(s): Funding by University(ies) – The project is funded by the Special Research Fund of Ghent University. There are no competing interests.
Trial registration number: NA.
Keywords: sperm donation, donor siblings, genetic relatedness, qualitative research

O-139 The effectiveness of psychosocial interventions that can be delivered by all staff members: a systematic review

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Study question: Which psychosocial interventions can be delivered by all staff members (i.e. physicians, nurses, midwifes, embryologists) as they do not require a mental health professional, and what is their effect on the psychosocial wellbeing (e.g. stress, marital satisfaction, social concerns) of fertility patients?
Summary answer: Evidence indicates positive effects of some psychosocial interventions that can be delivered by all staff members. High quality trials, not contradicted by other trials evaluating similar interventions, proved the benefit of self-administered leaflets with preparatory information for patients' individual wellbeing and of self-administered online cognitive-behavioural-therapy for patients' social wellbeing.
What is known already: Fertility problems and treatments have a negative impact on patients' psychosocial wellbeing. Historically, patients have been referred to mental-health professionals (e.g. psychologists, social workers) if this was deemed required to ensure their psychosocial wellbeing. Recently, enabling all staff members to fulfil the need for psychosocial care of all their patients was suggested. A review of research on the nature and effectiveness of psychosocial interventions that can be administered by all staff members was missing.
Study design, size, duration: This systematic literature review followed a predefined protocol replicated by two reviewers. In August 2014 four electronic databases were searched for (randomized) controlled trials evaluating psychosocial interventions (potentially) delivered by all staff members (i.e. not requiring mental health professionals) and their effect on patients' psychosocial wellbeing assessed with reliable questionnaires.
Participants/materials, setting, methods: Self-administered interventions were differentiated from interpersonal interventions. Psychosocial wellbeing was operationalized as individual, relational and social wellbeing. We extracted data about the sample, nature of the intervention, study design, questionnaires' reliability, statistical tests and effect sizes. The trials' quality was rated and a meta-synthesis of the data was conducted.
Main results and the role of chance: Seven randomized controlled trials evaluated self-administered interventions and nine trials (all controlled, seven randomized) evaluated interpersonal interventions. Trials had high (n = 3), moderate (n = 9) or low (n = 4) quality. Evidence on similar interventions was contradictory. Four in seven trials found an effect of self-administered interventions (i.e. online psycho-education, expressive writing, information leaflet, online cognitive-behavioural-therapy) on individual wellbeing (i.e. stress, self-efficacy). One in two trials found an effect of self-administered interventions (i.e. online psycho-education) on relational and social wellbeing (i.e. sexual/social concerns). Six in eight trials found an effect of interpersonal interventions (i.e. complex interventions or group information sessions) on individual wellbeing (i.e. depression, anxiety, self-efficacy, adjustment, life-satisfaction). One in two trials found an effect of a complex intervention on relational wellbeing (i.e. marital and sexual satisfaction).
Limitations, reason for caution: The reliability of the findings of this review is restricted as only three trials received high quality ratings. Many interventions integrated multiple components (e.g. information provision combined with training in coping skills) and therefore it is not possible to specify which of their components were affecting wellbeing.
Wider implications of the findings: Although patient-centred care requires all staff members to show empathy with patients during all clinical procedures and interactions, high-level evidence on whether staff members other than mental health professionals can deliver effective psychosocial interventions is missing. Psychosocial interventions that can be delivered by all staff members with a theory-led rational should be tested while allowing assessing the individual impact of each of their components.
Study funding/competing interest(s): Funding by University(ies) – Cardiff University; Academic Medical Centre Amsterdam; Leuven University.
Trial registration number: NA.
Keywords: nursing, midwifery, patient-centred care, randomized controlled trials, psychosocial wellbeing

O-140 Understanding the preferred role in infertility treatment decision making among Chinese infertile women in Hong Kong

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Study question: What are the treatment decision making preferences in Chinese women undergoing fertility treatments in Hong Kong?
Summary answer: Results revealed majority of Chinese infertile women preferred relying on healthcare professionals and took minimal role in treatment decision-making. The findings align with qualitative reports from local healthcare professionals, highlighting a need to empower and facilitate Chinese women in fertility treatment decisions in order to improved clinical outcomes.
What is known already: Past studies have found treatment decision-making as central part of patient participation in healthcare which relates to patient autonomy and empowerment. In a local qualitative study professionals at local ART clinics described fertility patients in Hong Kong as unassertive, compliant and largely reliant on physicians in different aspects of treatment decisions, especially on whether to continue or terminate treatment. However, without recruiting patients as informers, little is known about their actual experience and preference.
Study design, size, duration: A cross-sectional survey was conducted with subfertile women currently undergoing IVF treatment in a local public hospital. Information was collected on infertility diagnosis, treatment history, their preferred decision making role, and demographic characteristics. The sample size is 198.
Participants/materials, setting, methods: Women undergoing IVF treatment were approached in a local hospital fertility clinic on the day of pregnancy test. Pregnant women and non-Chinese women were excluded. Of 465 eligible participants, 283 agreed to participate, 205 completed questionnaires were eventually returned. Data analysis were run on 198 valid set of response.
Main results and the role of chance: On average, participants were 37.0 years (SD = 3.5), married for 7.4 years (3.7), and suffered subfertility for 4.1 years. The majority of them received tertiary education and had full-time job. Most of them preferred shared by leaning passive role (41.9%) or total passive role (40.4%). 89.4% of them thought doctors should best decide diagnosis and evaluate treatment options, and only 10.1% preferred sharing responsibility. No one assumed an autonomous role. Similarly, most women preferred minimal role and relied heavily on physicians in deciding whether to continue or terminate further treatments (84.3%), only a small proportion preferred sharing responsibility (7.1%) or asserting autonomy (8.6%). Chinese infertile women appeared to be more compliant in making treatment decisions as compared to their Western counterparts.
Limitations, reason for caution: Self-selection bias was inevitable in questionnaire survey, and the cross-sectional nature of the study did not permit causal inferences. Only infertile women were recruited in the study, so the decision

making preference for men experiencing fertility problems in Hong Kong is yet to be investigated.
Wider implications of the findings: This study adds to our understanding of Hong Kong Chinese women's role preference and level of involvement in infertility treatment decision making by providing quantitative evidence from patients' experience. It highlights the importance of physicians and nurses in facilitating informed decision making.
Study funding/competing interest(s): Funding by University(ies) – None.
Trial registration number: NA.
Keywords: preferred role, treatment decision making, infertile women, Chinese, Hong Kong

INVITED SESSION

SESSION 39: TOWARDS SAFER AND BETTER IVF

Tuesday 16 June 2015 **14:00–15:00**

O-141 Towards an OHSS free clinic

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Over the last 20 years substantial progress has been accomplished in the field of reproductive medicine with an increase, not only in the annual number of treatment cycles, but also in the cumulative live birth rates following IVF/ICSI. Nevertheless, in spite of this progress, and despite that more than 5 million babies have been born from IVF/ICSI, we must not forget we treat "healthy infertile women" and we must deliver "healthy pregnant women". Ovarian hyperstimulation syndrome (OHSS) is the most serious complication for women undergoing IVF treatment. According to the 2010 annual report of ESHRE European IVF-monitoring (EIM) Consortium, 1500 cases of OHSS were recorded in 25 out of the 31 countries with an incidence of 0.3%. Although its incidence has reduced over the last decade, these figures may be underestimated, if we consider that OHSS frequency in well-designed randomized controlled trials is approximately 3%. Taking into account that, even after 25 years since the introduction of ovarian stimulation, OHSS is still present and considering that it can be a severe complication, strictly associated with high morbidity and potential mortality, we are obliged to aim for a "safer and better" IVF practice and work towards an "OHSS free clinic". The concept of OHSS free clinic, which has been introduced in 2010, appears to be more relevant than ever. Such a concept includes the use of GnRH antagonists for pituitary downregulation and ovulation triggering with a GnRH agonist, followed by segmentation of IVF treatment with elective embryo cryopreservation and embryo transfer in a subsequent cycle. This presentation aims to address the evidence supporting the use of the GnRH antagonist protocol with the use of GnRH agonist triggering for final oocyte maturation as a key strategy towards an OHSS free clinic. In addition it aims to address the safety and efficacy of a fresh embryo transfer following this concept and finally discuss whether segmentation of IVF treatment and embryo transfer in a subsequent cycle might be the future for all patients undergoing IVF treatment.
Keywords: OHSS, GnRH agonist triggering, GnRh antagonist, freeze-all, segmentation of IVF

O-142 Towards a "freeze all" policy

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The first pregnancy described after a frozen–thawed embryo transfer (FET) dates back to 1983 in Australia, and the first live birth dates back to 1984 in the Netherlands. Since that time, embryo cryopreservation techniques have dramatically improved and the number of FETs has increased. In 2012, around one-third of all assisted reproductive technology (ART) births reported by the Society for Assisted Reproductive Technology (SART) originated from FET. Nowadays, although fresh embryo transfer (ET) is the norm in most *in vitro* fertilization (IVF) centers, there is increasing interest in the freeze-all policy. In this strategy, the entire cohort of viable embryos is electively cryopreserved and a delayed FET is performed. The potential advantage of this strategy is that the embryo transfer is performed in a more favorable intrauterine environment, possibly improving IVF outcomes and overall safety. Although controlled ovarian stimulation (COS) is considered an essential step during IVF treatments, there are increasing concerns about the adverse effects of COS over the endometrial and uterine environment, as well as regarding the safety of COS in pregnancies that have originated from ART. Many genes related to endometrial receptivity are regulated by hormones, and COS may alter the gene expression of more than 200 genes related to implantation when compared to natural cycles without hormone stimulation. The supraphysiologic levels of estradiol and progesterone during COS could lead to morphologic and biochemical modifications, and would consequently impair endometrial receptivity. Conversely, endometrial development and priming are controlled more precisely during frozen–thawed cycles when compared to COS with gonadotropins, and this could be related to better endometrial receptivity, thus favoring those patients adopting the freeze-all policy. It is known that uterine contractions (UC) at the time of ET adversely affect IVF outcomes, and that supraphysiologic hormone levels may increase these UC. Some studies found that uterine contractility is much higher in stimulated cycles than in natural cycles. Considering all of the possible side effects of COS on endometrial receptivity, as discussed above, recent studies have shown better IVF outcomes when performing elective FET. Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic, potentially lethal, and major complication encountered during COS in IVF. The prevention of OHSS is the most important aspect of its management. The freeze-all strategy virtually eliminates the onset of early and late OHSS. It seems that ectopic pregnancy occurs more frequently in pregnancies that result from IVF treatments when compared to natural pregnancies. This higher risk would be related to increased UC and supraphysiologic hormone levels during COS. Some observational studies have suggested that pregnancies that occurred after FET seem to have better obstetric and perinatal outcomes when compared to fresh ET, suggesting an advantage in performing FET over fresh ET. The COS and supraphysiologic hormone levels may be related to altered placentation, leading to an increased risk of pre-eclampsia, low birth weight, prematurity, small size for gestational age, antepartum hemorrhage, and perinatal death. Regardless of the growing evidence favoring the freeze-all strategy, there are few data concerning the cost-effectiveness of elective FET when compared to fresh ET. There is still a lack of higher-quality randomized controlled trials regarding the freeze-all policy and its relationship to IVF, obstetric and perinatal outcomes, the best developmental stage for embryo cryopreservation when applying this strategy, and the best endometrial priming method to perform FET. This presentation will evaluate the available evidence in the literature comparing fresh ET and the freeze-all policy regarding the possible interference of COS in implantation and endometrial receptivity, IVF safety, and obstetric and perinatal outcomes.
Keywords: freeze-all policy, elective frozen–thawed embryo transfer, delayed frozen-thawed embryo transfer

INVITED SESSION

SESSION 40: POWER BALANCE IN THE IVF LABORATORY

Tuesday 16 June 2015 **14:00–15:00**

O-143 Scientist vs. technology in determining success

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The definition of success in IVF has not remained constant over the past 35 years. Although originally designed to be a potential solution for women whose infertility was ascribed to tubal disease, advances in IVF quickly allowed it to become applicable to a wide range of indications. More recent advances have led to outcomes such as the time taken to achieve pregnancy, the overall/cumulative efficiency of a treatment cycle and the safety of any resultant pregnancy for both the mother and fetus, becoming significant considerations that have driven contemporary clinical practice. What have remained constant, however, are the critical laboratory aspects associated with

achieving success. In that context, the IVF laboratory has been charged with overseeing the formation, development, selection, and storage of human embryos ex vivo. The increased success of clinical IVF since its inception, is attributable to a familiar scientific process involving the interaction of the scientist and technology. Science is, by definition, the systematic acquisition of knowledge and the scientist has acquired this knowledge in part by developing technology to aid in the quest. The scientific knowledge gained has, in turn, driven the development of further technology for two reasons: a) to increase insight into the biological processes involved in human conception in vitro and b) to apply the scientific knowledge in attempting to achieve improved clinical outcomes. The latter aspect then involves the scientist as a practitioner whose skill is an important component of successful treatment. Examples of this interdependence can be seen in improvements at all levels of IVF treatment. For example, scientific knowledge on the physiology of fertilisation led to the introduction of sperm microinjection techniques culminating in the clinical introduction of ICSI which revolutionised the treatment of male infertility and dramatically increased the population of patients suitable for in vitro-based treatments over 20 years ago. The technology used for ICSI has also generated new knowledge on the critical events associated with fertilisation of the human oocyte. At a practitioner level, the skill required for optimal performance of ICSI is a crucial component of the scientist's contribution to clinical success. Basic scientific knowledge, some of it gleaned well before the introduction of clinical IVF, was instrumental in forming our current view of the nutritional/ metabolic requirements of the preimplantation mammalian embryo in vitro. The work of a number of notable scientists, building on the foundations laid by early experimental embryologists, resulted in this scientific knowledge being applied to the design of culture systems that resulted in dramatic increases in the viability of human embryos grown in vitro. A similar pattern can be observed in the knowledge, generated by cryobiologists over many decades, being applied to the development of optimal cryopreservation systems for human embryos. Scientific advances in these two areas have been pivotal in the trend towards a reduced number of embryos in transfer procedures and the growth of single embryo transfer (SET). Accurate prediction of embryo viability remains a major challenge to scientists in the field and, again, basic scientific knowledge of preimplantation genetics and, more recently, morphokinetics has led to the development of new technologies that hold much promise for the future. In summary, improvements in every facet of the IVF laboratory have been underpinned by scientific knowledge, acquired by scientists. This has in turn, led to the development of technology (specific tools and devices) that has allowed the knowledge to be applied and achieve optimal clinical outcomes. So, the scientist has been, and is, the key player in both development and utilisation of the technology responsible for success in IVF. It is unlikely that this pattern will be different in the future.

Keywords: scientist, technology, IVF laboratory

O-144 Embryology vs. clinic – the balance in decision making

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All decision making in modern assisted reproduction with the aim of assisting the couples or the women in achieving a live birth of a healthy child must be based on all available information concerning the women, the man, the sperm, the oocyte, the uterus and their interaction. It is a decision of whether to treat, how to treat or not to treat at all. Decision making is about predicting outcome in a scenario of variable biology based on physiological indicators and previous responses. The tools are our knowledge and experience – mixed with and/or distorted by our wish to fulfill patient expectations and dreams. Real life decisions are much more than just selecting the best embryos for the patient. The challenge is to rank the different aspects and arguments. Should we transfer a poor quality embryo in order to provide closure to the patient after a series of failed treatments? Should we inject non-motile sperm cells into the oocytes from an ICSI couple who don't want a sperm donor? Should we proceed with the transfer or cryopreserve all the top quality embryos in a patient at risk of hyperstimulation? Should we cryopreserve poor prognosis embryos thereby risking that the patient have to go through numerous FER cycles in vain? In many situations there simply is no standard answer to these types of questions and the only way forward involves evaluating the individual couple, their history, their gametes and embryo and their wish and priorities. Only when including evidence based embryology and evidence based clinic will we be able to make balanced decision to the benefit of the patients. But what do we do when decisions are based on other things than clinic and embryology? Too often suboptimal or irrelevant aspects get in the way of an objective assessment and thus the best decision for the patients. This can be due to political, cultural, religious, financial or personal ethical issues. It can be due to lack of continued education both medical and biological or it can be financial considerations taking precedence over medical judgments. Would any of us accept to pay extra for the surgeon to use a sharp scalpel during surgery? The answer is of course "NO". When receiving medical treatment ourselves we – like everybody else – expect the highest professional standards when seeking cure for a health problem. In assisted reproduction we are the highest professional standard. How can we claim to make decisions with the intention to balance or optimize the chances for the patients and at the same time try to sell supplementary high cost services – often without any documented effect? Examples are numerous. With the growing praxis of fertility clinics selling these kinds of "additionals" the underlying decisions taking is no longer medical or biological but financial. And the patients have no chance to evaluate if these options are value for money. They expect us to provide expert recommendations for how to cure their problem – having a child. Unfortunately, our field of medicine may be moving in a direction that makes us less health professionals and more businessmen. Maybe the title for this talk should have been "*Finance department vs. Clinic – the balance in decision making"* because there is no "versus" between embryology and clinic.

Keywords: embryology, clinic, decision making

INVITED SESSION

SESSION 41: PATIENT SESSION – SOCIAL INFERTILITY VERSUS MEDICAL INFERTILITY

Tuesday 16 June 2015 **14:00–15:00**

O-145 Psychosocial differences between medical and social infertility

D. Guerra Diaz[1]
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In our countries (Portugal, Spain) there is little understanding of what conception by Assisted Reproduction Techniques is and even less knowledge of Gamete Donation, which can make the experience even more difficult for prospective parents.

Before and during pregnancy, patients have various concerns and fears regarding the personal and genetic health of the donor and the perception of family and friends and what to tell them.

The role of the mental health professional in counselling prospective donor gamete parents is to maintain a neutral stance with patients in order to facilitate their exploration of the pros and cons of an action, rather than giving direct advice or recommendations. With this in mind, an examination of the factors involved needs to be done to clear up recommendations.

All potential gamete recipients (sperm, eggs or both) who undergo first donation treatment at our centres completed a questionnaire regarding their emotional state, fears and opinions on the release of information about the manner of the conception, whether to others (peers, family etc..) and / or their children. All patients in the sample group received a booklet with frequently asked questions and some educational material along with the initial questionnaire.

The aim of this study was to examine Spanish potential gamete recipients' emotional state and attitudes towards the treatment, and their intentions to disclose. It was also intended to investigate differences between males and females and between couples and single or lesbian women.

Differences between single or lesbian couples and heterosexual couples will be explained, in order to enhance the understanding of factors that contribute to learn how counselling should benefit these patients.

This qualitative study highlights the factors that influence the feelings and decisions of patients undergoing anonymous gamete donation.

It also underscores the scope for the desirable modification of patients' emotional states, anxieties and plans regarding disclosure by use of educational materials and psychological counselling intervention.

The findings have implications in relation to the use of pre-treatment counselling and raise a number of issues which merit further investigation.

O-146 Are there other choices? Attitudes towards social freezing, gamete donation and surrogacy in young adults in reproductive age

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Study question: What do childless young adults in reproductive age think about gamete donation, surrogacy and social freezing?
Summary answer: Participants show positive attitudes towards the use of gamete donation and surrogacy, not only regarding significant others' use but also regarding their own.
What is known already: Delaying childbirth is becoming increasingly common, particularly among higher educated women. Due to age-related fertility decline this has significant health and fertility implications. Moreover, research has highlighted a lack of fertility awareness in general population. Young adults tend to overestimate the probability of achieving spontaneous pregnancy as well as IVF-pregnancy rates. Social and legal acceptability of third-party reproduction diverges among countries. In Portugal, gamete donation is allowed within the regulatory framework but surrogacy is not.
Study design, size, duration: Cross-sectional study. Data were collected between February and April of 2015.
Participants/materials, setting, methods: A total of 480 childless, individuals between the ages of 18 and 40 years old completed an online questionnaire specifically developed for this study.
Main results and the role of chance: According to our results, 48,6% assume they would consider donating gametes and about 80% agree that if they were unable to get pregnant using their own gametes, recurring to gamete donation would make them happy by helping them achieving parenthood. About 83,7% also indicated that they would support or highly support friends decision of recurring to gametes donation. A total of 32,8% would consider the possibility of using surrogacy if all other possibilities were to be excluded. Results also showed that in case participants knew that they would have fertility problems in the future, they were most likely to freeze their own gametes for use in the future.
Limitations, reasons for caution: The online format and recruitment methods (snow ball sampling) may limit the generalizability of the findings.
Wider implications of the findings: Men and women in the current study indicated willingness to consider the use of third third-party reproduction treatments. Even in a legal framework that does not consent surrogacy, they would consider this possibility, maybe through cross-border reproductive care. They also express their willingness to freeze their gametes in order to prevent eventual fertility problems in the future.
Study funding/competing interest(s): There is no conflict of interests.
Trial registration number: The study was not a trial.

O-147 Missing the fertility train? Italian lifestyle, social structure, economy and how the country that loves kids is making it difficult to have them

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This qualitative analysis of the fertility issue in Italy explores the personal side of infertility and how specific socio-economic factors contribute to the larger context of what has now come to be referred to as Social Infertility. Informal interviews with individuals and couples going through, or who have recently completed treatment highlight the obstacles and challenges they face in their quest for having a family. Not surprisingly, besides the often talked about decision to "put career before family," those interviewed cited the added stress of uncertainty resulting from an ongoing financial crisis which has produced high levels of unemployment as a significant factor in postponing family plans. This uneasy feeling about the future manifests itself as a source of anxiety and affects larger life decisions like whether and when to leave the family home. In this way, Italy, which has long been considered an idyllic environment for children to grow up with a strong sense of family, has become instead an increasingly difficult place in terms of meeting the needs of young people which allow them to become independent adults with promising futures and family possibilities. In other words, results indicate that the overall country situation is itself contributing to an increasing wave of Social Infertility.

Other factors included specifically:

1. An increase in educational expectations (staying in school longer than in the past).
2. The Desire to have "all ducks in a row" which translates to staying in the family home until able to buy a home of one's own with or without a partner.
3. Extended financial dependence on parents.
4. prolonged transition to adulthood/independence due to family dynamics.
5. Economic uncertainty as financial crisis continues to adversely affect Italian families.
6. Unemployment rates highest in 20–40 age range.
7. decrease in the number of permanent work contracts, especially for women.
8. lower salaries and little job security.

O-148 The late lessons of delayed motherhood – from a patient's perspective

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When does social infertility turn into medical infertility? Is age related inability to conceive still infertility?

Is media coverage of advanced-age celebrity moms sending a wrong message to the women?

The personal story of a woman who postponed her pregnancy till her late thirties for social reasons reveals the typical path of turning the social infertility into a medical condition. There are some things in life that just can't be postponed. Sharing patient's experience with delayed motherhood, the presentation is trying to find the reasons why so many women misread the signs of the biological clock.

INVITED SESSION

SESSION 42: PARAMEDICAL INVITED SESSION: LABORATORY

Tuesday 16 June 2015 **14:00–15:00**

O-149 Molecular networks of embryo-endometrium crosstalk during implantation

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A prerequisite for successful embryo implantation is the adequate preparation of receptive endometrium and the establishment and maintenance of a viable embryo. The endometrium is receptive to blastocyst implantation only during a spatially and temporally restricted period in the secretory phase of the menstrual cycle, known as 'window of implantation'. During this window, ovarian estrogen and progesterone induce the endometrial cells to proliferate, differentiate, and secrete molecules that influence trophoblast development. Meanwhile, the presence of an embryo in the uterus triggers specific molecular and cellular responses within the endometrium. The success of embryonic implantation further relies upon a two-way dialogue between the blastocyst and the endometrium, which involves cell-cell and cell-extracellular matrix interactions, mediated by integrins, matrix-degrading enzymes and their inhibitors, a variety of growth factors and cytokines, and their receptors and modulator proteins. Disturbances in this bidirectional crosstalk are believed to represent a major reason why over 60% of all pregnancies are terminated at the end of the periimplantation period. As it is ethically and practically extremely difficult to study implantation process in humans *in vivo*, many studies have been performed on animal models in order to improve the understanding of the molecular mechanisms involved in embryo-maternal crosstalk. Animal models do provide important clues to the processes regulating implantation, nevertheless the results cannot always be extrapolated to

humans. With the development of 'omics' technologies, numerous transcriptome studies of the human endometrium have revealed hundreds of simultaneously up- and down-regulated genes that play a role in endometrial receptivity. Information concerning the molecular basis of human preimplantation development is steadily growing. Several molecules and molecular pathways have been found to have a role in the endometrium and/or in the embryo at the time of implantation, nevertheless the molecular basis of the reciprocal embryo-endometrium interactions still remains largely unknown. The novel findings and approaches to investigate the embryo-endometrium crosstalk in humans will be discussed.
Keywords: implantation, endometrial receptivity, embryo-endometrium interactions

O-150 Maternal metabolic disorders, oocyte quality and longterm offspring health

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Obesity currently affects over one-third of reproductive-age women in the United States leading to overall health and metabolic disorders, including reproductive function. Obese women experience subfertility and infertility at greater rates but more importantly, when they do get pregnant, their offspring suffer significant health consequences. Offspring of obese women are more likely than those born to normal-weight women to be obese at one year of age; to have metabolic syndrome, hypertension, and liver disease as young children; and to experience developmental delay, intellectual disabilities, and autism spectrum disorder. Given the severity and intractability of the obesity epidemic, we must identify effective means of intervention to prevent these detrimental effects of maternal obesity; this requires determination of the underlying mechanisms. In this presentation, using a mouse diet-induced obesity model, I will discuss the rationale for attributing these offspring abnormalities to the maternal oocyte, specifically due to a metabolic aberration originating in the oocyte mitochondria. I will also present data to suggest that this process is not reversible by dietary changes or exercise in the mothers. Finally, I will introduce possible mechanisms to explain the intergenerational transfer of these maternal metabolic abnormalities to the offspring.
Keywords: obesity, developmental programming, spindle abnormalities, metabolism, oocyte

SELECTED ORAL COMMUNICATIONS

SESSION 43: OPTIMIZING BLASTOCYST CRYOPRESERVATION

Tuesday 16 June 2015 **15:15–16:30**

O-151 Vitrification of single blastocyst on day 5 or day 6 of development using the Rapid-I closed system: clinical outcome of 327 cycles

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Study question: To investigate the utility of the Rapid-I closed system for vitrification of blastocyst on day 5 and day 6 of development and to assess the implantation and pregnancy rate following single blastocyst transfer (SBT) of warmed day 5/day 6 blastocysts.
Summary answer: High survival rates after warming were achieved for both day 5 and day 6 blastocyst vitrified using the Rapid-I closed device. Similar pregnancy and implantation rate were achieved as those following SBT in fresh cycles. There was no significant difference in outcome following SBT of day 5 vs day 6 vitrified blastocyst.
What is known already: Vitrification is a highly efficient technique for the cryopreservation of human blastocysts, but there are a large number of commercial devices available, many of which allow contact between specimen and liquid nitrogen, thereby increasing the risk of cross contamination. This study aims to test the utility of the Rapid-I closed system for vitrification on day 5 and day 6, as well as assess the role of SBT in reducing multiple pregnancies.
Study design, size, duration: A two year retrospective study of frozen single blastocyst transfer (SBT) was carried out. The survival, pregnancy and implantation rate was compared for blastocysts vitrified on day 5/day 6 in 327 frozen SBT cycles. Miscarriage and multiple pregnancy rate was also compared between the two groups
Participants/materials, setting, methods: Embryo were cultured in sequential medium (Vitrolife G series) in low oxygen atmosphere. All good quality blastocyst were vitrified on day 5 or day 6 using Irvine Vitrification medium and Rapid-I device (Vitrolife). After warming blastocysts were cultured in G2 medium (Vitrolife) supplemented with 20% HSA for 2 h before the transfer.
Main results and the role of chance: The overall survival rate of vitrified blastocyst was 90.2% (295/327) and there was no significant difference in survival between the two groups: 89.9% (197/219) and 90.7% (98/108) respectively. 295 surviving blastocysts were transferred in 295 patients (all SBT). The implantation rate (fetal sac: IPR) and clinical pregnancy rate (fetal heart at 7 weeks: CPR) were 49.8% (147/295) and 39.0% (115/295) respectively. A higher IPR and CPR were achieved following transfer of day 5 vs day 6 blastocysts : IPR 52.3% (103/197) vs 44.9% (44/98) day 5/day 6 respectively and CPR 40.1% (79/197) vs 36.7% (36/98) day 5/day 6. Miscarriage rate were similar in two groups: 8.6% (17/197) on day 5 and 7.1% (7/98) on day 6. These differences were not statistically significant. Multiple pregnancy rate was 0.6% (2/295), both in day 6 group.
Limitations, reason for caution: Fewer Blastocysts were frozen on day 6 so the group sizes are not equal and further data is required to corroborate the results. In addition live birth rates should also be investigated to see if there is a difference in final outcome between the groups.
Wider implications of the findings: This study shows that vitrification using a closed system (Rapid-I) followed by a single blastocyst transfer can be considered an effective and practical method for cryopreservation of human blastocyst and may be a beneficial approach to reduce multiple pregnancies in ART. Embryos reaching the blastocyst stage on day 6 could be considered developmentally retarded and yet a reasonable pregnancy rate was achieved following transfer, demonstrating their clinical utility. Further studies are required to confirm this data.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by Edinburgh Reproductive Endocrine Centre, RIE, Edinburgh
Trial registration number: NA.
Keywords: Blastocyst Vitrification, Closed System, Multiple Pregnancy Rate, Single Blastocyst Transfer

O-152 Seeking the optimal point for the vitrification and warming of blastocysts

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Study question: In which degree do pregnancy outcomes of warming cycles are affected by the expansion stage of the blastocysts before vitrification?
Does warming point before embryo transfer affects pregnancy outcomes? Increasing culture duration after warming, does it improve implantation potential for certain embryos like younger blastocysts and morulae?
Summary answer: A linear increase of pregnancy outcomes was observed, between day 5 morulae and expanded blastocysts, followed by a decline for hatching and fully hatched vitrified blastocysts.
On the other hand, increasing the duration of post warming culture was only beneficial for morulae and young blastocysts, while the opposite was observed for the more expanded blastocysts.
What is known already: It is considered that the efficiency of vitrification may depend on the expansion of the blastocyst, with better survival in morulae or early blastocyst stages compared to full or expanded blastocysts, while the later, upon survival, demonstrate higher implantation probabilities. On the other hand, although supported with few published data, vitrification of fully hatched blastocysts is not a preferred situation. The importance of post warming culture duration has not been widely discussed. A duration of 3–4 h is generally adopted.
Study design, size, duration: A retrospective, observational study, based on 1291 vitrification/warming cycles, performed from 1/1/2012 to 30/10/2014. Embryo transfers were divided into six groups according to the expansion rate of blastocysts before vitrification. The number of cases that were included was 121,199, 279, 321, 306, 65 for Group 1–6, respectively. Each group was further divided into two subgroups according to the selection of warming point; one day before (18–20 h) or the day of the embryo transfer (4 h).

Participants/materials, setting, methods: Embryos from an oocyte donation program of a private ART clinic, ranging on day 5 from the morulae to the fully hatched stage, of good to top quality, were vitrified using the FertiPro vitrification kit combined with a closed carrier system (VitriSafe), by placing a maximum of 3 similar growth embryos per straw.
Main results and the role of chance: Heading from Group 1 (morulae and early cavitating blastocysts) to Group 6, (hatched and fully hatched blastocysts), the clinical pregnancy rates were 35.2%, 40.1%, 40.9%, 44.8%, 45.2% and 37.3% for the six groups respectively. Increasing the post warming duration of culture had a positive impact for the corresponding sub group of embryos in Group 1 (38.6% vs. 29.4%) while had a negative impact for the sub groups of blastocysts in groups 2–6, where short post warming culture lead to higher clinical outcomes. Most notably, in Group 6, long versus short post warming culture duration resulted in clinical pregnancy rates of 12.5% vs. 44.1%.
Limitations, reason for caution: The number of cases included in each group was not of equal strengh. Vitrification was performed on a standard time in the morning of day 5, approximately 115 h post ICSI. Additionally the allocation of the cycles to the early warming or not was based on the work load of the clinic, the day of the week, the need for an early morning transfer and various other parameters, but in general it cannot be considered as a random process.
Wider implications of the findings: Further studies need to be conducted in the way of investigating whether cryopreservation of the embryos strictly at an advanced stage, like the expanded blastocyst, even by increasing the duration of culture for those that are delayed, can improve the clinical outcomes of vitrification/warming cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – NA.
Trial registration number: NA.
Keywords: blastocyst, vitrification, warming

O-153 Highly effective vitrification of human blastocysts using hyaluronate supplemented, non-DMSO containing solutions and the validation of a modified microSecure vitrification procedure

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Study question: After the discontinuation of CBS 0.3 ml Embryo straws (containing a hydrophobic plug), in August 2014, could traditional CBS 0.3 ml semen straws with cotton/PVA plugs be effectively modified to support optimal vitrification and warming outcomes, as monitored by blastocyst survival and development?
Summary answer: MicroSecure vitirification, incorporating an inner plug seal modification, proved to be effective with no difference in vitrification outcomes observed in blastocyst recovery, survival or post-warming development compared to control straws. Overall, this simple, highly repeatable aseptic closed method will continue to provide unparalleled success in recovering embryos and sustaining viability.
What is known already: MicroSecure VTF was developed in 2008 as an inexpensive, non-commercial, FDA compliant method which optimized quality control aspects of vitrification. It is unique by offering tamperproof internalized, dual colored labeling in a ionomeric-resin straw that completely weld seals. In combination with hyaluronate containing, non-DMSO vitrification solutions, vitrification has proven to be highly effective. However, due to the competitive marketing of commercial vitirification devices, the original CBS Embryo storage straw was eliminated from the marketplace.
Study design, size, duration: Since 2012, the overall clinical effectiveness of hyaluronate (HA) supplemented, non-DMSO containing vitrification solutions (Innovative Cryo Enterprises) with or without PGS treatment (n = 531 cycles) were retrospectively evaluated by age groups (<38 yo, 38–43 yo, donor egg). Furthermore, we prospectively validated the vitrification effectiveness of our straw modification using 70 blastocysts.
Participants/materials, setting, methods: Traditional microSecure vitrification was performed until 2015, straw modifications were then implemented. Using new CBS 0.3 ml Embryo/Semen straws, an internal seal is created to maintain separation between the vitrification tips and cotton plug. In addition, 2-stainless steel ball bearings are added to the shorter 30 mm ID rods to prevent buoyancy.
Main results and the role of chance: We warmed 1341 vitrified blastocysts between 2012 to June 2014, with 1341 embryos recovered (100%) and 1316 completely survived (98.1%). Biopsied blastocysts experienced over 99.5% survival. When transferring PGS-tested euploid embyo(s), clinical pregnancy (80.5–81.4%) and live birth rates (75.9% to 81.4%) were unaffected by age group (38 yo, respectively). Conversely, age related pregnancy differences (P < 0.01) were observed in VET cycles using untested blastocysts (clinical: <38 yo–64.7%, >38 yo–50%; live birth: <38 yo–55.3%, >38 yo–33.3%), being higher than fresh ET outcomes in the older age groups. Interestingly, vitrified blastocyst cycles using donor eggs (n = 85; 60% clinical) have been less successful than fresh ET (n = 48; 92.5% clinical). In validating our modified storage straws for use in 2015, we experienced 100% recovery and 100% survival with 70 re-vitrified aneupolid blastocysts.
Limitations, reason for caution: Innovators of effective, non-commercial devices can be subject to politics, when the outstanding performance of their low cost system competes with the marketshare of another VTF device (e.g., HSV). Until we can have a comparable embryo storage straw manufactured, we'll continue to effectively adapt the new semen/embryo straws for mS-VTF.
Wider implications of the findings: Sodium hyaluronate addition to solutions is a logical improvement to increasing viscosity and cell membrane stabilization during vitrification. Furthermore, since the cellular toxicity of DMSO has been debated for decades, its effective removal seemingly improves the overall safety of the vitrification process. Finally, the ability to easily modify conventional 0.3 ml CBS straws facilitates the continued use of microSecure vitrification as an inexpensive, aseptic closed system that eliminates recovery failure while optimizes post-warming survival and viability.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Southern California Institute for Reproductive Sciences.
Trial registration number: None.
Keywords: vitrification, blastocyst, hyaluronate, non-DMSO solutions

O-154 Randomised study on the effect of artificial blastocyst collapsing before vitrification on their behaviour after thawing: cinematographic and morphometric analysis and comparison of transfers outcome

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Study question: What is the impact of blastocoelic fluid reduction before vitrification on blastocyst survival rate, intensity of their shrinkage after thawing, speed and progressiveness of blastocoelic re-expansion, morphology of their trophectoderm and inner-cell-masses and implantation ability in comparison with blastocysts having no intervention before vitrification?
Summary answer: Although collapsed blastocysts showed in average more intensive shrinkage and faster and more regular re-expansion after thawing in comparison with unshrunk counterparts, they had the same morphological scores at the time of transfer and after transfer resulted pregnancies in the same percentages as unshrunk blastocysts from the control group.
What is known already: Several studies reported that an artificial shrinkage of blastocysts before vitrification improves survival rate and increases clinical success of blastocyst cryopreservation programme. Most of these reports are from earlier period of today well established human blastocyst vitrification. Blastocysts can be shrunk by using different methods: microneedle or laser puncture, blastocoel aspiration or pipetting of blastocysts with tight pipettes. To date the advantage of using this method is not evidence based.
Study design, size, duration: Prospective quasi-randomised study. From April 2012 surplus blastocysts from each patient were split. Half of them were artificially shrunk before vitrification. Blastocysts for thawing were selected by alternation: once shrunk, once nontreated. The study included single transfers of thawed blastocysts (118 in study, 119 in control group) till July 2014.
Participants/materials, setting, methods: Expanded Day-5 and Day-6 blastocysts were either left intact or shrunk with laser pulse on trophectoderm. They were then equilibrated for 10 min in DMSO/ethylene-glycol before vitrification in closed straws. The recovery of warmed blastocysts was recorded by time-lapse camera before transfer and the changing of their diameters was analysed.
Main results and the role of chance: Shrunk and control blastocysts survived vitrification in similar proportions (100% and 99.2%). Although the intensity of shrinkage was greater in the study group (65.3 ± 16.8 vs. 50.7 ± 15.3 μm; p < 0.001) they re-expanded faster (14.5 ± 12.5 μm/h vs. 5.5 ± 14.0 μm/h; p < 0.0001). One hour after thawing both groups had similar dimensions and

comparable morphology scores. Re-expanding blastocysts of control group reached zona faster (82.3 ± 38.9 min vs. 97.0 ± 31.2 min; $p = 0.03$), but hatching occurred more frequently in a group of shrunk blastocysts (53.4% vs. 16.9%; $p < 0.001$). The implantation rates (26.3% vs. 28.6%) did not differ between groups. Continuous monitoring of re-expansion shows curves with different patterns. Mean curves of study and control groups fit linear regressions ($Y_{study} = 9560 + 62.83*X$ vs. $Y_{control} = 12100 + 31.01*X$; $p < 0.05$) with significantly different slopes.

Limitations, reason for caution: There is a missing data about the proportion of hatching blastocysts before vitrification in both groups. Any opening in zona allows cryoprotectants to act immediately and directly on trophectoderm and theoretically more efficiently dehydrate the blastocyst. Hatching blastocysts from control group could therefore behave similarly as artificially collapsed blastocysts.

Wider implications of the findings: Our findings could not confirm previously published observations about beneficial effect of artificial collapsing before vitrification on clinical outcome. By using time-lapse photography we detected differences in thawed blastocysts behaviour during recovering *in vitro*. Many new parameters for description of blastocyst re-expansion after thawing were introduced, but none of them was predictive for implantation. Their prediction could probably increase, if blastocysts were cultured for a longer period before transfer.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the Slovenian Research Foundation (P3-334-0327). All authors declare no conflict of interest.

Trial registration number: NA.

Keywords: blastocyst, artificial collapsing, laser, vitrification, time-lapse

O-155 Clinical outcomes of Day-2, Day-3 and Day-5 embryos vitrified with a closed system

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Study question: To assess outcomes achieved after closed system vitrification of Day-2, Day-3 and Day-5 embryos and to test whether the day of embryo vitrification or the treatment for endometrial preparation has an impact on pregnancy outcomes.

Summary answer: Although survival rate were similar after warming of Day-2, Day-3 and Day-5 vitrified embryos, higher implantation and delivery rates were achieved after warmed blastocyst (Day-5) transfer. Similar implantation rates were obtained when embryos were transferred in natural cycle or after hormonal replacement treatment.

What is known already: Vitrification is an established technique to cryopreserve surplus embryos after embryo transfer and for cryopreservation because of a risk of hyperstimulation syndrome (OHSS) or impaired endometrial pattern. Closed and open vitrification devices are routinely used for this purpose. However, there have been concerns with closed vitrification devices that slower cooling rate may affect embryo developmental potential compared to open vitrification systems. Few large scale studies have evaluated the clinical outcomes after closed system vitrification.

Study design, size, duration: Retrospective observational study designed to analyze implantation and delivery rates according to the day of vitrification.

From 2008 till 2014, 4770 embryos were vitrified using a closed system. 2185 embryos were transferred (1239 Day-2, 590 Day-3 and 356 Day-5 embryos) in 1905 replacement cycles (59.8% natural and 40.2% after hormonal replacement treatment). The mean number of embryo replaced per transfer was of 1.1 and comparable between the three groups.

Participants/materials, setting, methods: Good quality surplus embryos (mean score 4.4 ± 0.6 on a 1–6 scale) were vitrified using the Irvine vitrification kit and High Security straws (CryoBiosystems) on the day of embryo transfer (Day-2, Day-3, Day-5). 5% of the embryos were obtained from donors in all three groups. After warming, embryos were cultured for 24 h before transfer in the replacement cycle.

Main results and the role of chance: Patients and cycles characteristics were similar for cleavage stage embryos and blastocysts warming cycles (mean age of the patients: 33.6 ± 5.2, 34.3 ± 5.3, day-5: 33.3 ± 5.2 years for Day-2, Day-3 and Day-5 respectively).

Embryo survival rates were similar whatever the stage of the embryo (92.1% at Day-2, 95.6% at Day-3 and 90.4% at Day-5). Implantation rates were significantly higher after transfer of warmed blastocyst (32.5%) compared to Day-2 embryos (18.2%) or Day-3 embryos (23.9%) ($P < 0.05$). Higher delivery rates were observed for Day-5 (22.7%) versus Day-2 (12.9%) and Day-3 (14.7%) vitrified embryos. Replacement of embryos in natural cycle or after hormonal treatment had no significant impact on the outcomes.

Limitations, reason for caution: This study was performed retrospectively on an unselected patient's population. However, the three groups had similar cycle characteristics, reducing potential selection bias.

Wider implications of the findings: Vitrification with a closed system allows the successful preservation of Day-2/Day-3 cleavage stages embryos and of Day-5 blastocysts and ensures high implantation rates after warming and transfer. When enough good quality embryos are available, it may be advisable to prolong culture till Day-5 and vitrify blastocysts.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hôpital Erasme.

Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 44: ADVANCES IN OVARIAN STIMULATION

Tuesday 16 June 2015 **15:15–16:30**

O-156 Development of an orally active positive allosteric modulator (PAM) of follicle stimulating hormone receptor for infertility treatment in assisted reproductive technology

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Study question: Can an orally active small molecule FSH receptor agonist or PAM (molecular weight < 650) be developed for human infertility treatment? This molecule will mimic the cellular and *in vivo* response of recFSH, with pharmaceutical properties compatible with a more convenient and safer treatment for patients undergoing controlled ovarian stimulation.

Summary answer: We have developed a novel PAM that selectively stimulates FSHR with similar estradiol production in rat and human granulosa cells as recombinant FSH. This molecule induces similar follicular development in rats as recombinant FSH, when administered orally. The molecule is undergoing preclinical safety and toxicity studies to enable clinical trials.

What is known already: Several approaches have been attempted to increase convenience and efficacy of ovarian stimulation for infertility treatments in women and men. Gonadotropins with modified glycosylation profiles to increase the half-life have been one approach that has met with moderate success, but they still have safety concerns. There have been attempts from pharmaceutical companies to develop oral FSH agonists, however none of them have been successful in demonstrating efficacy in humans sufficient for clinical use.

Study design, size, duration: The work reported here is conducted in preclinical models or in human granulosa cells obtained from discarded follicular fluid following oocyte retrieval during IVF procedure. Estradiol production in rat or human granulosa cells is measured after 24 or 48 h, respectively. Stimulation of follicular development is assessed in immature rats.

Participants/materials, setting, methods: This study was performed in a drug discovery setting. Initial selection of compounds were done by high through put screening (HTS) followed by rational drug discovery effort to identify suitable molecule for clinical development.

Main results and the role of chance: A chemical library of 750,000 compounds were screened by HTS with CHO cells expressing FSHR to measure cAMP production. Non-selective compounds were eliminated by counter screening molecules positive in HTS using CHO cells expressing, thyroid stimulating

hormone receptor (TSHR) and leutenizing hormone receptor (LHR) to identify 3 chemical series as 'hits'. Hits were further fine-tuned through extensive medicinal chemistry iteration to identify a compound (TOP00001) with similar efficacy in estradiol production as recombinant FSH in human and rat granulosa cells. TOP00001 stimulated follicular development in a dose dependent manner in immature rats upon oral administration with maximal effect at 50 mg/kg. Further profiling of the compound against 55 GPCR-ion channels, 211 kinase and 11 phosphodiesterases demonstrated the compound to be very selective to FSHR.

Limitations, reason for caution The compound is in the early phase of safety and toxicology evaluation prior to initiating GLP studies for IND filing. Safety and efficacy of the compound in humans is yet to be demonstrated.

Wider implications of the findings: Currently, a significant number of women drop out of infertility treatment following failure in OI with clomiphene due to the stress of multiple injections involved in IVF cycle. An oral therapy, like the TOP drug, would provide a more convenient treatment for all patients undergoing ovarian stimulation without compromising the efficacy. Furthermore, the convenience of this PAM is ideally suited for evaluation of efficacy in idiopathic oligospermic male patients to improve sperm production, and quality.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – TocopheRx Inc., EMD Serono.

Trial registration number: NA.

Keywords: Oral FSH agonist, Female infertility, COH, OI, Male infertility

O-157 Kisspeptin-54 safely and effectively triggers oocyte maturation in women at high risk of the ovarian hyperstimulation syndrome (OHSS)

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Study question: We have recently reported that kisspeptin-54 triggering induces high rates of oocyte maturation in women with normal ovarian reserve undergoing IVF treatment. We therefore evaluated whether kisspeptin-54 could be used to safely trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS).

Summary answer: We demonstrate for the first time that kisspeptin-54 can be used to safely and effectively trigger oocyte maturation in women at high risk of OHSS. Low rates of OHSS and high clinical pregnancy rates were observed using kisspeptin-54 triggering following a standard recombinant FSH/GnRH antagonist protocol.

What is known already: IVF is an effective treatment for infertility, however triggering with hCG can result in life-threatening complications such as OHSS. Kisspeptin is a recently discovered naturally occurring hormone that safely stimulates gonadotrophin release without known adverse effects. We have recently reported that kisspeptin-54 triggering induces high rates of oocyte maturation in women with normal ovarian reserve, however the safety and efficacy of kisspeptin-54 in women at high risk of OHSS has not previously been evaluated.

Study design, size, duration: This was a single-centre prospective clinical trial. Sixty women at high risk of OHSS underwent an FSH/GnRH antagonist protocol using kisspeptin-54 to trigger oocyte maturation followed by fresh embryo transfer and intensive luteal phase support for 12 weeks. Patients were randomised to kisspeptin-54 doses between 3.2 and 12.8 nmol/kg (adaptive design, $n = 5–20$ per dose).

Participants/materials, setting, methods: IVF cycles were performed at Hammersmith Hospital, London. Inclusion criteria were: age <35 years; body mass index < 30 kg/m^2; serum anti-Mullerian hormone >40 pmol/L or antral follicle count >23. The primary outcome was presence of mature (metaphase II) oocytes retrieved 36 h following kisspeptin-54 injection. All women were screened for early and late OHSS.

Main results and the role of chance: Oocyte maturation was observed in 95% (57/60) of women following kisspeptin-54 administration. Embryo formation did not occur in 3 women, and a clinical decision was made to electively freeze embryos in a further 3 women due to very high risk of OHSS prior to kisspeptin-54 trigger administration; thus 51 of 60 women had fresh embryo transfer. Biochemical and clinical pregnancy rates per transfer at all tested doses of kisspeptin-54 were 63% (32/51) and 53% (27/51), respectively. Highest pregnancy rates were observed following 9.6 nmol/kg of kisspeptin-54, which resulted in a 77% (10/13) clinical pregnancy rate per transfer. There were two cases of mild OHSS, but no cases of moderate, severe or critical OHSS, and no medical admissions or interventions for OHSS.

Limitations, reason for caution: Kisspeptin-54 can safely trigger oocyte maturation in women at high risk of OHSS achieving good clinical pregnancy rates and low rates of OHSS. Further work is required to directly compare the efficacy of kisspeptin-54 with other currently used triggers of oocyte maturation in women at high risk of OHSS.

Wider implications of the findings: We found that kisspeptin-54 safely and effectively triggers oocyte maturation in women at high of OHSS as identified by high AMH and antral follicle count on ultrasound. Good clinical pregnancy rates and no cases of moderate-severe OHSS were observed following kisspeptin-54 triggering. Kisspeptin-54 may therefore offer an entirely novel trigger for oocyte maturation during IVF treatment, especially in women at high risk of OHSS.

Study funding/competing interest(s): Funding by national/international organization(s) – Medical Research Council UK, National Institute for Health Research, Wellcome Trust. The authors have no competing interests.

Trial registration number: ClinicalTrials.gov Identifier: NCT01667406.

Keywords: kisspeptin, trigger, oocyte maturation, OHSS

O-158 The impact of high progesterone level prior to oocyte retrieval on endometrial morphology and uNK cell count in the peri-implantation period

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Study question: Does high progesterone level prior to oocyte retrieval have any impact on the morphological development and uNK cell count of the endometrium in the peri-implantation period (seven days after hCG administration) in women undergoing IVF treatment?

Summary answer: High progesterone level prior to oocyte retrieval produced advancement in endometrial development as well as increased number of uNK cell count.

What is known already: High progesterone level prior to oocyte retrieval has been reported to adversely affect endometrial receptivity, resulting in reduced implantation rate. The exact molecular mechanism by which high progesterone level prior to oocyte retrieval affect endometrial development is still unclear. We were particularly interested in the impact of high progesterone on the uNK cell count because the latter has been reported to be elevated in women with recurrent miscarriage and recurrent implantation failure.

Study design, size, duration: This was a single-center, prospective cohort study carried out in a university-affiliated reproductive center between June 2013 and December 2013. Endometrial biopsy was obtained from 119 subjects

Participants/materials, setting, methods: Women undergoing IVF who did not proceed to have fresh embryo transfer were included. All subjects had blood sample taken for estrogen and progesterone measurement on the day of hCG and the day after hCG administration. Endometrial sample was obtained 7 days after hCG administration for histological dating and uNK cell measurement.

Main results and the role of chance: Multiple regression analysis showed that (1) progesterone level on the day after hCG administration was the only significant variable affecting the results of histological dating. The endometrial development in women with high progesterone level was significantly ($P < 0.001$)

more advanced than that of women with normal progesterone; and (2) progesterone level on the day of hCG administration was the only significant variable affecting uNK cell count. The median (range) of uNK cell count of 9.2% (1.3–21.6%) in women with high progesterone was significantly ($P < 0.001$) higher than the median (range) of uNK cell count of 6.3% (1.4–18.7%) in women with normal progesterone.

Limitations, reason for caution: Immunohistochemistry analysis for CD56 + uNK cell counting are semi-quantitative methods. The hypothesis could be further verified by genomic study in the future.

Wider implications of the findings: The adverse effect of high progesterone prior to oocyte retrieval in IVF treatment cycle may be mediated via its action on the uNK cell.

Study funding/competing interest(s): Funding by national/international organization(s), the National Natural Science Foundation of China (No. 81270657), the Major Science and Technology Projects of Zhejiang (No. 2011C13037) and the Natural Science Program of Zhejiang (No. Y14H040012).

Trial registration number: None.

Keywords: progesterone, endometrium morphology, uNK cell, IVF

O-159 Pharmacogenetic study of CYP19 in polycystic ovary syndrome patients undergo intrauterine insemination cycles

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Study question: Are polycystic ovary syndrome (PCOS) patients with different *CYP19* genotypes respond differentially to ovulation induction drugs such as Clomiphene Citrate (CC) or Letrozole compare to control group?

Summary answer: PCOS patients with AA genotypes (rs2414096) in intron 2 were poor responders to ovulation induction treatment. Although CC is the first line treatment, we demonstrated that Letrozole was more effective than CC in PCOS patients.

What is known already: Aromatase (encoded by *CYP19)* is a member of cytochrome P450 family. The role of aromatase is estrogen biosynthesis. A considerable amount of literature has been published on *CYP19* polymorphisms but none of them were about pharmacogenetic aspects of it. Previous studies have reported common polymorphisms of *CYP19* in PCOS patients. Base on literature three polymorphisms were nominated including (rs2414096 located in Intron2, rs700519 in Exon 7, TTTA repeat and TCT insertion on intron 4).

Study design, size, duration: One hundred and forty two young intrauterine-insemination (IUI) candidates (61 PCOS & 81 patients without ovulatory problem) referred to Royan institute during 2012–2014, were selected for this case-control study. PCOS patients were selected as a Definition in Rotterdam consensus. Both groups received CC or Letrozole plus gonadotropins for ovulation induction.

Participants/materials, setting, methods: DNA was extracted from blood; exon 7, Intron2, Intron4 and promoter PII of *CYP19* were amplified via PCR. Restriction fragment length polymorphism (RFLP) and sequencing was performed to investigate polymorphisms. Antral follicles were monitored by ultra-sonography and realtime PCR was perfomed to investigate gene expression in granulosa cells of patients.

Main results and the role of chance: Our study revealed no correlation between rs700519 (exon7), and average number of follicle >18 mm ($p = 0.9$), there were also no significant difference between TTTA repeats or TCT insertion (located in intron 4) and drug response ($p = 0.4$ & 0.2). No variation in PII region has been found. Average follicle count per cycle in cases with GG, GA, AA genotype in rs2414096 (intron 2), was 1.79, 1.6 and 1.04, respectively that represents AA genotype as the poor responders to drug. Multi variant analysis demonstrated that although PCOS patients bearing AA genotype were poor responders to both CC and letrozole, however they respond better to letrozole ($p = 0.008$) than CC. We also demonstrated that *CYP19* in PCOS paitents has a significantly lower gene expression compare to control group ($p = 01$).

Limitations, reason for caution: The results need to be confirmed by raising the number of IUI patients and functional analysis.

Wider implications of the findings: For the first time our study showed the probable role of aromatase gene in PCOS from personalized medicine view. Although sample size was 142, however they were distributed in two groups homogenously. Groups were matched by age ($p = 0.16$) and CC as the first line treatment was not superior to Letrozole in drug response ($p = 0.14$). It seems rs2414096 (AA) can be a proper candidate for more research in field of personalized medicine.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by a grant from the Royan institute, Reproductive biomedicine group, Tehran, Iran [91000262].

Trial registration number: There is no trial.

Keywords: PCOS, Aromatase, Pharmacogenetic, CYP19, Personalized medicine

O-160 Fertility outcomes in women with hypopituitarism who undergo ovulation induction: a new study of 17 women

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Study question: What are the fertility outcomes of women with hypopituitarism (HP) who undergo ovulation induction (OI)?

Summary answer: Reasonable OI rates and pregnancy rates can be obtained in patients with HP. However, high miscarriage rates mean that the live birth rate is significantly lower. Assuming no other contraindications, it is reasonable to consider vaginal delivery in these women who are likely to deliver normal weight babies at term.

What is known already: Studies in our unit have published poor outcome both in terms of pregnancy rates and pregnancy outcome (Hall, 2006; Overton, 2002). de Boer et al. also reported poor pregnancy outcomes in 13 women with growth hormone deficiency (DeBoer, 1997). These studies are still the only ones concerning the outcomes of ovulation induction in this group of patients. In addition, literature search has found a high level of obstetric complications in HP patients (Kübler, 2009).

Study design, size, duration: A local database was used to identify 17 women with HP not included in our previous study who underwent 70 cycles of OI at University College London Hospitals. This included patients between 1998 and 2013. Their notes were retrospectively reviewed along with audit information inputted contemporaneously into the the database.

Participants/materials, setting, methods: 17 hypopituitary patients treated with OI using human menopausal gonadotrophin in The Reproductive Medicine unit at University College London Hospital. Their notes were retrospectively reviewed along with audit information inputted contemporaneously into the database. The data was inputted onto a data collection tool and analysed.

Main results and the role of chance: Of 70 cycles 72% were ovulatory, 29% resulted in pregnancy and 16% in live birth. Ovulation was achieved in 82% of patients, 65% conceived and 56% had a live birth. There was only one multiple pregnancy; a triplet pregnancy which was selectively reduced to a twin pregnancy and resulted in a twin live birth. There were 7 miscarriages, 1 ectopic pregnancy and 1 termination for trisomy. 2 of the live births were by vaginal delivery, the rest by caesarean section, of which 7 (78%) were elective with the reason given in all being their HP. All the babies were born at term, with an average birth weight of 3.66 kg. Due to the rarity of the disease the study sample was small.

Limitations, reason for caution: Due to the rarity of the disease the study sample was small. The study is retrospective and is therefore potentially subject to bias and loss or absence of data. Caution should be taken as the results differ from a similar sized study published previously by our unit.

Wider implications of the findings: We hope that this study will go some way in helping to guide clinicians and patients in choosing the correct form of assisted conception for this rare group of patients. The findings will help manage expectations of treatment success and pregnancy outcome. The presence of successful vaginal births in this group is relatively novel and opens the door to

consideration of this mode of delivery. The findings suggest several ideas for possible further research.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University College London Hospital.
Trial registration number: NA.
Keywords: hypopituitary, ovulation induction, fertility, pregnancy, outcome

SELECTED ORAL COMMUNICATIONS

SESSION 45: FOR BETTER IVF RESULTS

Tuesday 16 June 2015 **15:15–16:30**

O-161 Mechanical stimulation of the endometrial lining to improve subsequent IVF cycle outcome: an analysis of 7 years of experience

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Study question: To assess the effect of mechanical endometrial stimulation (MES) on IVF treatment outcome in unselected infertility patients.
Summary answer: MES prior to-IVF did not result in improved clinical pregnancy, ongoing pregnancy, or implantation rates. Moreover, stratification of women according to repeated IVF cycles had no impact on treatment outcome when compared with control. Finally, in a stepwise Logistic regression analysis EMS was not associated with improved fertility outcome.
What is known already: Various randomized studies have been published on this topic. Most studies show improvement in cycle outcomes but lack power and uniformity in patient selection. Meta-analysis and systematic reviews have shown benefit from this procedure but the quality of studies included and the uniformity of treatment protocol have been criticized. Lately few larger prospective randomized studies have shown no improvement in subsequent cycle outcome after performing MES compared with controls.
Study design, size, duration: Retrospective matched case control study. Analyzing patients undergoing fresh IVF cycles at a tertiary center between January 2006 and December 2012.The study and control groups consisted of 238 patient cycles in each group. Study patients received MES in a natural cycle preceding the IVF cycle analyzed controls did not.
Participants/materials, setting, methods: Study patients were compared to controls on a 1 to 1 ratio. Patients were matched based on age, number of previous IVF attempts and number of embryos transferred. Only first time MES patients were included in the study group. Only one cycle was analyzed per patient in the control group.
Main results and the role of chance: Comparison of baseline and cycle characteristics showed no difference in: age (32.6 ± 4.5 vs.32.6 ± 4.5, $p = 0.11$, matched), number of previous cycles (0.96 ± 1.6 vs.0.96 ± 1.96, $p = 0.9$, matched), number of oocytes collected (8.7 ± 4.5 vs.8.13 ± 5.03, $p = 0.8$), estradiol level on hCG day (5027.9 ± 2904 vs. 4960 ± 2797 pMol/L, $p = 0.69$), number of embryos transferred (1.3 ± 0.46 vs. 1.29 ± 0.46,$p = 0.8$, matched) and quality of embryos transferred (1.21 ± 0.91 vs.1.13 ± 0.87, $p = 0.13$) in the study and controls respectively.
Clinical and ongoing pregnancy rates were 34.03% and 40.33% ($P = 0.18$) and 18.48% and 28.99% ($P = 0.33$) for the study and control group respectively.Implantation rates were respectively similar (28.06% vs. 30.08%, $p = 0.8$). Logistic regression performed to assess the weight of influence of various variables on pregnancy rates found no significant association between EMS and pregnancy rate.
Limitations, reason for caution: This is a retrospective analysis and as such harbors various limitations. The study and control groups are composed of un-selected infertility patients. Even though we found no improvement in outcomes when results were analyzed after stratification to number of previous IVF attempts, the number of patients in t in each subgroup of previous cycles are not sufficient to reach definite conclusions.
Wider implications of the findings: MES in un-selected sub fertile population has no advantage in improving IVF outcome, thus, it should not be used routinely as a means to improve fertility in un-selected women during IVF treatments. Further prospective studies are warranted to evaluate possible benefits in different subsets of patients such as high order implantation failures.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kaplan Medical Center (internal funding).
Trial registration number: Retrospective analysis of patients charts in a tertiary IVF referral center. No registration was required.
Keywords: IVF, outcome, biopsy, endometrium

O-162 "I will use my own": until what age?

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Study question: In women ≥38 years, what is the real efficiency of an IVF cycle plus the frozen embryo transfer (FET) resulting from the fresh cycle? What is the expected cumulative live-birth rate (CLBR) according to women's age and the number of oocytes retrieved?
Summary answer: CLBR significantly decreased with increasing age (23.6% in 38–39 years, 1.3% in ≥44 years). CLBR significantly rose with increasing oocyte yield. In women 38–39 years 5 oocytes provided a 20% CLBR, in ≥44 years CLBR of 3% was never reached (regardless of oocyte yield) and thus IVF with their oocytes should be discouraged.
What is known already: IVF is increasingly applied to advanced-aged women (in Spain >60% of IVF cycles are in ≥35 years). Live birth rates (LBR) decrease exponentially after 37 years due to diminished ovarian reserve and oocyte quality but little is known concerning CLBR in advanced-aged women.
The possibility of predicting the CLBR according to patients' age and number of oocytes retrieved is of great interest in order to counsel advanced-aged women willing to do IVF with their oocytes.
Study design, size, duration: Retrospective study carried out in a University-affiliated fertility clinic from 2000 to 2012, including 4195 women and 5841 IVF cycles.
Four age groups are distinguished: Group 1 (G1): 38–39 years; Group 2 (G2): 40–41 years; Group 3 (G3): 42–43 years; Group 4 (G4): ≥44 years.
Participants/materials, setting, methods: 4195 women ≥38 years underwent 5841 cycles (G1 = 2119; G2 = 1883; G3 = 1159; G4 = 680 cycles).
Fresh, frozen and cumulative live-birth rates are analyzed for each group. χ^2 and ANOVA tests were used for comparisons. CLBR is analyzed according to age and oocyte yield (Generalized Additive Model, software R).
Main results and the role of chance: Fresh LBR significantly decreased with increasing age (G1 = 20.3%; G2 = 13.2%; G3 = 6.1%; G4 = 1.2%).
The embryo cryopreservation rate and the number of embryos frozen significantly decreased with increasing age. 1384 frozen cycles were performed and LBR decreased as age increased ($p < 0.001$). Cryopreservation significantly increased LBR in the fresh cycle (compared to cycles that did not cryopreserve) but the extra benefit of the frozen cycles was limited.
CLBR were: G1 = 23.6%; G2 = 15.6%; G3 = 6.6%, G4 = 1.3% ($p < 0.001$).
GAM model revealed significantly higher CLBR with increasing number of oocytes. For a given number of oocytes, differences of CLBR among groups were significant (12 oocytes: 36% in G1 and 2% in G4). Irrespective of the number of oocytes, G4 did not reach 3% of CLBR.
Limitations, reason for caution: Our results are limited by the retrospective nature of the study.
Wider implications of the findings: This is the largest study analyzing CLBR (fresh cycle + subsequent FET) in women ≥38 years. Embryo cryopreservation significantly increased LBR in fresh cycles but added limited benefit after frozen cycles.
Women of ≥44 years should be advised against doing an IVF with their own oocytes. Women in the other age groups should be counselled regarding CLBR according to their age and oocyte yield. Being able to predict ovarian response in advance is of crucial interest.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study was performed under the auspices of Cátedra d'Investigació en Obstetricia i Ginecología of the Department of Obstetrics and Gynecology, Hospital Universitario Quirón Dexeus, Universitat Autónoma de Barcelona.
Trial registration number: NA.
Keywords: Ovarian response, IVF, frozen embryo transfer, cumulative livebirth rate, advanced-aged women

O-163 Increased pregnancy rates following luteal GnRH agonist addition in natural thawed cleavage-stage embryo transfer cycles: a prospective, randomized, placebo-controlled study

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Study question: Does the addition of a single luteal GnRH agonist administration in natural thawed cleavage-stage embryo transfer cycles (NC-FET) influence pregnancy rates?
Summary answer: Luteal-phase GnRH agonist administration significantly increased pregnancy rates in NC-FET.
What is known already: In fresh IVF cycles luteal phase support is well proven to be associated with a significantly higher live birth rate. In contrast, the physiological rational of luteal phase support in NC-FET is less obvious. Indeed, the effect of such luteal phase support was examined in several studies, yielding inconsistent results.
A meta-analysis showed significantly higher clinical pregnancy rate, multiple pregnancy rate and live birth rate following GnRH agonist addition to the luteal support scheme in fresh ICSI/IVF-embryo transfer cycles. There are no data in the literature regarding the effect of GnRH agonist administration during the luteal phase in NC-FET.
Study design, size, duration: A prospective, randomized, placebo-controlled, assessor blinded study was performed including NC-FET cycles of at least two cleavage stage embryos, transferred 3 days after detection of the endogenous LH surge. All patients were treated with 4 injections of 2,500 I.U. hCG every 3 days starting on the day of ET. Patients were randomly assigned to receive a single injection of GnRH agonist 3 days following ET (triptorelin acetate 0.1 mg) or placebo (saline). NC-FET cycles performed between September 2013 and December 2014 at a university-based hospital were included for analysis.
Participants/materials, setting, methods: A total of 91 NC-FET cycles were included for analysis, of them 47 cycles were randomized to the GnRH-agonist group and 44 cycles were randomized to the placebo group. Maternal and paternal ages, maternal BMI, maternal gravity and parity, main cause of infertility, cycle rank, number and quality of embryos transferred, freezing method, maximal estradiol levels and maximal endometrial thickness were taken into account. Pregnancy rates were the primary outcome parameter.
Main results and the role of chance: The two groups did not differ in mean age, gravidity, parity, BMI, cycle number, maximal endometrial thickness and estradiol levels, number of embryo transferred per patient, freezing method and embryo quality. Administration of GnRH agonist significantly increased pregnancy rate compared to controls (57.4% versus 31.8% respectively, $P = 0.02$), clinical pregnancy rate per ET (51% versus 27.2% respectively, $P = 0.029$), and implantation rate (25.2% versus 13.6% respectively, $P = 0.036$). A multivariable logistic regression analysis showed a single significant predictor for pregnancy, namely luteal GnRH-agonist administration (OR 3.2, CI = 1.2–8.3, $P = 0.016$).
Limitations, reason for caution: The conclusions are limited by the study size, and lack of data about live births, as some of the pregnancies are still ongoing.
Wider implications of the findings: Luteal-phase GnRH agonist administration significantly enhances pregnancy rates in natural frozen-thawed cleavage-stage embryo transfer cycles, possibly by a combination of effects on the embryo, endometrium and the corpus luteum.
The beneficial effect of luteal GnRH-agonist on pregnancy rates in NC-FET cycles may imply a possible beneficial effect of luteal GnRH-agonist in other fertility treatments, such as ovulation induction, controlled ovarian hyperstimulation etc.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Assaf Harofeh Medical Center.
Trial registration number: NCT01933009.
Keywords: frozen-thawed embryo transfer, GnRH-agonist, natural cycle, luteal support

O-164 Diagnostic accuracy of saline infusion sonography in asymptomatic patients prior to IVF

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Study question: Is saline infusion sonography an accurate test for diagnosing unexpected intrauterine pathology before starting IVF?
Summary answer: Saline infusion sonography (SIS) has a high specificity in diagnosing intrauterine abnormalities, but sensitivity is limited.
What is known already: SIS is highly accurate in diagnosing intrauterine abnormalities in subfertile women with suspected uterine pathology, based on clinical symptoms. However, in a substantial part of asymptomatic subfertile women intrauterine abnormalities are detected at hysteroscopy. Current literature suggests that these intrauterine abnormalities might have a negative impact on IVF outcome. Screening for such abnormalities is usually done by hysteroscopy. Studies investigating the role of SIS in diagnosing unsuspected intrauterine abnormalities before starting IVF are lacking.
Study design, size, duration: Between 2011 and 2013, we performed a multicenter, prospective cohort study as part of a RCT in 5 of 22 hospitals in the Netherlands, that was positioned in a nationwide consortium for studies in women's health. Eligible were asymptomatic subfertile women with a normal transvaginal ultrasound and no history of recurrent miscarriage.
Participants/materials, setting, methods: All women underwent SIS (index test) and hysteroscopy (reference test) before starting the first IVF treatment. SIS and hysteroscopy were performed in the early follicular phase of the menstrual cycle. The hysteroscopy examiner was blinded for the SIS results. We calculated sensitivity, specificity, positive and negative predictive value (PPV and NPV).
Main results and the role of chance: In this substudy of the larger INSIGHT trial, we included 139 women, of whom 14 (10%) had intrauterine abnormalities detected at SIS. Hysteroscopy showed an abnormality in 37 (27%) of the women. Sensitivity and specificity of SIS for diagnosing intrauterine abnormalities were 0.38 (95% CI: 0.21–0.54) and 0.98 (95% CI: 0.96–1.00), respectively, resulting in positive and negative predictive values of 0.86 (95% CI: 0.67–1.04) and 0.84 (95% CI 0.87–0.91). For diagnosing endometrial polyps the sensitivity of SIS was 0.38 (95% CI: 0.20–0.64) and the specificity was 0.97 (95% CI: 0.94–1.00).
Limitations, reason for caution: In one center, for practical reasons, video recordings of the hysteroscopy procedure were assessed by an independent examiner blinded for the SIS results.
Wider implications of the findings: These results show a high false negative rate for SIS in diagnosing unsuspected intrauterine abnormalities. The clinical relevance of such small abnormalities not visualized by SIS must be questioned. SIS could possibly be an appropriate screening tool to detect more prominent abnormalities that may negatively affect pregnancy prospects. Further research is needed.
Study funding/competing interest(s): Funding by national/international organization(s) – ZonMW, the Dutch Organization for Health Research and Development.
Trial registration number: NCT01242852.
Keywords: SIS, Hysteroscopy, Infertility, Diagnostic accuracy

O-165 Supplementation of recombinant LH to poor responders in mid-follicular phase along with recombinant FSH results in better blastocyst formation and implantation rate in antagonist IVF cycles

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Study question: Does addition of exogenous recombinant LH (r-LH) to an IVF/ICSI stimulation protocol with recombinant FSH (r-FSH) improve pregnancy rates in poor responders irrespective of age factor?

Summary answer: Women with poor ovarian reserve classified on the basis of their low AMH levels and antral follicle count (AFC) undergoing GnRH antagonist IVF/ICSI cycles when supplemented with recombinant LH during the late follicular phase show higher blastocyst formation, clinical pregnancy and implantation rates.

What is known already: Two of the several randomized studies using a GnRH agonist protocol have indicated a clinical benefit of r-LH supplementation in patients of advanced age (36 years or older). however, recent prospective RCTs conducted in mainly women of advanced age did not show a significant effect of LH supplementation in controlled ovarian stimulation for IVF/ICSI cycles with GnRH antagonists on pregnancy rates in patients of 35 years or older.

Study design, size, duration: In a randomized control trial (RCT) performed between 2012 and 2014, 106 poor responder women (basal serum AMH <1.2 ng/ml; AFC ≤ 6) undergoing IVF/ICSI irrespective of age, received ovarian stimulation with r-FSH (Gonal-F 150 IU/day) starting from cycle day 2 and GnRH antagonist (Cetrotide 0.25 mg/day) from stimulation day 6.

Participants/materials, setting, methods: On day 6 of stimulation, randomization was carried out such that along with GnRH antagonist (Cetrotide 0.25 mg/day) 54 women received both r-FSH (150 IU daily) and r-LH (Luveris 75 IU/day) whereas 52 women received only r-FSH (150 IU daily). 42 women in each group received day 5/6 blastocyst transfer.

Main results and the role of chance: There were no demographic or clinical differences between the two study groups. However, the r-LH supplementation group showed significantly higher blastocyst formation rate (50.0 vs. 32.53%; $p = 0.02$), top quality blastocysts (3.7 ± 0.8 vs 3.1 ± 0.6; $p = 0.0003$), clinical pregnancy rates (42.86 vs. 23.81%; $p = 0.03$) and embryo implantation rates (38.71 vs. 21.4%; $p = 0.02$) than with group receiving only r-FSH. Implantation rate was calculated as: Total number of embryo sacs × 100/ Total number of blastocysts transferred.

Limitations, reason for caution: A limitation of our study is its small number of cases. Secondly, only cycles involving day 5/6 blastocyst transfers have been taken into consideration.

Wider implications of the findings: Although Cochrane review indicates that LH supplementation has no benefit on ongoing pregnancy rates in elderly women ≥35 years of age, our study demonstrates that LH supplementation may immensely benefit poor responder patients if poor ovarian response is defined on the basis of basal serum AMH level and AFC rather than just on the basis of age. This study also indicates that proper classification of patients is required to analyze response to various drugs.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Self funded by our own IVF clinic: Vaunshdhara Clinic and assisted Conception Centre, Nagpur.

Trial registration number: NA.

Keywords: r-LH, r-FSH, antagonist, implantation, poor responder

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SELECTED ORAL COMMUNICATIONS

SESSION 46: OPTIMIZING EARLY PREGNANCY MANAGEMENT

Tuesday 16 June 2015 **15:15–16:45**

O-166 Trends in ectopic pregnancy rates following assisted reproduction technologies. A 12 year nationwide analysis on 160.000 pregnancies

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Study question: What is the evolution of ectopic pregnancy (EP) rates following ART over time and how is this affected by the advancement of ART techniques and changes in the incidence of specific causes of infertility?

Summary answer: Ectopic pregnancy rates following ART have progressively decreased, practically halving, over a period of 12 years. This decrease may be partially due to both the progressive proportional reduction of tubal factor infertility as an indication and several optimizations of ART (such as reduction of embryos transferred and extended embryo culture).

What is known already: Ectopic pregnancy rates following ART are significantly increased compared with natural conception. Although the reasons behind this increased incidence of EP following ART are not completely understood, and several mechanisms have been proposed, no study up to date attempted to evaluate whether progress made over time in the optimization of the ART techniques and changes in the incidence of specific causes of infertility had any impact on the overall EP rate.

Study design, size, duration: Nationwide population-based analysis of anonymized data from the HFEA database including all pregnancies following ART cycles carried out in the UK between 2000 and 2012.

Participants/materials, setting, methods: Overall, data from 161.967 treatment cycles resulting in a pregnancy were included in the analysis. Among them 8852 pregnancies were derived through IUI and 153.115 pregnancies through IVF/ICSI.

Main results and the role of chance: During a period of 12 years (2000–2012) approximately 1.4% (2 244) of all pregnancies following ART were EPs. According to unadjusted analysis, EP was borderline significantly higher after IVF/ICSI (1.4%) compared with IUI (1.1%), $p = 0.043$.

Overall, EP rates decreased over time, crude odds ratio OR 0.96 per year, CI 95% 0.95–0.97 and results remained significant even after adjustment for potential confounders (aOR 0.97 per year, CI 95% 0.95–0.98).

However, analysis according the type of ART procedure demonstrated that the incidence of EP significantly decreased over time only in IVF/ICSI cycles (OR 0.96 per year, CI 95% 0.94–0.97), but not in IUI cycles (OR 0.96 per year, CI 95% 0.90–1.02).

When analysis was pertained only in pregnancies following IVF/ICSI, the major risk factor for EP was the presence of tubal infertility (aOR 2.23, CI 95%1.93–2.58), followed by the increased number of embryos transferred [(2 vs.1 embryo, aOR 2.23 CI 95% 1.10–1.48) and (3 or more vs.1 embryo transferred aOR 1.67 CI 95% 1.34–2.09)]. Finally the presence of unexplained infertility also increased the risk of EP.

On the contrary, the use of extended embryo culture to day 3 or day 5 significantly reduced the risk of EP compared with the transfer of early cleavage (day 1/2) embryos, aOR (95% CI) 0.85(0.76–0.94) and 0.73(0.63–0.84), respectively. Finally the use of ICSI compared with IVF resulted in a lower EP risk (aOR 0.78 CI 95% 0.70–0.87).

Surprisingly, and contrary to smaller previous studies, the use of exogenous ovarian stimulation or the use of frozen compared to fresh embryos had no impact on the EP rates following IVF/ICSI.

Limitations, reason for caution: Owing to the use of registry data, well established risk factors of EP such as smoking habits or even uterine surgery could not be assessed. As nationwide anti-smoking campaigns with very high success rates have been implemented in the UK over the last decade, it is highly likely that reduction in smoking prevalence would have had a substantial impact on the progressive decrease in the incidence of EP over time which we were not able to assess.

Wider implications of the findings: Our findings, for the first time, suggest that EP rates following IVF/ICSI progressively decreased over a period of 12 years. This decrease appears to be strictly associated with the reduction in the incidence of tubal factor infertility and the increased use of lower number of embryos transferred and extended embryo culture. Consequently, implementation of national programs aiming to reduce the incidence of tubal infertility, such as the National Chlamydia Screening Programme, should be further reinforced. In addition, campaigns towards the widespread introduction of single embryo transfer should be promoted since this may not only reduce the incidence of multiple pregnancies, but also the incidence of EP following IVF/ICSI.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Vrije Universiteit Brussel(VUB)/Universitair Ziekenhuis Brussel (UZ Brussel).

Trial registration number: NA.

Keywords: ectopic pregnancy, ART, nationwide population based analysis, IVF/ICSI, tubal factor infertility

O-167 Subclinical hypothyroidism and live birth rate in women with unexplained recurrent miscarriage

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Study question: Is subclinical hypothyroidism (SCH) associated with a l ower live birth rate in women with unexplained recurrent miscarriage (RM)?

Summary answer: SCH in women with unexplained RM did not lower live birth rate. Ongoing pregnancy and miscarriage rates were not different between women with SCH compared to euthyroid women with RM.

What is known already: SCH is associated with adverse pregnancy outcomes including perinatal mortality, placental abruption and pre-eclampsia. The evidence of an association between SCH and miscarriage is controversial. Most of the published studies were cohort studies applying different TSH cut off levels. In overt hypothyroidism, low TSH is associated with miscarriage, fetal or neonatal death. The influence of SCH on live birth rate in women with RM is less well defined.

Study design, size, duration: All 1341 women were prospectively included in the database. The present study was defined retrospectively, and analyses were performed retrospectively. Primary outcome measure was live birth rate (LBR). Secondary outcome measures were ongoing pregnancy rate and miscarriage rate.

Participants/materials, setting, methods: Women with RM, 18–40 years, who visited a tertiary recurrent miscarriage clinic (Liverpool Women's Hospital) from 2000 to 2011. Women with TSH > 3.8 mU/L (95th percentile) and normal fT4 were classified as having SCH. The association between SCH and LBR was determined by logistic regression adjusted for age and previous miscarriages.

Main results and the role of chance: Of 1341 women with recurrent miscarriage, 68 (5.0%) had SCH, 1264 (94.2%) were euthyroid and 9 (0.7%) had an abnormal fT4 with a normal TSH. Baseline characteristics were comparable (age and previous live births). Previous miscarriages were lower in SCH compared to euthyroid women (3.0 vs. 3.4, $p = .03$).

The LBR was 33.8% in women with SCH and 34.1% in euthyroid women (OR 0.95, 95% CI 0.6–1.6). The ongoing pregnancy rate was 42.6% in women with SCH and 45.5% in euthyroid women (OR 0.9, 95% CI 0.5–1.5). The miscarriage rate was 22.1% in women with SCH and 18.2% in euthyroid women (OR 1.3, 95% CI 0.7–2.3). Comparable results were found when SCH was defined as TSH > 4.8 mU/L (97.5th percentile) and normal fT4.

Limitations, reason for caution: Data are prone to selection bias, since this is a cohort study. Only the most important potentially confounding variables were available. Couples had different follow-up times, therefore the actual live birth rates in both groups might be different. Missing data on outcome occurred in 36% of cases.

Wider implications of the findings: No difference in live birth rates, ongoing pregnancy and miscarriage rates were seen in women with unexplained RM with SCH compared to euthyroid women. This study supports current practice guidelines, that screening of women with RM for detecting SCH is not necessary.

Study funding/competing interest(s): Funding by University(ies) – Academical Medical Centre Amsterdam.

Trial registration number: NA.

Keywords: Recurrent miscarriage, Thyroid, Subclinical hypothyroidism

O-168 Stress and depression at referral do not entail a lower chance of live birth/ongoing pregnancy for women with recurrent pregnancy loss

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Study question: Is emotional stress or depression at referral associated with lower chance of live birth/ongoing pregnancy one year after referral to a tertiary Recurrent Pregnancy Loss (RPL) Unit?

Summary answer: High scores on stress and depression scales are not associated with a lower chance of live birth/ongoing pregnancy after 12 weeks' gestation one year after referral. Women with a live born child/ongoing pregnancy have lower scores on depression and stress scales at follow-up.

What is known already: We have recently established that high stress levels and moderate/severe depression frequently occur among women with RPL (Kolte AM et al, Human Reproduction, in press) in concordance with other studies. However, to our knowledge, this is the first longitudinal study of emotional distress and RPL.

Study design, size, duration: A prospective study of self-reported stress and depression at referral and one year after was performed from 2010 to 2014. 301 women completed an online baseline questionnaire and of these, obstetrical outcome was confirmed for 287 women (95%). 185 women completed a follow-up questionnaire.

Participants/materials, setting, methods: The study was conducted in the Danish RPL Unit. Psychological stress was evaluated by the Perceived Stress Scale (PSS) and depression by the Major Depression Inventory (MDI). We used logistic regression, paired samples T-test and independent samples T-test for comparisons.

Main results and the role of chance: Of the 287 women, 167 (58%) had a live birth in the year after referral or an ongoing pregnancy at follow-up. High scores on the PSS or the MDI at referral were not associated with a negative prognosis; OR 1.00 (95% CI 0.99; 1.05) and OR 1.00 (95% CI 0.97; 1.00), respectively. Women with a subsequent live birth/ongoing pregnancy showed a significant decrease in emotional distress from referral to follow-up. PSS scores: mean 16.94 versus 12.47, difference -4.47 (95% CI -5.94; -3.00). MDI mean: 13.59 versus 10.12, difference -3.47 (95% CI -5.95; -1.00). This was not observed for the remaining patients. PSS mean: 16.86 versus 15.49, difference -1.37 (95% CI -2.82; 0.08). MDI mean: 12.65 versus 12.92, difference 0.27 (95% CI -2.07, 2.61).

Limitations, reason for caution: Although both scales are validated and widely used, face-to-face interview data would have strengthened the conclusions. It would also have been interesting to include the women's partners in the study.

Wider implications of the findings: Feelings of guilt and self-blame are integral elements in depressive disorders and prominent among women with RPL. The results from this prospective study of emotional distress in RPL can be actively used in the care for women with RPL to assure patients that feelings of stress or depression do not lead to a poorer prognosis in the first year after referral.

Study funding/competing interest(s): Funding by hospital/clinic(s) – University Hospital Copenhagen, Rigshospitalet.

Trial registration number: NA.

Keywords: recurrent pregnancy loss, stress, depression, prospective cohort study

O-169 Does dilatation and curettage (D&C) increase the risk of preterm birth in the subsequent pregnancy? A systematic review and meta-analysis

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Study question: Does Dilatation and Curettage (D&C) for a miscarriage or induced abortion increase the risk of preterm birth in subsequent pregnancy?

Summary answer: This meta-analysis shows that D&C is associated with increased risk of subsequent preterm birth.

What is known already: D&C is a frequently used procedure in obstetrics and gynecology. The procedure is generally considered to be safe and easy to perform, but serious adverse effects, e.g., cervical tears, bleeding, infection, perforation of the uterus, bowel or bladder and Asherman syndrome, may occur.

As suggested by some, preterm birth in subsequent pregnancies might also be an adverse effect of D&C.

Study design, size, duration: We conducted a systematic review and meta-analysis of cohort and case-control studies.

Participants/materials, setting, methods: We searched OVID MEDLINE and OVID EMBASE form inception until May 2014. We selected cohort studies comparing subsequent preterm birth in women who had a D&C for miscarriage or IA and a control group, and case control studies assessing a history of D&C among women with and without D&C.

Main results and the role of chance: We included 21 studies reporting on 1,853,017 women. In women with a history of D&C, the odds ratio (OR) for preterm birth (<37 weeks) was 1.29 (95% CI 1.17; 1.42), while for very preterm birth ORs were 1.69 (95% CI 1.20; 2.38, <32 weeks) and 1.68 (95% CI 1.47; 1.92, <28 weeks). The risk remained increased for D&C when the control group was limited to women with a medically managed miscarriage or induced abortion: OR 1.19 (95% CI 1.10; 1.28). For women with a history of multiple D&Cs, the OR for preterm birth (<37 weeks) was 1.74 (95% CI 1.10; 2.76). When the analysis was limited to spontaneous preterm birth subsequent to D&C the OR was 1.44 (95% CI 1.22; 1.69).

Limitations, reason for caution: There were no randomized controlled trials comparing women with and without a history of D&C and subsequent preterm birth. Furthermore confounding influence the results since included studies were either cohort or case control studies and not all studies corrected their results for possible confounding factors.

Wider implications of the findings: This meta-analysis shows that D&C is associated with increased risk of subsequent preterm birth. Although confounding cannot be excluded, these data warrant caution in the use of D&C for miscarriage and induced abortion. This conclusion should contribute to the implementation of misoprostol as a non-invasive treatment option for both miscarriage and induced abortion.

Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by ZonMw, a Dutch governmental organization for Health Research and Development. Project number 80-82310-97-12066.

Trial registration number: NA.

O-170 Curettage versus expectant management in women with an incomplete evacuation of the uterus after treatment with misoprostol for miscarriage: the misorest trial (NTR 3310)

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[12]*University of Adelaide, The Robinson Research Institute, Adelaide, Australia*

Study question: What are the effects of curettage versus expectant management in women with incomplete evacuation of the uterus after misoprostol treatment for first trimester miscarriage?

Summary answer: Expectant management is effective in about 80% of cases. Surgical management (curettage) is effective in 93% of the women but has a higher risk of complications.

What is known already: Misoprostol is an inexpensive medical treatment for women with first trimester miscarriage. However, about 30% of women with a miscarriage treated with misoprostol are left with an incomplete evacuation of the uterus. Although these women are usually asymptomatic, curettage is often performed. While the effectiveness of curettage for these women is unknown, it might lead to intra-uterine adhesions and a higher risk of preterm birth in future pregnancies.

Study design, size, duration: Between June 2012 and June 2014, we performed a RCT in 27 hospitals positioned in the Dutch network for women's health research. Women with incomplete evacuation of the uterus after misoprostol for miscarriage were randomized to curettage or non-intervention. Women with a preference for a treatment were followed prospectively.

Participants/materials, setting, methods: We compared curettage (within three days after randomization) versus no intervention. Primary outcome was sonographic evidence of an empty uterus after six weeks. In case of missing sonography, uneventful clinical follow-up of 3 months was assumed to indicate an empty uterus. Complications were excessive blood loss, infection or thromboembolism.

Main results and the role of chance: We studied 259 women, of whom 59 were randomized (curettage (n = 30), expectant management (n = 29)), and 200 were treated according to their preference (curettage (n = 70), expectant management (n = 130)).

In the RCT the primary outcome occurred in 92% of women in the surgical group and 70% of women in the expectant group (RR 1.3, 95% CI 0.99–1.7). The rate of complications was 13% in the curettage group versus 9% in the expectant management group (RR 1.5, 95% CI 0.28–8.2). In the cohort the primary outcome occurred in 94% of women in the surgical group and 82% of the expectant group (RR 1.14, 95% CI 1.03–1.3), and there were more complications in the curettage group (12% versus 3%, RR 4.2, 95% CI 1.1–16).

Limitations, reason for caution: Because of strong preferences for expectant management, inclusions in the RCT were limited, thus affecting the power of the study. However, combining RCT and cohort participants resulted in sufficient power to answer the study question.

Wider implications of the findings: This study shows that in women with an incomplete evacuation of the uterus after misoprostol for miscarriage, expectant management is safe and effective. Curettage, being more effective, has a higher risk of complications. These findings might lead to a further implementation of misoprostol treatment for first trimester miscarriages.

Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by ZonMw, a Dutch organization for Health Research and Development. Project number 80-82310-97-12066.

Trial registration number: Dutch Trial Register NTR3310, http://www.trialregister.nl.

Keywords: miscarriage, misoprostol, incomplete evacuation, expectant management

O-171 Provision of intrauterine contraception at the time of abortion reduces subsequent abortions – first-year results of a randomized, controlled trial

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Study question: Can the need of subsequent induced abortion be reduced already during the first year following an induced abortion by routine provision of intrauterine contraception at the time of abortion (either medical or surgical)?

Summary answer: Provision of intrauterine contraception as an integral part of abortion service is safe and effective in reducing the number of subsequent abortions by more than half already during the first year after the index abortion.

What is known already: Women requesting induced abortion are typically 20–30 years old and of high fertility. The need of subsequent abortions is high, approximately 30–40%. In cohort studies the use of long-acting reversible contraceptives (LARC), especially intrauterine devices (IUD) or levonorgesterel-releasing intrauterine system (IUS) have association with reduced risk of repeat abortion.

Study design, size, duration: In this RCT, conducted between 2010 and 2013 at Helsinki University Hospital, we compared early vs. patient-controlled IUD provision. The sample size calculation (80% power and 5% significance level) assumed a 15% risk of subsequent abortion during a five-year follow-up time and a 50% risk reduction with the study intervention.
Participants/materials, setting, methods: Altogether 756 adult women undergoing abortion during the 1st trimester were randomized. The intervention-group (n = 377) received IUD at the surgical abortion or 2–3 weeks after medical abortion. In the control group (n = 376) post-abortal contraception provision was patient-controlled. Subsequent abortions were identified from Finnish Abortion Registry and hospital records.
Main results and the role of chance: During the first year of follow-up 24 (3.2%) women requested a subsequent induced abortion according to intention-to-treat (ITT) -analysis. Kaplan-Meier analysis showed 98.4 and 95.0% cumulative proportions of women without subsequent abortion at one year among the intervention group and the control group, respectively (p = 0.012). During the first follow-up year the study intervention decreased the need of subsequent abortion [2(0.6) vs. 18 (5.0), p = 0.001; per-protocol analysis]. Early IUD provision did not induce any insertion-related complications. None of the subsequent pregnancies were conceived during IUD use.
Limitations, reason for caution: This single-center study was conducted in Helsinki responsible for 15% of all Finnish induced abortions. The power calculation was based on 5-year follow-up. However, a highly significant decrease in need of repeat abortion was evident during this one-year follow-up. In Finland all induced abortions are reliably tractable from Abortion Registry.
Wider implications of the findings: The present results have public health impact – in order to decrease the need of subsequent abortion, provision of LARC, and especially IUD/IUS for post-abortal contraception should be part of high quality abortion services.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by national/international organization(s) – This study was supported by the Helsinki University Central Hospital Research funds and by research grants provided by Antti and Jenny Wihuri Foundation and by Yrjö Jahnsson Foundation. The City of Helsinki provided the IUSs and IUDs used.
Trial registration number: www.clinicaltrial.gov NCT01223521.
Keywords: Induced abortion, LARC, subsequent abortion, IUD, IUS

SELECTED ORAL COMMUNICATIONS

SESSION 47: SPERM DAMAGE: A MATTER OF CONCERN?

Tuesday 16 June 2015 **15:15–16:30**

O-172 Oxidative stress in semen is not related to semen parameters and does not affect reproductive outcomes in cycles with donor oocytes

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Study question: Does the level of oxidative stress detected in the ejaculate relate to semen characteristics or reproductive outcomes in assisted reproduction cycles with donor oocytes?
Summary answer: The level of oxidative stress detected in the ejaculate does not relate to either motility and proportion of normal spermatozoa, nor with the reproductive outcomes of assisted reproductive cycles carried out with the semen.
What is known already: A balance between reactive oxygen species (ROS) and antioxidants is required for efficient fertilization; an imbalance between these two factors in semen causes oxidative stress (OS). OS can harm fertilization by affecting the sperm membrane or by DNA damage. While oral treatment with antioxidant is currently being offered to patients with altered semen characteristics, the relationship between sperm OS and ART remains unclear, with conflicting data on its relationship to seminal characteristics and reproductive outcomes.
Study design, size, duration: This is a prospective cohort study, carried out in a fertility center between October 2013 and December 2014. The study was approved by the local IRB, and included 132 consecutive patients attending the clinic for IVF treatment with donated oocytes.
Participants/materials, setting, methods: Semen analysis was performed according to WHO guidelines. Ejaculate volume, concentration, motility (% of a + b forms) and morphology (% of normal spermatozoa) were measured. A colorimetric test based on nitro blue tetrazolium assay, which measures the level of O-2 in the ejaculate, was carried out within 1 h from semen collection to assess OS.
Main results and the role of chance: The OS assay classified samples into: very high (n = 2; 1.5%), high (n = 57; 43.2%), low (n = 40; 30.3%), and very low (n = 33; 25.0%). Overall values for seminal parameters were: volume (ml) = 4.2 (SD 2.1); motility (a + b%) = 47.7 (SD 18.0), and normal spermatozoa (%) = 8.2 (SD 5.1). Thirty (22.3%) of the patients did not start their IVF cycle at the time of analysis and there was 1 (0.8%) fertilization failure. Of the ninety-three cycles that reached embryo transfer, 55% evolved in biochemical, 45% in clinical, and 34% in ongoing pregnancy. We found no correlation between OS and seminal parameters, fertilization rate, or pregnancy outcomes. Additional regression analyses for pregnancy outcomes, adjusted for day of transfer and number of embryos transferred, showed no effect of OS level.
Limitations, reason for caution: This study was carried out in donation cycle and using ICSI, thus the high quality of the female gamete and the fertilization technique might have masked a partial functional damage to the sperm DNA. Caution should be exerted when extending the results to older women and classical IVF cycles.
Wider implications of the findings: The lack of relationship between sperm parameters and OS implies that prescribing antioxidant based on sperm characteristics might not be effective; reproductive results in ART with young oocyte and ICSI does not seem to be affected by OS levels in the ejaculate.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.
Keywords: oxidative stress, ICSI, oocyte donation, spermatozoa

O-173 Assessment of the impact of oxidative stress on frozen seminal plasma in fertile and infertile men by examining the total antioxidant capacity

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Study question: To explore the impact of oxidative stress on frozen seminal plasma in fertile and infertile men by examining the total antioxidant capacity
Summary answer: Total antioxidant capacity of the seminal plasma as measured by the luminometric assay is a reliable and simple test for the diagnosis and management of male infertility
What is known already: The most common cause of male infertility is defective spermatozoal function. It may result from testicular pathologies, genetic disorders, and exposure to drugs, toxins, or irradiations, or because of oxidative stress damage. The mechanism of action for loss of sperm function may be because of elevated levels of reactive oxygen species [ROS] beyond the available total antioxidant capacity in the semen. Low level of seminal total antioxidant capacity [TAC] has a key role in male infertility. Oxidative stress causes damage to the spermatozoa, oocyte, and embryos. Several reports relate low seminal plasma TAC levels to male infertility, as well as in embryo culture media from the oocytes, cumulus cell mass, and spermatozoa used for insemination in conventional IVF. The potential cellular sources of TAC in an intracytoplasmic sperm injection setting are the spermatozoa and the injected oocytes
Study design, size, duration: Seminal plasma from proven fertile men [n = 50] and infertile patients [n = 50] were examined for TAC level, semen paremeters as morphology, motility and concentration, and DNA integrity test.
Participants/materials, setting, methods: Infertile patients from male infertility clinic of various diagnoses and fertile mens
Interventions: Seminal plasma TAC measurement by luminometric assay using the TAC assay kit, semen analysis parameters, DNA integrity test
Main results and the role of chance: Fertile men showed higher TAC values [median and SD]: 1201 μM [SD ± 548]; compared with the infertile patients: 831 μM [SD ± 343]. The result from sperm morphology of fertile patients showed mean percentage of 4.8% [SD ± 1.68] and the percentage in infertile

group of 2.68% [SD ± 1.68]. The same group of samples, analyzed for DNA damage showed mean of DFI 10.38% [SD ± 5.17%] for fertile men and mean of DFI 17.22% [SD ± 7.22%] for infertile men

Limitations, reason for caution: No limitations

Wider implications of the findings: An oxidative stress test may accurately discriminate between fertile and infertile men and identify those with a clinical diagnosis of male-factor infertility who are likely to initiate a pregnancy if they are followed over a period of time. In addition, such a test can help select subgroups of patients with infertility in which oxidative stress is a significant factor, and those who may benefit from antioxidant supplementation. To assess seminal oxidants is concerning the inclusion of oxidative stress analysis as part of the routine diagnostic workup of an infertile male.

Study funding/competing interest(s): Funding by University(ies) – Scientific research project [no.3 -2013] of Medical University-Pleven.

Trial registration number: NA.

Keywords: ART, Semen, TAC

O-174 Fertilix, a novel antioxidant formulation designed to treat male infertility emanating from sperm oxidative DNA damage: Promising preclinical evidence from mouse models

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Study question: Does Fertilix, a novel antioxidant formulation designed for the male reproductive tract, reduce Sperm DNA Damage (SDD) and increase pregnancy rates in mouse models of sperm oxidative stress (SOS)?

Summary answer: Oral administration of Fertilix for a period of 2 weeks significantly reduces SDD in Glutathione Peroxidase 5 (GPX-5) knockout mice and restores pregnancy rates almost back to normal levels in mice subjected to Scrotal Heat Shock (SHS).

What is known already: Animal and human studies document the adverse effect of SDD on fertilization rate, embryo quality, miscarriage rates and the transfer of *de novo* sporadic mutations to the offspring. Semen samples of infertile men are known to be deficient in several key antioxidants relative to fertile counterparts. Antioxidants alone, or in combination, have consistently demonstrated a measure of efficacy against sperm oxidative stress or DNA damage in numerous human clinical trials.

Study design, size, duration: Fertilix efficacy was evaluated in two, well-established mouse models of SOS, SHS and GPX-5 knockout mice, each with $n = 12$, by independent laboratories. Mice were provided Fertilix in their drinking water for 2–4 weeks and compared with control groups for SDD and pregnancy rates.

Participants/materials, setting, methods: In SHS model, each male's fertility was tested by partnering with 3 females for 5 days. The percentage of pregnant females, number of vaginal plugs, resorptions per litter, and litter size were recorded. Sperm DNA oxidative damage was evaluated by immunocytochemical detection of 8-OHdG residues in GPX-5 KO mice.

Main results and the role of chance: 8-Hydroxy-deoxy Guanosine (8-OHdG) is a biomarker of DNA oxidation. The average background levels of 8-OHdG in WT mice is around 30%. This level doubles up to about 60% in transgenic mice deficient in the antioxidant enzyme GPX-5. Our results indicate that a 2 week pretreatment of GPX-5 KO mice with Fertilix provides complete protection of sperm DNA against oxidation. In mouse models of SHS, only 35% (19/54) female mice got pregnant resulting in 169 fetuses. This is in contrast to the Fertilix pretreated group where 74% (42/57) female mice got pregnant resulting in 427 fetuses. The role of chance in obtaining supporting results for the efficacy of Fertilix in both models is minimal.

Limitations, reason for caution: It was not possible to ensure that every mouse took 100% of the product for the treatment period.

Wider implications of the findings: The present situation is gravely concerning as clinical studies confirm moderate to severe SDD in about 60% of all men visiting IVF centers and about 80% of men diagnosed with idiopathic male infertility. These results, if confirmed in humans, will impact clinical fertility practice. Antioxidant supplementation will be an adjuvant therapy prior to undertaking ART procedures to improve fertilization rates, maintain a healthy pregnancy, and reduce *de novo* sporadic mutations being passed onto children.

Study funding/competing interest(s): Funding by University(ies) – The study was funded by the University of Clermont-Ferrand and the University of Madrid. The corresponding author, A.M., is an employee of CellOxess LLC, which has a commercial interest in the detection and resolution of oxidative stress. The author, *P*.G., is the Managing Director of CellOxess LLC, which has a commercial interest in the detection and resolution of oxidative stress.

Trial registration number: Not applicable. The local ethics committee authorized this study.

Keywords: DNA Damage, Male Infertility, Oxidative Stress, Antioxidant, Nutraceutical

O-175 The influence of paternal age on reproductive outcome in Assisted Reproductive Techniques

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Study question: Does advanced paternal age affect reproductive outcome in assisted reproductive techniques (ART)?

Summary answer: No significant influence of paternal age on ongoing pregnancy rate (OPR) in ART was found in this study.

What is known already: The effect of delayed male parenthood has not been of much social or biological interest, as it has been noted that the risks are negligible compared to the maternal effect. Little is known about the influence of paternal age on the reproductive outcome after ART.

Study design, size, duration: We performed a retrospective cohort study. A total of 7051 first ART cycles between 2001 and 2013 were evaluated with regard to: age of men and women at the time of ovum pick up, *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), embryo quality and pregnancy results.

Participants/materials, setting, methods: Age effects on OPR were analyzed, using 3 age classes for males and females, based on odds ratios estimated by logistic regression. First, we analyzed the effect of paternal age on OPR by controlling for maternal age. In a second model we used interaction terms for paternal and maternal age.

Main results and the role of chance: When maternal/paternal interaction was not taken into account, we did not found a significant paternal age effect. After including interaction terms we did found significant age effect in couples with: males ≤35 and females 35–40 (OR 0.53 CI 0.39–0.73); males 35–45 and females 35–40 years (OR 0.69 CI 0.60–0.79); males >45 and females 35–40 (OR 0.75 CI 0.56–0.99); males 35–45 and females >40 (OR 0.40 CI 0.28–0.56); and males >45 and females >40 (OR 0.36 CI 0.21–0.62). Regardless female age, no evident decline in odds ratios was seen towards a decrease in OPR in ageing men. In subgroups analysis, based on treatment or number of embryos transferred, similar results were found. No paternal age effect was found in biochemical pregnancy rate and embryo quality as well.

Limitations, reason for caution: The study is limited by the lack of information on live births and other factors as indication for treatment, and time to treatment. OPR was therefore chosen as major outcome.

Wider implications of the findings: No significant influence of paternal age on OPR in ART was found in this study. As increased paternal age is associated with disturbance of spermatogenesis, increased DNA damage in sperm and late development effects such neurological disturbances, we should consider the possible increase in *de novo* mutations or epigenetic alterations affecting the offspring.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Radboud university medical center.
Trial registration number: None.
Keywords: paternal age, ART, pregnancy rate

O-176 Predictive capacity of sperm DNA damage tests: A systematic review and meta-analysis

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Study question: Can sperm DNA damage test predict success of assisted reproductive techniques?
Summary answer: Currently used methods to test DNA damage seem to have limited capacity to predict pregnancy rates achieved with the use of assisted reproductive techniques.
What is known already: In men with poor semen quality, sperm DNA damage has been associated with reduced fertilization rates, embryo quality and pregnancy rates, and higher rates of spontaneous miscarriage. The tests used to detect sperm DNA damage are mainly the Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, the Sperm Chromatin Structure Assay (SCSA), the Single Cell Gel Electrophoresis (Comet) assay, the Chromomycin A3 (CMA3) staining and the sperm chromatin dispersion (SCD) test.
Study design, size, duration: Systematic review and meta-analysis. The electronic databases Pubmed and Embase were searched from inception to December 2014, the search will be updated to April 2015 for the ESHRE congress. Studies that allowed comparison of results of sperm DNA integrity tests to pregnancy outcomes were included in couples.
Participants/materials, setting, methods: Quality assessment was performed with the QUADAS 2 checklist. Summary Receiver Operating Characteristic (sROC) curves were estimated to assess the accuracy of sperm DNA damage in the prediction of pregnancy. In case sROC analysis was not feasible, meta-analysis was done to evaluate whether mean (SD) values for the tests differed between pregnant and non-pregnant women. Analyses were stratified for IUI, IVF and ICSI.
Main results and the role of chance: Out of 504 non-duplicate studies, 31 were included in the meta-analysis. Eight studies reported on the TUNEL assay, 11 studies reported on the SCSA, five studies reported on the Comet assay, six studies reported on the CMA3 staining and four studies reported on the SCD test. The estimated sROC curve indicated a modest discriminatory capacity of the COMET assay and absence of discriminatory capacity of the TUNEL assay and SCSA in the prediction of pregnancy. For CMA3 and SCD, ROC curve analysis was not feasible. Meta-analysis for the CMA3 and SCD tests did not result in evidence of a difference in mean test result between pregnant and non-pregnant women (weighted mean difference -4.26, 95% CI -9.21 to 0.70 and -2.15, 95% CI -6.54 to 2.25, respectively).
Limitations, reason for caution: The studies included used different cut-off points to determine whether the DNA was damaged or not and results were limited by the number of available studies.
Wider implications of the findings: The currently used methods to test DNA damage in sperm do not appear to be useful for the prediction of pregnancy rates achieved with ART. In view of the increasing health care costs and to prevent over-testing these DNA damage tests should not be used as part of the standard pre-ART male assessment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Academic Medical Center, Jeroen Bosch Hospital.
Trial registration number: NA.
Keywords: ART, pregnancy, DNA damage

SELECTED ORAL COMMUNICATIONS

SESSION 48: GENES IN MONOGENETIC DISEASE AND FERTILITY

Tuesday 16 June 2015 **15:15–16:30**

O-177 A novel preimplantation genetic diagnosis method for monogenic diseases by targeted sequencing

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Study question: Current preimplantation genetic diagnosis (PGD) methods for monogenic disease are either complex or expensive. To develop a simple, accurate and cost-effective PGD method for monogenic disease, we explored the applicability of targeted sequencing.
Summary answer: Our targeted sequencing based method is simple, accurate and cost-effective for PGD of monogenic disease. Particularly, it may be used for PGD by testing parents and embryos only.
What is known already: Current main PGD methods for monogenic disease are multiple fluorescence PCR (MF-PCR) and karyomapping. PGD by MF-PCR needs labor-intensive and time-consuming designing and optimizing for patient or disease specific test development, which is complex. Karyomapping has largely simplified PGD workflow. However, the costs of genome wide SNP arrays is high, particularly when testing of many embryos is needed. Moreover, both tests require analyzing parents together with child or other appropriate family members, besides embryos.
Study design, size, duration: Our methodology was based on haplotype linkage analysis and direct mutation detection. Disease causing genes together with thousands of polymorphic SNPs flanking each of the genes were analyzed for parents and their child. Data were generated by targeted sequencing with barcodes and analyzed by an in house-developed analysis pipeline.
Participants/materials, setting, methods: A family suffering from beta-thalassaemia major undergoing PGD was tested. Single blastomere WGA products together with genomic DNA from parents and child were subjected to targeted sequencing for PGD. Besides, a simulation analysis of PGD by testing parents and embryos using the same data set was carried out.
Main results and the role of chance: Results were provided directly by the integrated software analysis pipeline. An average of 380 informative SNPs flanking both sides of each targeted gene were detected, with an average SNP interval of 10.5 Kb. The haplotype linkage analysis and direct mutation detection results were consistent with those of the reference laboratory. All embryos carried a HBB mutation.
Multiple DNA copy number variation (CNV) and recombination in targeted area were detected and validated, which were not discovered by MF-PCR performed by the reference laboratory. In addition, simulation analysis of PGD by testing parents and embryos using the same data set in the study achieved accurate detection results of disease causing mutation, DNA CNV and recombination.
Chance plays little role in process of obtaining our results.
Limitations, reason for caution: CNV caused by inheritance of two identical haplotypes from one of the parents could not be detected by the method without quantitative analysis. Besides, for PGD by testing parents and embryos, the disease causing mutation should be possible to be detected directly and multiple embryos are available.
Wider implications of the findings: We describe here a simple, accurate and cost-effective method for PGD of monogenic disease that can be used by testing only parents and embryos. It may provide benefit to many patients requiring PGD for monogenic disease.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by Shenzhen Birth Defect Screening Project Lab (JZF No. [2011] 861), Guangdong Natural Science Funding (No. S2012010009176) and Key project of Science and information technology of Guangzhou (No.201300000097). The authors have no competing interests to declare.
Trial registration number: NA.

Keywords: preimplantation genetic diagnosis (PGD), targeted sequencing, massively parallel sequencing (MPS), haplotype linkage analysis, direct mutation detection

O-178 Types of abnormal embryos in inversion cases

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Study question: Does the outcome of inversion cases differ from translocation cases?
Summary answer: Our study shows differences in the proportion of abnormal embryos from inversion cases compared to those from translocation cases. These differences may point to a heightened interchromosomal effect (ICE) in inversion cases.
What is known already: Structural abnormalities are a cause of infertility as the segregation of the affected chromosomes has a greater chance of producing unbalanced gametes and affected offspring. Structural abnormalities may affect the segregation of other chromosomes not involved with the abnormality itself, also known as ICE. Most of the studied structural abnormalities are translocations. The scarcity of inversion makes its study more difficult, and as a result most of the publications are directed to single individual cases.
Study design, size, duration: This is a retrospective study involving 26 inversion carrier cycles with 127 embryos and 78 reciprocal translocation carrier cycles with 559 embryos. This study extended from cases tested between 6/5/2011 and 4/7/2014.
Participants/materials, setting, methods: Cycles from 47 centers across the USA and Canada. Embryos that underwent blastomere or blastocyst biopsies analyzed by aCGH.
Main results and the role of chance: a. 25.2% of embryos from inversion cases were normal or balanced compared to 17.9% of embryos from translocation cases, though not statistically different.
b. 29.9% of embryos from inversion cases are unbalanced compared to 57.4% of embryos from translocation cases ($p < 0.001$).
c. 33.1% of embryos from inversion cases were aneuploid for chromosomes not related to the inversion. 13.1% of embryos from translocation cases were aneuploid for chromosomes not involved with the translocation ($p < 0.001$).
d. The inversion group was split in subgroups by maternal age: under 35 and 35 or over. Both groups didn't show statistical differences.
e. In the inversion Group 71.1% of unbalanced embryos also carry another abnormality whereas in the translocation group this proportion it is only 51.1% ($p < 0.001$).
Limitations, reason for caution: Expanding the inversion group may show a statistical difference for the proportion of normal or balanced between inversion and translocation carriers.
It will allow us to compare further differences between the meiotic behavior of inversion and translocation cases
Wider implications of the findings: Maternal age may not be the only factor for the increase in aneuploidy of the inversion group because age subgroups had no differences. Therefore, ICE could be the other contributing factor.
The high proportion of aneuploid embryos and the lower proportion of unbalanced embryos may indicate Comprehensive Chromosome Screening as a better strategy in dealing with inversion cases as opposed to FISH technique focusing only on the affected chromosome.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – NA.
Trial registration number: NA.
Keywords: inversion, PGD, aCGH, meiosis, embryos

O-179 High cumulative success rate and excellent safety profile after trophectoderm biopsy, blastocyst vitrification and single thawed blastocyst transfer. A new era in PGD for inherited disorders

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Study question: What is the cumulative ongoing pregnancy rate after trophectoderm biopsy, blastocyst vitrification and single thawed blastocyst transfer for PGD?
Summary answer: This approach has a high cumulative success rate and has the potential to eliminate OHSS and multiple pregnancy
What is known already: PGD has traditionally been performed on cleavage-stage embryos followed by fresh embryo transfer. The limitations include short analysis time, risk of OHSS and high multiple pregnancy rate in order to maximize the pregnancy rate after fresh transfer
Study design, size, duration: This is a prospective observational study of 161 consecutive couples who underwent PGD from October 2013 to August 2014
Participants/materials, setting, methods: A cohort of 161 couples who were carriers of either monogenic diseases (n = 127) or chromosomal rearrangements (n = 34) underwent oocyte retrieval after controlled ovarian stimulation employing a GnRH antagonist protocol and GnRH agonist administration for final oocyte maturation. Trophectoderm biopsy was performed on day 5 or 6 blastocysts, followed by blastocyst vitrification and single thawed blastocyst transfer in a natural or programmed cycle. DNA analysis was performed by whole genome amplification followed by PGH for monogenic diseases or array CGH for chromosomal rearrangements. Only one retrieval cycle per couple during the study period was included
Main results and the role of chance: In total, 546 blastocysts were biopsied and vitrified, of which 534 (98%) were diagnosed. Of the 161 couples, 132 (82%) had blastocysts suitable for biopsy; 16 had no unaffected embryos and 118 (73%) had genetically suitable vitrified blastocysts for transfer. Those 118 couples underwent 154 single thawed blastocyst transfers, resulting in 85 pregnancies (53% per retrieval cycle and 55% per transfer) with an implantation rate of 48%. The survival rate of vitrified blastocysts after thawing was 93% (154/166). The cumulative live birth/ongoing pregnancy rate per retrieval cycle was 40% (41% for monogenic diseases and 39% for chromosomal rearrangements, P = 0.80). There were no cases of OHSS and only 1 monozygotic twin pregnancy (1.1% per pregnancy) in this cohort
Limitations, reason for caution: This is a single centre cohort study of PGD patients. Implementation of this PGD approach requires advanced biopsy and vitrification techniques, which may not be widely available
Wider implications of the findings: This approach to PGD has high cumulative success rate and excellent safety profile, and has the potential to eliminate OHSS and multiple pregnancy. It allows flexibility in cycle programming and efficient scheduling of genetic testing
Study funding/competing interest(s): Funding by hospital/clinic(s) – Guy's and St. Thomas' Hospital NHS Foundation Trust.
Trial registration number: NA.
Keywords: PGD, Trophectoderm biopsy, vitrification

O-180 Carrier screening: an analysis of observed carrier rates among a European population from an expanded panel

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Study question: Based upon observed carrier rates among patients of self-reported European ancestry tested with an expanded genetic carrier screening panel, which autosomal recessive genetic diseases beyond Cystic Fibrosis, but with similar severity and frequency, may be appropriate for broad carrier screening among European patients?
Summary answer: Our analysis has identified several high-impact diseases with high carrier rates (greater than 1/100) which may be appropriate for screening; we also found that some diseases have higher carrier frequencies in our population than reported in the literature for patients of European ancestry, and may therefore be under-reported.
What is known already: Within the United States, professional societies provide carrier screening guidelines for a select few recessive genetic diseases based on ancestral background. Technological advancements in genomics now allow carrier screening to be performed for over 200 pan-ethnic genetic diseases in a

cost-effective and high-throughput manner. Carrier rates may be an impactful way to determine appropriate incorporation of diseases into such expanded panels, particularly for areas in which guidelines are not currently in place.
Study design, size, duration: Our retrospective analysis examined genotype data from an expanded carrier screening panel run on over 4000 patients over the past two years. Patients were referred for carrier screening by reproductive endocrinologists, obstetricians, and genetic counselors. Documented informed consent was obtained to use genotype information in a de-identified manner.
Participants/materials, setting, methods: The Illumina Infinium HD Custom Genotyping platform was used to identify 1,679 mutations associated with 213 autosomal recessive and X-linked genetic diseases. Carrier rates for each disease were calculated for the general population and by ethnic group and then compared with the literature. Diseases were also analyzed for severity.
Main results and the role of chance: American professional medical societies recommend screening for diseases with an impact on quality of life and high carrier frequency, e.g., Cystic Fibrosis. Our results identified several diseases with high carrier rates among patients of European ancestry and a significant effect on quality of life, but which are not currently screened for broadly. Identified diseases include but are not limited to syndromes such as Smith-Lemli-Opitz (observed rate of 1/47). Such diseases may be appropriate for screening in a European population to allow for the greatest reduction of reproductive risk. We also identified diseases with higher carrier rates among European patients than reported in the literature, such as Bardet-Biedel syndrome (1/168 vs. 1/376), highlighting the importance of continual carrier rate observation and comparison.
Limitations, reason for caution: Our study is limited by the self-reported nature of patients' ancestral backgrounds. It is possible that patients may be unaware of, or not identify with, their genetic ethnicity. However, this limitation highlights the importance of pan-ethnic carrier screening to accurately diagnose carrier states.
Wider implications of the findings: Our data identified several high-impact genetic diseases with carrier rates higher than 1/100. These results suggest that carrier states of such diseases may be under-diagnosed and indicate that the lack of broadly implemented screening programs within Europe may need to be re-evaluated. Proper observation and calculation of carrier rates, along with services such as genetic counseling, will be crucial to the effectiveness of carrier screening among all populations.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Recombine.
Trial registration number: NA.
Keywords: genetics, genetic testing, carrier screening

O-181 Mammalian Fertility Decoded: Over 3000 genetic loci implicated in female fertility potential

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Study question: The primary objective of this study was to compile and collate data implicating genes in female fertility, including where possible, data demonstrating how particular genes contribute to mechanisms of female reproductive physiology.
Summary answer: We have collated data for 3,000 genetic loci implicating them in mechanisms of female fertility in animal models, humans, or both. Our growing dataset includes cross-referenced details of gene expression in female reproductive tissues, biochemical data, phenotypes of genetic deletion/mutation, and meta- and bioinformatics analyses that collectively characterize the role of each loci in female fertility.
What is known already: Association studies in humans and targeted experiments in animal models have linked particular genes and genetic mutations to various aspects of reproduction and fertility. As the polygenic nature of infertility becomes clearer, our understanding of how different genetic elements **collectively** contribute to female reproductive biology becomes crucial. This is only possible with a fully comprehensive, cross-sectional understanding of the different levels of data describing the molecular mechanisms of female reproductive physiology.
Study design, size, duration: We collected and cross-referenced human- and animal model- specific expression data, phenotypic and genotypic data for more than 5,000 genetic loci.
Participants/materials, setting, methods: Data were iteratively cross-referenced and analyzed using meta-analyses and bioinformatics tools to demonstrate roles in particular reproductive processes.
Main results and the role of chance: Our developing understanding of the genetic regions involved in fertility currently encompasses 3,047 loci. Inclusion is based on: (1) Expression in reproductive tissues in humans and/or animal models; (2) Association with infertility or reproductive mechanisms in humans; (3) Infertility phenotypes in animal models and/or; (4) Meta- and bioinformatics analyses integrating data from 1 to 3. To systematize this dataset with respect to gene function, genetic loci were grouped according to their inferred and/or reported function(s) in reproductive physiology. For example, 318 genes are linked to mechanisms of oogenesis, while 320 genes are involved in endocrinological processes. Particular genes from both of these sets are among the 566 genes linked to mechanisms placentation. Thus, our big data approaches facilitate the identification of non-obvious associations among different genetic loci and a variety of fertility-related processes.
Limitations, reason for caution: Data relating particular genes and variants to reproduction in mammalian models or humans may have yet to be identified.
Wider implications of the findings: By using a big data approach to comprehensively classify genetic loci according to their role in mechanisms of reproduction and female fertility, our growing dataset helps to clarify how particular genes and genetic variants functionally contribute to the pathophysiology of infertility disorders. By highlighting genetic loci for which relatively little existing information links them mechanistically to human infertility, we provide a route for hypothesis driven translational research that could lead to clinically actionable molecular targets.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Celmatix Inc.
Trial registration number: NA.
Keywords: Female infertility, Genetics, Reproduction, Gene network

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SELECTED ORAL COMMUNICATIONS

SESSION 49: REPRODUCTIVE ENDOCRINOLOGY

Tuesday 16 June 2015 **17:00–18:00**

O-182 The usefulness of intrauterine hCG administration prior to blastocyst transfer in IVF-patients ≥38 years

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Study question: Implantation rates decline with female age. Besides an increasing incidence of aneuploid embryos, alterations in implantation promoting factors such as human chorionic gonadotropin (hCG) are considered to contribute to IVF failure. Therefore, we evaluated whether intrauterine injection of hCG improves embryo implantation in women of advanced age.
Summary answer: We found that intrauterine infusion of 500 IU hCG prior to blastocyst transfer does not have a sustained effect on implantation-; pregnancy-; miscarriage- or birth rates in women at advanced age.
What is known already: Embryo-secreted hCG is crucially involved in the implantation process, including the modulation of endometrial receptivity and the attachment and invasion of the embryo into the decidualized uterus. Previous studies postulate a supportive effect of intrauterine hCG infusion on embryo implantation. Serum concentrations of hCG in early pregnancy in women at advanced age were shown to be reduced, probably due to functional impairment of trophoblast cells. Thus, hCG application might be helpful for older IVF-patients.
Study design, size, duration: This clinical trial was designed as a randomized patient-blinded single-center study. We included 480 stimulation cycles of women between 38 and 43 years with fresh embryo transfer (ET) on day 5. Primary outcome was pregnancy rate (PR), clinical pregnancy rate (cPR), miscarriage rate (MR) and delivery rate (DR).
Participants/materials, setting, methods: For controlled ovarian hyperstimulation (COH) the GnRHa long protocol was applied. Fertilized oocytes (IMSI) were cultured in single-step medium. Only IVF-cycles with fresh ET on day 5 were included. Just prior to transfer, patients received intrauterine injection of hCG (500 IU) or culture medium (control). Clinical outcome was compared.

Main results and the role of chance: Mean age of patients was 40.4 years (control) and 40.3 years (hCG group). Mean duration and stimulation dose (HMG) were 11.8 days and 3276 IU (control) and 11.8 days and 3230 IU (hCG group). No statistically significant differences were found in cause and type of infertility, endometrium build-up, as well as the number of retrieved oocytes per ovarian pick-up and the number and quality of transferred blastocysts. In the control group PR was 41.6%; cPR 36.9% and DR 30.2%; MR was 6.7%. In patients receiving hCG prior ET a PR of 44.0%; cPR 33.7%; DR 26.7% and a MR of 7.1% were found. No statistically significant differences in clinical outcome between hCG administration and control group were observed.
Limitations, reason for caution: This study does not encompass frozen transfers or transfer of cleavage stage embryos. For COH only the GnRH agonist (long) protocol was used. Thus, these findings might be only applicable for fresh blastocyst transfers and not for hormonal stimulation using the GnRH agonist protocol.
Wider implications of the findings: The detailed molecular mechanisms of hCG signalling are still not fully understood. Controversial reports underline that simple intrauterine administration might be insufficient to create a receptive endometrium. There might be molecular differences between embryo-secreted hCG vs. administered hCG. This trial indicates that the applied intrauterine injected hCG has no benefit for IVF-patients at advanced age undergoing blastocyst transfer in a fresh cycle.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study was not externally funded.
Trial registration number: CRT:355.
Keywords: Intrauterine hCG application, implantation, blastocyst quality, pregnancy rate, advanced maternal age

O-183 The optimal dose of medroxyprogesterone acetate (MPA) to prevent premature LH surge during controlled ovarian hyperstimulation in normal ovulatory women

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Study question: Which is the optimal dose of MPA during progestin-primed ovarian stimulation (PPOS) to prevent premature LH surge in normal ovulatory women?
Summary answer: MPA might be an effective oral alternative for the prevention of premature LH surge in woman undergoing controlled ovarian hyperstimulation (COH). 10 mg/d of MPA might be more effective to prevent premature LH surge than 4 mg/d MPA in woman undergoing COH.
What is known already: Premature LH surge is a major cause for cycle cancellation during COH. High levels of progesterone, such as during the luteal phase, is known to block the estrogen-induced GnRH/LH surge in the ewe. Our previous researches demonstrated consistent LH suppression in the luteal phase, with no spontaneous LH surge. The follow-up of over 500 children born from luteal-phase COH showed high progesterone status didn't increase the risk of congenital malformations compared with the short protocol.
Study design, size, duration: A prospective controlled study including 300 women undergoing ovarian stimulation for the treatment of infertility during August to November in 2014 was conducted. All women were recruited consecutively and allocated to the 10 mg group or the 4 mg group.
Participants/materials, setting, methods: MPA 4 mg/d or 10 mg/d were co-administered with hMG beginning on cycle day 2–3.Ovulation was induced with hCG or co-triggered by hCG and GnRH agonist when dominant follicles matured. Transvaginal ultrasound–guided oocyte retrieval was conducted 32–36 h later. Viable embryos were cryopreserved for later transfer in both protocols.
Main results and the role of chance: The number of oocytes retrieved in 10 mg group was similar to those in 4 mg group (9.81 ± 6.26 vs. 9.61 ± 5.94, $P > 0.05$). No differences were found in number of viable embryos, mature oocyte rates, fertilization rates, cleavage rates and viable embryo rate per oocyte retrieved between the two groups. No premature LH surge occurred in either group. The rate of cycle cancellation in 10 mg group was significantly lower than that in 4 mg group (4.7% (7/150) vs. 12.7% (19/150)). Logistic regression analysis found that the cycle cancellation rate was negative correlated to daily dose of MPA and positive correlated to patients' age.
Limitations, reason for caution: As a new regimen, a control group should be contained besides the two study groups such as the short protocol.
Wider implications of the findings: PPOS using MPA has the advantages of an oral administration route, easy access and more control over LH levels. It might become a new non-down-regulation regimen for ovarian stimulation in combination with embryo cryopreservation. 10 mg/d of MPA could help to achieve the best pregnancy outcome and reduce the probably adverse complications.
Study funding/competing interest(s): Funding by national/international organization(s) – National Nature Science Foundation of China (grant numbers: 31071275), Natural Science Foundation of Shanghai (grant number: 14411964300).
Trial registration number: ChiCTR-ONRC-14005127.
Keywords: LH surge, medroxyprogesterone acetate, controlled ovarian hyperstimulation

O-184 Effect of Dehydroepiandrosterone (DHEA) on ovine intra-follicular response prior and after controlled ovarian stimulation

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Study question: Does *in vivo* DHEA supplementation affect intra-follicular steroidogenesis and gonadotrophin receptor expression before and after ovarian stimulation in sheep?
Summary answer: DHEA stimulated antral follicle number in pre-stimulated ovaries ($P < 0.01$) but this effect was not associated with changes in either ovarian steroidogenesis or gonadotrophin receptor mRNA expression. However, higher intrafollicular progesterone levels and lower gonadotrophin receptor expression levels ($P < 0.05$) were observed in DHEA-treated animals after ovarian stimulation.
What is known already: DHEA has been used worldwide to improve ovarian response in women with diminished ovarian reserve undergoing IVF treatment. DHEA stimulates primordial follicles initiation and preantral/early antral follicular development in the gonadotrophin responsive stages by improving granulosa cell proliferation and modulating local growth factors, e.g., AMH. It is believed that DHEA increase ovarian susceptibility to FSH stimulation at the later stage but there has not been an established evidence to support at the follicular/molecular level.
Study design, size, duration: An animal experiment was conducted in 12 mature ewes which were equally divided into treatment and control group. In the treatment group, all 6 animals received DHEA implants for a 12-week period. One ovary was then removed to examine pre-stimulation effects of DHEA prior to ovarian stimulation for 5 days.
Participants/materials, setting, methods: Ovarian stimulation utilized recombinant human FSH (rhFSH, Gonal-F®) and GnRH-antagonist (Cetrotide®). Animals were sacrificed after hCG injection to harvest the remaining ovary. Follicles were dissected from both before and after-stimulation ovary for hormonal evaluation (fluid) and FSH/LH receptor mRNA expression (tissue).
Main results and the role of chance: DHEA treatment resulted in an increase in the number of antral follicles in pre-stimulation ovaries (19 ± 2 vs. 15 ± 2; $P < 0.01$). In pre-stimulation follicles, DHEA levels tended to be higher but there was large variation among follicles (107 ± 35 vs. 43 ± 8 ng/ml; $P = 0.84$). Levels of androstenedione, oestradiol, and progesterone showed no difference. Likewise, utilising the real-time PCR, FSH/LH receptor mRNA expressions in the follicular tissue were similar in both groups. After FSH stimulation, mean follicular DHEA and progesterone concentrations were significantly higher in the treatment group (76 ± 28 vs. 23 ± 7 ng/ml and 114 ± 13 vs. 83 ± 7 ng/ml, $P < 0.05$). No significant difference in follicular oestradiol was observed. However, there were approximately 40% and 30% reduction in both FSH and LH receptor mRNA expressions in the treated follicular tissue compared to the control (mean fold change 0.63, 95% CI 0.20–1.07 and 0.71, 95% CI 0.40–1.01, respectively; $P < 0.05$).
Limitations, reason for caution: Although sheep are a large mono-ovulatory species, direct interpretation of experimental findings to humans should be done so with caution, especially as experimental animals were of normal fertility. In addition, further studies to evaluate an impact of reduction in both FSH and LH protein receptor expression are required.

Wider implications of the findings: While evidence of using DHEA in clinical setting is currently uncertain, this study shows that DHEA can enter ovarian follicles and further modify the intrafollicular microenvironment. The detrimental effect of DHEA on gonadotrophin receptor mRNA expression warrants concern over quality of individual oocyte despite its positive influence on early folliculogenesis.

Study funding/competing interest(s): Funding by University(ies) – University of Nottingham

Trial registration number: NA.

Keywords: Dehydroepiandrosterone (DHEA), Controlled ovarian hyperstimulation (COH), Ovarian folliculogenesis

O-185 Ovarian Hyperstimulation Syndrome, Pregnancy, Live Birth and IVF Cycle Failure by Risk Category

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Study question: Among women undergoing ovarian stimulation with corifollitropin alfa (CFA) or recombinant follicle-stimulating hormone (rFSH) in a gonadotropin-releasing hormone (GnRH) antagonist protocol, did rates of ovarian hyperstimulation syndrome (OHSS), pregnancy, live births and *in vitro* fertilization (IVF) cycle failure differ between groups stratified by risk category for OHSS?

Summary answer: Rates of moderate or severe OHSS were significantly higher ($P < 0.001$) among the high-risk group versus the non-high-risk group. Pregnancy and live birth rates were slightly higher among the high-risk group versus the non-high-risk group. Rates of IVF cycle failure were similar in both groups.

What is known already: Prevention of OHSS, especially in high-risk populations, is a major goal in assisted reproductive technology. The use of a GnRH agonist trigger is almost 100% effective in eliminating early-onset OHSS, but may result in inferior pregnancy rates compared with hCG triggers with fresh autologous transfers.

Study design, size, duration: Analyses of data from ENGAGE (1506 women, aged 18–36 years) randomized controlled trial. Patients received 150 µg CFA ($n = 756$) or daily 200 IU rFSH ($n = 750$) from stimulation days 1–7, followed by ≤200 IU/d rFSH until criteria for hCG were met.

Participants/materials, setting, methods: Subjects were classified into high-risk and non-high-risk groups based on a threshold number of follicles [19 or more follicles of 11 mm or more in diameter on the day of hCG]). Rates of moderate or severe (Grades II and III) OHSS, pregnancy, live births and IVF cycle failure were compared between high-risk and non-high-risk groups and treatments.

Main results and the role of chance: Of the 1506 subjects, 391 (26%) were high-risk, 241 (31.9%) in the CFA group and 150 (20.0%) in the rFSH group. Rates of moderate or severe OHSS were significantly higher ($P < 0.001$) among the high-risk (28/391 [7.2%]) versus the non-high-risk group (23/1115 [2.1%]); the rate difference was consistent between treatments. Pregnancy and live birth rates were slightly higher for the high-risk versus the non-high-risk groups, respectively: 43.5% versus 38.1% (5.4% higher) for vital pregnancies, 42.2% versus 37.3% (4.9% higher) for ongoing pregnancies, and 38.1% versus 33.9% (4.2% higher) for live births; none of these differences achieved a descriptive *p*-value below 0.067 (Cochran–Mantel–Haenszel [CMH] test, controlling for treatment). Rates of IVF cycle failure were 63.2% for the high-risk group and 58.6% for the non-high-risk group ($P = 0.111$, CMH test).

Limitations, reason for caution: This was a retrospective analysis of patients. The women represent a subset of the normal patient population, as they were participants in a large clinical trial with pre-specified inclusion /exclusion criteria. Use of this cut-off threshold would not prevent all cases of OHSS as 23 cases occurred in the non-high-risk group.

Wider implications of the findings: Although pregnancy and live birth rates were slightly higher in the high-risk group with more follicles at day of hCG, the elevated rates of OHSS in this group may present an unfavorable benefit-risk profile for these patients. Use of a GnRH agonist trigger for those with >19 follicles ≥11 mm on the day of hCG would likely have avoided 28 cases of OHSS.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck & Co., Inc., Kenilworth, NJ, USA.

Trial registration number: NCT00696800.

Keywords: agonist trigger, corifollitropin alfa, recombinant FSH, ovarian hyperstimulation syndrome

SELECTED ORAL COMMUNICATIONS

SESSION 50: OOCYTE MARKERS AS PREDICTORS OF OUTCOME

Tuesday 16 June 2015 **17:00–18:00**

O-186 Do multiple IVF cycles increase the chance of having a euploid embryo in females of advanced age?

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Study question: What is the impact of female age on the incidence of aneuploidy during preimplantation development? How does this affect the probability of finding no euploid embryos after a cycle of preimplantation genetic screening (PGS)? Can batching of embryos from multiple IVF cycles increase the likelihood of identifying euploid embryos?

Summary answer: Increasing age adversely affects chromosome segregation. As expected, the risk of having no euploid embryos depends on the number of embryos suitable for biopsy and the aneuploidy rate. Patients batching embryos from more than one cycle display a 2- to 3-fold reduction in the risk of having no euploid embryos.

What is known already: Recent randomized controlled trials have shown increased implantation and/or ongoing pregnancy rates following transfer of euploid embryos after comprehensive chromosome screening. Importantly, provided that a euploid embryo was identified for transfer, high pregnancy rates were achieved regardless of maternal age. However, the likelihood of producing a euploid embryo in a single cycle is reduced for some patients due to elevated aneuploidy rates and/or an insufficient number of embryos generated.

Study design, size, duration: The effect of female age on aneuploidy rates, number of abnormalities generated, and the specific chromosomes affected during preimplantation development, was assessed. Furthermore, the probability of finding no euploid embryos for transfer in a single or multiple cycle(s) was evaluated in relation to female age and number of embryos produced.

Participants/materials, setting, methods: Microarray-CGH was applied to cells biopsied from 1780 cleavage-stage embryos and 1213 blastocysts generated by 262 (age range: 20–46, average: 38.6) and 234 (age range: 28–45, average: 38.2) couples, respectively. Twenty two couples having analysis at the cleavage-stage and 12 with blastocyst testing underwent multiple (2–4) treatment cycles.

Main results and the role of chance: In line with expectation, aneuploidy was correlated with advancing age at both cleavage and blastocyst stages ($p = 0.0006$ and $p < 0.0001$). Highly abnormal embryos (>3 errors) also became significantly more common ($p < 0.0001$, $p = 0.0035$). Increasing aneuploidy was associated with elevated risk of having no euploid embryos in a single cycle at both embryonic stages ($p = 0.0002$, $p = 0.0005$). An increased likelihood of having no euploid embryos was also apparent in younger females (20–32 years) generating ≤3 cleavage-stage embryos. At the blastocyst-stage a euploid embryo was always produced by women ≤32, but for patients ≥33 decreased embryo count was associated with increased probability of having no euploid embryos to transfer. Multiple cycle patients displayed a 3- and 2-fold increase in the chance of having euploid embryos at the cleavage- and blastocyst-stage.

Limitations, reason for caution: In order to firmly establish a beneficial effect of multiple treatment cycles on euploid embryo rates in females of different age, results need to be verified in a larger population study, ideally as part of a randomised trial.

Wider implications of the findings: Generation of an increased number of embryos by combining multiple treatment cycles significantly improves the chances of having a euploid embryo for transfer, even in women >41, an important fact given the rapidly declining fertility in this patient group. Younger patients with a poor response to ovarian stimulation may also benefit from

multi-cycle strategies. Another consideration is that batching of embryos may lead to reduced PGS costs, since samples from multiple cycles are analysed simultaneously.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Reprogenetics UK.
Trial registration number: NA.
Keywords: Preimplantation Genetic Screening, Multiple IVF Cycles, Euploid Embryo Transfer

O-187 Cohorts of donated oocytes showing smooth endoplasmic reticulum clusters produce clinical outcomes similar to normal cytoplasm oocytes cohorts

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Study question: Should cohorts of donated oocytes containing at least one oocyte with smooth endoplasmic reticulum clusters (SERC) be considered for oocyte donation?
Summary answer: Cohorts of donated oocytes with at least one oocyte presenting SERC may be safe to be used in oocyte donation programs.
What is known already: Several studies in the past reported that the presence of SERC in autologous oocyte cohorts affects fertilization, embryo development and pregnancy rates as well as neonatal data. However recent studies suggest comparable clinical and neonatal outcomes when employing normal and affected cohorts.
Study design, size, duration: A retrospective study was performed from January 2011 to September 2014 in a private IVF clinic that included 1092 oocytes obtained from 82 donors divided in two groups according to the presence or absence of at least one oocyte showing SERC. These were evaluated only in terms of clinical outcomes.
Participants/materials, setting, methods: 95 embryo transfers were divided as follows: **Group A:** 45 cycles with at least one SERC positive oocyte in the cohort; **Group B:** 50 cycles with SERC negative oocytes. Only cases with normal sperm parameters (WHO 2010) were included. Chi square and Student's t-test were used for data analysis.
Main results and the role of chance: 131 of 3178 cycles of ICSI with donor oocytes (4.0%) presented SERC in at least one oocyte; 13 out of 82 (15%) presented SERC in their oocyte cohorts at least twice during the period of this study, and 994/1092 (91%) oocytes were mature. No statistical significance ($P > 0.05$) was observed when comparing Group A vs. B regarding mean of recipients (42.2 ± 2.2 vs. 42.5 ± 3.4) fertilization rate [353/492 (71%) vs. 368/502 (73%)], pregnancy rate [31/45 (69%) vs. 36/50 (72%)], Transferred blastocyst (2.1 ± 0.25 vs 2.0 ± 0.18), Implantation (36.2 vs. 36.8%) and miscarriage rates [12% vs 15%]. Likely, there was no statistical difference between the SERC+ vs. SERC- oocytes in terms of fertilization [70/99 (70%) vs. 368/502 (73%)], cleavage [90/99 (91%) vs. 345/368 (93%)], and blastocyst formation [39/90 (43%) vs. 158/345 (45%)] rates.
Limitations, reason for caution: Taking into account that this retrospective study did not analyze the neonatal outcomes and that fresh donor oocyte cohorts have not been previously studied, the relevance of this findings must be taken with caution, even though it has been suggested that SERC oocytes produce healthy babies.
Wider implications of the findings: This study contributes to demonstrate that cohorts of donated oocytes presenting at least one SERC+ oocyte avoiding to transfer embryos derived from SERC+ oocytes may be safe to use in IVF cycles without an impact in clinical outcomes. This study is consistent with some previous reports suggesting the lack of impact of SERC+ oocyte cohorts in success rates derived from autologous oocytes. More studies should be performed to demonstrate the safety of SERC+ cohorts on neonatal outcomes.
Study funding/competing interest(s): No financial support was received for this study, and there are no potential conflicts of interest.
Trial registration number: NA.
Keywords: oocyte, SERc, ICSI

O-188 Is oocyte meiotic spindle morphology associated with embryo ploidy? A prospective cohort study

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[4]*IVF Australia, Scientific Director, Sydney, NSW, Australia*

Study question: Is there an association between oocyte meiotic spindle morphology visualised by polarised light microscopy (PLM) at the time of Intracytoplasmic sperm injection (ICSI) with the ploidy of the resulting embryo?
Summary answer: Oocyte spindle morphology is associated with the resulting embryo's ploidy. Oocytes with normal spindle morphology are significantly more likely to produce euploid embryos compared to oocytes with meiotic spindles that are translucent or not-visible.
What is known already: Embryo ploidy contributes significantly to successful reproduction. Aneuploid embryos fail to implant, lead to miscarriage or genetically abnormal offspring. A significant proportion of aneuploidy is maternal in origin, resulting from chromosome misalignment and errors in segregation. Current embryo morphological markers are not reliable predictors of euploidy or aneuploidy while pre-implantation genetic diagnosis (PGD) is an invasive procedure. Hence there is a need for additional non-invasive methods that will allow better prediction of embryo euploidy.
Study design, size, duration: PGD patients (n = 113 patients, 135 cycles, 745 embryos) of a private IVF clinic between June 2011 and July 2014 were included prospectively. The oocyte meiotic spindles were assessed by PLM and classified at the time of ICSI as normal, dysmorphic, translucent, telophase and not-visible.
Participants/materials, setting, methods: All embryos with suitable development were biopsied on Day 3 of culture following laser zona dissection. Biopsied blastomeres were analysed by array CGH. Spindle morphology and embryo ploidy association was evaluated by generalised estimating equation analysis while accounting for non-independence of data.
Main results and the role of chance: The frequency of euploidy in embryos derived from oocytes with normal spindle morphology (30.4%) was significantly higher than all other spindle classifications combined (18.4%) odds ratio (OR): 1.93 [95% confidence interval (Cl): 1.33–2.79], translucent spindle morphology (9.8%) OR: 0.24 (95% CI: 0.12–0.49) and not-visible spindle morphology (13.5%) OR: 0.35 (95% CI: 0.19–0.64). There was no significant difference between normal and dysmorphic spindle morphology (24.5%) OR 0.73 (95% CI: 0.49–1.08) while no telophase spindles resulted in euploid embryos (n = 11). Assessment of spindle morphology was also found to be independently associated with embryo euploidy after controlling for embryo quality (OR: 1.85, 95% CI: 1.26–2.71), i.e., regardless of embryo quality, the chances of a euploid embryo increased by 85% when spindle morphology was normal.
Limitations, reason for caution: The oocyte is not the only contributor to the embryo's ploidy. Paternally derived aneuploidy occurs less frequently but is still a risk to the outcome of the embryo. Hence, assessment of oocyte spindle morphology should be regarded as an additional useful tool for the non-invasive identification of euploid embryos.
Wider implications of the findings: A non-invasive method that can aid in the selection of euploid embryos has significant implications for patients. Visualisation of the meiotic spindle by PLM is an early marker that can impart additional information regarding the genetic integrity of an oocyte and aid in the selection of a euploid embryo for transfer, hence optimizing the chances of a live birth of a chromosomally normal infant.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Australia.
Trial registration number: None.
Keywords: spindle, polarised light microscopy, euploidy, preimplantation genetic diagnosis, oocyte

O-189 Characterization of the localization of some key factors of the spindle assembly checkpoint (SAC) during human oocyte's nuclear maturation

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Study question: Are Bub1 and BubR1, key known kinases involved in the spindle assembly checkpoint functionality, correctly expressed and localized during human oocyte meiosis?
Summary answer: Through this analysis, we show that Bub1 and BubR1 proteins are expressed and localized on the kinetochores of oocytes's chromosomes at the metaphase I stage. As anaphase I takes place they are delocalized from these kinetochores. These observations suggest for the first time that SAC functionality is preserved during human first meiotic division (meiosis I).
What is known already: The SAC is the major molecular mechanism involved in accurate chromosome segregation in meiotic division and is being widely studied in mouse models. Considering the fact that the majority of human aneuploidies is due to errors arising during meiosis I in oocytes and increase with female age, it is important to transpose at least a part of these studies to human oocytes, what has not been done so far.
Study design, size, duration: One year study on immature oocytes from patients undergoing intra-cytoplasmic sperm injection (ICSI).
Participants/materials, setting, methods: Immature oocytes are collected after ovarian stimulation and fixed a few hours after punction and cumulus cells removal. Oocytes are then subjected to indirect immunofluorescence labelling and observed under a confocal Leica SP5 microscope.
Main results and the role of chance: Immunofluorescence analysis showed that Bub1 and BubR1 proteins are nearly undetectable at the germinal vesicle stage of oocyte's maturation. After germinal vesicle breakdown, Bub1 and BubR1 proteins strongly localized to kinetochores. This strong staining is lost as homologous chromosomes undergo segregation between the ooplasm and the first polar body. These results are correlated with those obtained in mouse models and strongly suggest that the SAC impairs precocious chromosome's separation and needs to be delocalized from kinetochores in order to allow the ending of the first meiotic division to take place in human oocytes.
Limitations, reason for caution: Very few human oocytes are available for research. This first study needs to be confirmed by more oocytes' staining.
Wider implications of the findings: These initial results will allow us to go further in understanding the molecular mechanisms that regulate the SAC in human meiosis. Further functional experiments may shed new light on the occurrence of women aneuploidy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CHU Arnaud de Villeneuve, Montpellier.
Trial registration number: None.
Keywords: Spindle Assembly Checkpoint, Human oocytes, Kinetochores, Aneuploidy

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SELECTED ORAL COMMUNICATIONS

SESSION 51: GAMETE DONATION AND PATIENT EXPERIENCES
Tuesday 16 June 2015 **17:00–18:00**

O-190 Online sperm donation: a survey of men seeking to donate sperm via a connection website

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Study question: What are the demographic characteristics, motivations, expectations and preferences regarding information sharing and contact with recipient families of men who choose to donate their sperm via a connection website?
Summary answer: These men had diverse demographic characteristics and a variety of reasons for using the website (e.g., access to a greater range of recipients than at clinics; ability to choose, and communicate directly with, recipients); approximately one third favoured anonymous donation, the large majority of whom were heterosexual.
What is known already: Although substantially more sperm donors are registered on connection websites than with clinics, there has been very little research on this population. A common concern is that men may have financial or sexual motivations for using these websites. Studies of clinic donors highlight both altruistic and financial motivations for donating and find most prefer anonymity, although willingness to be identifiable may vary demographically; e.g., identity-release donation is associated with a rise in older, married donors.
Study design, size, duration: An online survey was completed by 398 men registered as sperm donors with Pride Angel, a UK-based website that facilitates contact between donors and recipients of sperm. All members of Pride Angel were invited to participate via an email invitation and the survey was live for 7 weeks.
Participants/materials, setting, methods: The survey comprised multiple choice and open-ended questions to obtain data on participants' demographic characteristics and motivations, expectations and experiences of online sperm donation, including preferences regarding contact with recipients and offspring. Data were analysed to examine differences according to participants' sexual orientation, marital and parental status, and age.
Main results and the role of chance: Most participants (77.4%) were heterosexual, 10.1% were gay, 8.8% were bisexual; ages ranged 18–69 years (mean 37.1, SD 9.6). Approximately half had a partner (46.5%) and children (51.8%); significantly more gay/bisexual men were partnered and/or childless than heterosexual men. Participants reported various reasons for using the website, including greater choice and communication with recipients than at clinics. The majority expressed altruistic reasons for donating. A greater proportion of gay/bisexual men desired open-identity donation ($p < 0.005$) and contact with recipients and offspring ($p < 0.005$) than heterosexual men; there were no significant differences by participants' age, marital or parental status. Seventy (17.6%) participants' donations had produced ≥1 successful pregnancy; of these, a minority (28.1%) were in contact with the child, comprising significantly more gay/bisexual than heterosexual men ($p = 0.001$).
Limitations, reason for caution: Although this study presents data from the largest sample of online sperm donors to date, a key limitation is that members of only one website participated. The findings may not be representative of all potential sperm donors using connection websites.
Wider implications of the findings: Since the removal of donor anonymity in many countries, concerns have arisen about the sharp increase in 'private' donors and falling numbers of clinic donors. This is the first in-depth study of donors who connect with recipients on the internet, including several who have successfully donated. The findings indicate that this is a diverse group and that sexual orientation may play a greater role in men's preferences regarding donation than marital and parental status.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by the Wellcome Trust [097857/Z/11/Z]. Erika Tranfield is Director and Co-Founder of Pride Angel and assisted with recruitment for this study.
Trial registration number: NA.
Keywords: sperm donor, connection website, internet, anonymous and open-identity donation, sexual orientation

O-191 Psychosocial counselling in donor sperm treatment: exploring parents' expectations

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Study question: In donor sperm treatment (DST)**,** what are parents' expectations on psychosocial counselling, especially concerning the decision making process towards disclosure to their child**.**
Summary answer: All 24 parents had the intention to disclose donor conception to their child before they received psychosocial counselling. They felt that counselling had no influence on their decision making on disclosure, but they

were concerned about when and how to disclose and lacked professional and trustful advice on this.

What is known already: Psychosocial counselling is recommended before DST for coming to terms with issues such as psychosocial implications of donor conception and decision-making on disclosure. Parents' expectations on counselling and whether their needs are met is unknown.

Study design, size, duration: A qualitative study was performed from July 2012 until August 2013 with 24 Dutch parents, who had conceived children after DST between 2000 and 2012.

Participants/materials, setting, methods: Semi-structured in-depth interviews were held with heterosexual and lesbian couples and single mothers. The interviews were fully transcribed and analysed using the constant comparative method of grounded theory.

Main results and the role of chance: Psychosocial counselling before DST had not influenced parents' decision-making to disclose. The intention to disclose donor conception to their child was already a matter of fact before psychosocial counselling. In retrospect they lacked professional advice on when and how to disclose and how to handle future donor contact. Reading and hearing about trustful experiences on disclosure of other parents was considered important. Another important finding was that when psychosocial screening for DST was combined with psychosocial guiding and support in the same counselling session, parents felt very vulnerable and reluctant to address certain issues.

Limitations, reason for caution: Only parents who already disclosed DST to their children or intended to disclose volunteered for the study. This selection bias is a limitation of this study, and may be due to the sensitive nature of sperm donation.

Wider implications of the findings: The period of availability of psychosocial support and advice for parents of a donor child should be extended and be especially available after childbirth for guiding parents on when and how to disclose and how to handle future contact of their child with the donor.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Academical medical centre, Amsterdam

Trial registration number: None.

Keywords: donor sperm treatment, counselling, disclosure, parents, donor offspring

O-192 Tailored expectant management in couples with unexplained infertility does not negatively influence patients' experiences with care

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Study question: How does a tailored expectant management of 6–12 months (TEM) in couples with unexplained infertility and a good prognosis affect their experiences with fertility care compared to couples who are exposed to overtreatment, i.e., start treatment within 6 months after fertility-workup?

Summary answer: Adhering to TEM of at least 6 months in couples with unexplained infertility does not negatively influence their experience with care compared to couples who started treatment too soon.

What is known already: In couples with unexplained infertility and a good prognosis of natural conception (>30%) within one year, expectant management of 6–12 months, is equally effective as an immediate start of medically assisted reproduction. Therefore, TEM is recommended by various national clinical guidelines. However, implementation of TEM is still poor because of several barriers on both patient and professional level. Moreover, how adherence to TEM actually affects the couples' experiences with care is unknown.

Study design, size, duration: This cross-sectional study is nested within a large cluster randomized trial, which assessed methods to improve guideline adherence to TEM. A survey with written questionnaires was performed among all infertile couples who participated in a cluster randomised trial of guideline adherence on TEM in 25 Dutch clinics.

Participants/materials, setting, methods: Couples eligible for TEM after fertility work-up were included. We used questionnaires to collect data on couples' experiences with care (patient-centeredness of care and patients' trust in their physician) and on determinants for their experiences. Multilevel regression analyses were performed to investigate associations between adherence to TEM and care experiences.

Main results and the role of chance: Out of the 544 couples that were approached, 384 couples responded (response rate 71%). Couples who adhered to TEM scored equally on patient-centeredness of care (PCQ-Infertility, scale 0–3) as couples with overtreatment (2.28 vs. 2.29, OR 1.00; 95% CI 0.92–1.08). The couples' trust in their physician (Wake Forest Trust Scale, scale 0–5) was not significantly different between couples who adhered to TEM and couples with overtreatment (4.1 vs. 4.9, OR 2.17; 95% CI 0.5–9.2). Determinants that positively influenced patient-centeredness of care were; the fulfilment of the childwish, a higher fertility related quality of life (FertiQol score), attention for TEM during the first visit to the clinic, and education on psychological consequences of medically assisted reproduction. We did not find any determinants for the patients' trust in their physician.

Limitations, reason for caution: Since the questionnaires were collected retrospectively, recall bias might have occurred. For some participants the time between their first visit to the clinic and completing the questionnaire was 1.5 year. However, we expect the effect of the recall bias to be equal between couples with overtreatment and couples that adhered to TEM.

Wider implications of the findings: Our results underline that TEM does not lead to less patient-centeredness of care or patients' trust in their physician. However, physicians should be aware of patients' mental health status (e.g., FertiQol score) and a good education and counseling on the consequences of fertility treatments. Therefore, more research on improving counseling couples for TEM should be encouraged and educational tools should be developed to help professionals and patients.

Study funding/competing interest(s): Funding by national/international organization(s) – Netherlands Organisation for Health Research and Development (ZonMW).

Trial registration number: www.trialregister.nl. NTR3405.

Keywords: Unexplained infertility, Expectant management, Patient-centredness, Guideline adherence, Education and counseling

O-193 Tailored education improves fertility knowledge and awareness: a randomized controlled trial

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Study question: Which educational methods improve the most fertility knowledge and awareness in women attending a fertility center to donate oocytes?

Summary answer: The delivery of tailored oral information related to fertility significantly increased women's knowledge score (+2.4) compared to non-tailored information (+1.29) or no information (+0.46) (p = 0.003), as measured in a 10-point self-rating questionnaire (SRQ) completed just before and 1–8 months after the intervention.

What is known already: Fertility knowledge and awareness are insufficient in reproductive-aged women. Awareness is mainly lacking in reproductive life span, risk factors for infertility, and limitations of assisted reproduction

treatments. Consequently, this could lead to involuntary permanent childlessness. In contrast, it is known that relatively simple educational interventions can be used to increase knowledge and to promote reproductive health, but, no head to head intervention has been tested so far in the context of a fertility center.
Study design, size, duration: RCT with two parallel blind educational interventions versus control, powered to detect a difference ≥2 points after the intervention. A SRQ was administered at first consultation, before the intervention, and again at oocyte pick-up (1–8 months later). From 201 enrolled women and after eligibility evaluation, 85 completed the donation cycle.
Participants/materials, setting, methods: Participants were candidates for oocyte donation who were on average 25.3 years old (SD 4.7), 30.8% university educated, and 64.1% without children. There were 3 study arms: 31 tailored oral intervention (T = brochure plus review of wrong answers to the questionnaire), 31 untailored intervention (U = brochure only), and 24 control (C = no intervention).
Main results and the role of chance: Fertility knowledge score at baseline was poor (mean score = 3.7/10). After the intervention, the score remained low for U (4.9/10) and C (4.6/10), while significantly increased for T (6.37/10) ($p = 0.003$). We observed that the score after the intervention was positively correlated to the study group ($p < 0.001$), the pre-test score ($p = 0.012$), and the woman's age ($p = 0.004$), but not to the educational level ($p = 0.76$) or having children ($p = 0.12$). Tailored information related to woman's most fertile age and infertility risk factors were the most efficient, with 80% ($p = 0.027$) and 79% ($p = 0.004$) of correct answers after the T intervention. We found a higher proportion of women with children in T ($T = 44\%$, $U = 27\%$, $C = 37\%$), however it does not seem to affect fertility knowledge at either pre-test ($p = 0.70$) or post-test ($p = 0.12$).
Limitations, reason for caution: The target population was women between 18 and 35 years old, so caution should be exerted when generalizing the results to younger or older women and men from the general population, as information needs might be different and alternative communication channels might need to be identified.
Wider implications of the findings: This study highlights the value of providing tailored oral information about fertility lifespan and infertility risks factors to reproductive age women, regardless of education level and parity. Further studies are needed to evaluate the long-term effect of educational interventions and if there is an effect on family planning (advancement of planned age for bearing the first and last children).
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: Study registered in Clinicaltrials.gov, but NCT not yet assigned.
Keywords: fertility knowledge, patient education, age-related infertility

SELECTED ORAL COMMUNICATIONS

SESSION 52: EPIGENETIC PATTERN IN OOCYTE AND EMBRYO

Tuesday 16 June 2015 **17:00–18:00**

O-194 H3K4me3-dependent epigenetic memory regulates transcriptional reactivation in the oocyte

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Study question: How does the oocyte regulate its transcriptional activity in light of its prolonged meiotic arrest?
Summary answer: A histone methylation-mediated epigenetic memory programed by the demethylase KDM5 is required for the correct temporal reactivation of the oocyte's transcriptional activity.
What is known already: During oogenesis oocytes transit from stages of transcriptional activity to those of transcriptional quiescence, and such transitions are believed to be essential for proper gamete formation. Although the temporal regulation of these transitions has been well documented across diverse organisms, the molecular mechanisms underlying these processes remain largely unknown.
Study design, size, duration: Basic research using the *Drosophila melanogaster* (fruit fly) model organism. The Drosophila ortholog of the human KDM5 gene family (hereafter referred to as dKDM5) was down-regulated specifically in the female germline by *in vivo* RNAi (knockdown efficiency: 97%). Outputs were compared to that of a mock RNAi.
Participants/materials, setting, methods: Transcriptional activity in the oocyte was temporally measured by an ex vivo ovary incorporation assay (Click-iT assay). Oocyte chromatin structure was analyzed and quantified by confocal microscopy after staining for DNA and H3K4me3. dKDM5 localization was analyzed by substituting the endogenous gene by a HA-tagged genomic construct.
Main results and the role of chance: Germline-specific dKDM5 knockdown results in severely reduced female fertility. Oocytes display precocious transcriptional reactivation and an equally precocious chromatin remodeling, leading to the premature acquisition of an open chromatin configuration. Both effects are a possible consequence of the significant up-regulation of H3K4me3 levels in the ovary, particularly in the meiotically-arrested oocyte. Increased H3K4me3 levels seem to solely impact the transcriptional status of the oocyte, as no evidence for either the activation of the DNA damage checkpoint or meiotic maturation defects were recorded. dKDM5 is evicted from the oocyte's chromatin by early oogenesis, indicating that the transcription defects recorded approximately 24 h later are the possible consequence of a H3K4me3-based epigenetic memory mechanism.
Limitations, reason for caution: The functional consequences of the reported transcriptional deregulation need to be fully elucidated.
Wider implications of the findings: Our results provide novel insight into the epigenetic mechanisms employed by the oocyte to regulate its transcriptional activity during the prolonged meiotic arrest. Given the significant evolutionary conservation of both dKDM5 and H3K4me3, it is likely that the in pre-ovulatory oocytes of our species the transition from a transcriptionally active to an inactive state is under equivalent epigenetic control. The deregulation of this process can therefore be an underlying cause of infertility in patients with low oocyte quality.
Study funding/competing interest(s): Funding by national/international organization(s) – Partly funded by Fundação para a Ciência e a Tecnologia.
Trial registration number: NA.
Keywords: meiosis, histone methylation, oocyte, transcription

O-195 Histone H4 lysine 12 (H4K12) acetylation state as epigenetic marker of quality of oocytes from in vitro maturation (IVM)

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Study question: Can H4K12acetylation state be used as oocyte quality marker to improve IVM?
Summary answer: H4K12acetylation was low in *in vivo* ovulated (IVO) mouse metaphase-II oocytes and high in IVM denuded oocytes (DOs). Cumulus-enclosed (COC) and stimulated physiological oocyte maturation (SPOM) decreased acetylation. Most normal status was achieved after 3D-follicle culture. Therefore H4K12acetylation appears to be a marker of oocyte quality, useful to improve IVM.
What is known already: The activity of histone deacetylases (HDACs /sirtuins) is relevant for epigenetic remodeling of chromatin during maturation. While H4K12 is hyperacetylated in mature GV oocytes and hypoacetylated at the metaphase-II (MII) state, it becomes reacetylated in zygotes and preimplantation embryos. The level of H4K12 acetylation is increased in oocytes of older female mice, after postovulatory aging and in diabetic mice. Increased H4K12acetylation is linked to age-related aneuploidy in human oocytes.
Study design, size, duration: Denuded or cumulus-enclosed murine MF1 GV oocytes underwent IVM for 16 h or SPOM for 21 h. Preantral follicles from C57/Bl6xCBA/Ca-mice were cultured for 13d or 10d under adherent (2D) or non-attachment (3D) conditions. H4K12acetylation pattern was analyzed in IVO and IVM MII oocytes by semiquantitative immunofluorescence.
Participants/materials, setting, methods: MII oocytes from IVO and IVM from COCs ± SPOM, COCs from SPOM ± dbcAMP (dibutyryl-cAMP), SPOM ± carbenoxolone, and from 2D and 3D follicle culture were fixed after zona removal and stained by specific antibody to H4K12ac. Image analysis was by ImageJ software comparing ratiometric mean values between IVO and IVM oocytes.

Main results and the role of chance: The highest H4K12acetylation state was recorded in the DO group followed by decreasing signal intensity in the SPOM groups, and was similarly decreased in 2D follicle culture. dbcAMP did not lead to a further reduction in acetylation of H4K12 in SPOM. MII oocytes from 3D follicle culture had very low acetylation signal, most similar to the signal intensity found in IVO MII oocytes. Since oocytes from SPOM, 2D and especially 3D follicle culture had lowest acetylation levels and most closely resembled the IVO group, and studies by other groups have shown improved oocyte quality in these IVM conditions, H4K12acetylation appears to be a promising marker of oocyte quality and might be helpful to improve IVM conditions.
Limitations, reason for caution: H4K12acetylation is just one of the multiple posttranslational modifications accompanying oocyte maturation. Further studies are required to assess histone deacetylase activities and compare different histone patterns and developmental potential of MII oocytes from IVM to confirm that H4K12acetylation can serve as marker for oocyte quality in mouse and other species.
Wider implications of the findings: Stage specific alterations in chromatin conformation and gene expression characterize oocyte growth, maturation and have impact on developmental potential. Although we have no information on the relevance of H4K12acetylation for the developmental competence, aberrant acetylation patterns associated with aging and metabolic diseases imply that it reflects the activities of HDACs and oocyte quality. Our studies suggest that it can be used as marker to improve *in vitro* culture conditions, e.g., in ART.
Study funding/competing interest(s): Funding by national/international organization(s) – Deutsche Forschungsgemeinschaft (DFG, FOR 1041).
Trial registration number: None.
Keywords: Epigenetic, IVM, oocyte quality, histone acetylation

O-196 Altered expression of imprinted genes in cord blood but not placenta from babies conceived via assisted reproductive technologies

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Study question: Do children conceived by the assisted reproductive techniques of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) have altered DNA methylation at imprinted regions or alterations of imprinted gene expression in placenta and/or cord blood samples compared to naturally conceived (NC) controls?
Summary answer: Our findings indicate that the imprinted *PLAGL1* gene may be susceptible to epimutations in the ART population as alterations in DNA methylation and gene expression in cord blood samples from ART infants were observed. Further, imprinted gene *KCNQ10T1* displayed altered expression in cord blood from IVF pregnancies.
What is known already: Studies in both animal and human models have indicated that ART is associated with increased rates of rare imprinting disorders and low birth weight. Altered DNA methylation and gene expression of imprinted genes has been found in ART infants; however results have been conflicting and inconclusive. Aberrant expression of *PLAGL1* has been linked to transient neonatal diabetes mellitus (TNDM), intrauterine growth restriction (IUGR) and numerous malignancies.
Study design, size, duration: Placental and umbilical cord samples from 271 infants were collected at birth for this prospective cohort study. Samples were divided into three experimental groups based on conception mode: (A) *in vitro* fertilization (IVF) (n = 105) (B) intracytoplasmic sperm injection (ICSI) (n = 79) and (C) naturally conveived (NC) controls (n = 87).
Participants/materials, setting, methods: DNA methylation at *PLAGL1*, *KvDMR1* and *PEG10* differentially methylated regions (DMRs) and *LINE-1* repetitive element expression levels were analyzed by bisulfite pyrosequencing. Quantative PCR (qPCR) was used in order to analyze imprinted gene expression levels in cord blood and placenta from ART and NC pregnancies.
Main results and the role of chance: We discovered a significant increase in DNA methylation at the DMR of the tumor suppressor *PLAGL1* in cord blood from IVF pregnancies (P = 0.0007) compared to NC controls, as well as decreased levels of *PLAGL1* gene expression in cord blood from both ART groups (IVF: P = 0.0013; ICSI: P = 0.016). We also found significantly altered gene expression levels of *KCNQ1OT1* (P = 0.0002) in IVF cord blood despite a lack of changes in DNA methylation. No significant differences were observed in DNA methylation or gene expression levels of analyzed regions in placental chorionic villi of ART pregnancies compared to NC controls (P > 0.05).
Limitations, reason for caution: As underlying subfertility has been shown to be associated with imprinting errors, it would be beneficial to control for fertility issues of the parents. However, in this study that clinical information was not obtained. Furthermore, we could not rule out the possible effects of ART techniques on aberrant imprinting.
Wider implications of the findings: These findings indicate that the imprinted *PLAGL1* tumour suppressor gene may be susceptible to epimutations in the ART population and could potentially lead to increased risk of imprinting disorders, IUGR and cancer susceptibility. Imprinted gene expression may be altered in ART pregnancies, despite a lack of change in DNA methylation.
Study funding/competing interest(s): Funding by national/international organization(s) – None of the authors has any competing interests. This study was funded by the Canadian Institute of Health Research (grant number MOP-77549 to S.M.)
Trial registration number: NA.
Keywords: Epigenetics, DNA methylation, Gene expression, Genomic Imprinting, ART pregnancies

O-197 Genome-wide epigenetic evaluation of cord blood from in vitro-conceived babies

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Study question: Are there genome-wide epigenetic differences in cord blood between babies conceived *in-vitro* and *in-vivo*?
Summary answer: No significant changes in DNA methylation profiles of cord blood could be detected between *in-vitro* and *in-vivo* conceived babies, neither in the number of epimutations nor in their genomic localization.
What is known already: An increased incidence of rare imprint-associated disorders has been reported by epidemiological studies in babies born from human assisted reproduction technologies (ART). However, evidence for an association between ART and an altered DNA methylation status of the conceived babies are insufficient and principally focused on imprinted genes without taking into account the rest of the epigenome. No study with a genome-wide approach has been published so far.
Study design, size, duration: This was a prospective observational study conducted between December 2011 and July 2014. Women with a single pregnancy were enrolled at the 20th gestational week. Babies conceived by Intracytoplasmatic Sperm Injection (ICSI) (n = 18) were enrolled in the study as cases, while babies from natural conceptions (n = 29) represented the control population.
Participants/materials, setting, methods: DNA extracted from cord blood was bisulfite converted and the methylation status of more than 485.000 CpGs was analyzed using the Illumina Infinium HumanMethylation450K BeadChip. Methylation levels of each CpGs, global number of rare and stochastic epigenetic differences (epimutations) and their genomic localization were compared between cases and controls.
Main results and the role of chance: Hierarchical clustering did not demonstrate different methylation profiles in cord blood between cases and controls. None of the 485.000 CpGs had a significantly altered methylation status despite the 80% power to detect differences larger than 15%. Assuming that ART effects could be stochastic, we developed a specific analysis aimed at identifying subject-specific epigenetic alterations. No difference in the median number of epimutations was observed between cases and controls when the analysis was conducted using, as reference to detect outliers, population data from cord blood samples from an online database (n = 44, 1153 vs.1553, p > 0.05), peripheral blood samples from a general population (n = 167, 1242 vs. 1486, p > 0.05) and our study population (640 vs. 656, p > 0.05). No localized epimutation enrichment (lesion) was found even in imprinted genes.
Limitations, reason for caution: This study does exclude the presence of differences in methylation status smaller than 15%, but the biological relevance of such differences is doubtful. Moreover, only ICSI babies were evaluated and differences linked to other techniques, like cryopreservation, could not be excluded.

Wider implications of the findings: This study demonstrates that the ovarian stimulation, ICSI and the embryo culture are 'epigenetically safe', supporting the idea that factors associated with the diagnosis of infertility itself might underlie the causes of the imprinting disease enrichment in ART. The new developed analysis aimed at identifying rare and stochastic epigenetic differences could also be implemented in the safety evaluation process of new drugs and techniques potentially useful for ART procedures.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Fondazione IRCCS Ca'Granda, Ospedale Maggiore Policlinico, Milan, Italy, IRCCS San Raffaele Scientific Institute, Milan, Italy, Istituto Auxologico Italiano, Milan, Italy.

Trial registration number: NA.

Keywords: DNA methylation, imprinting, epigenetics, genome-wide, ICSI

SELECTED ORAL COMMUNICATIONS

SESSION 53: FROM OOCYTE TO CHILD HEALTH – IMPACT OF TECHNOLOGY

Tuesday 16 June 2015 **17:00–18:00**

O-198 Understanding the impact of Assisted Reproductive Technologies (ART) on embryo health, child health and disease and longevity in later life

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Study question: Can gene expression microarray data from different stages of human embryo development be used to investigate the expression of metabolic and epigenetic pathways contributing to embryo health? Such key pathways are likely to be affected by ART factors such as maternal oocyte age, embryo cryopreservation and prolonged embryo culture.

Summary answer: Several novel genetic and epigenetic regulatory pathways have been identified as important in embryonic genome activation and blastocyst formation. Expression of some of these are altered by ART factors. Further analysis into interacting partners of the novel genes reveal unique developmental networks important for healthy blastocyst formation.

What is known already: Many studies have shown the relationship between the maternal environment and offspring health, using animal models and epidemiological data. Mouse embryos removed from a diabetic environment before embryo replacement have an increased risk of developing obesity, diabetes and metabolic syndrome in later life and other studies demonstrate the importance of epigenetic DNA methylation stability during embryonic development. However few studies have attempted to clarify the link between the environment/ART technology and human embryo and future offspring health.

Study design, size, duration: Gene expression microarray data and QPCR analysis of amplified cDNAs from individual embryos and isolated blastomeres from different stages of human embryo development (n = four × oocytes, four × 4 cell, three × 8 cell, eight individual 8 cell blastomeres and ten × blastocyst stage embryos).

Participants/materials, setting, methods: Embryos were donated by patients undergoing IVF at St. Mary's hospital, Manchester, UK. cDNA was amplified via polyAPCR and analysed using the Affymetrix microarray HG U133 plus 2 chip, at the Paterson cancer Institute, Manchester. Arrays undergo rigorous AQM analysis within R, statistical analysis within Partek, Cytoscape and Ingenuity.

Main results and the role of chance: We identified a large epigenetic gene list which correlated with maternal age with two clear groups of gene expression profiles emerging, in embryos from women aged 35 and under, versus those aged >35. We detect a strong up-regulation of a selection of oxidative stress pathways as the embryo remains in extended culture; in particular the NRF2 mediated oxidative stress response. A more systems based network analysis resulted in the generation of novel embryonic developmental expression networks, network modules and module/gene hierarchy and causal networks. Data mining revealed many interesting genes, amongst them Zscan4, previously shown to restore developmental potency of mouse embryonic stem cells, identified as a key gene during EGA. A network of transcription factors and the epigenetic regulatory genes GATA2, TRIM8 and DNMT3L are significantly increased in the blastocyst and may represent important changes occurring during the period of *in vitro* culture in IVF.

Limitations, reason for caution: The number of samples per developmental stage was restricted in order to focus on individual embryos. Embryos are inherently different to one another, e.g., in genetic background and quality and therefore detailed analysis needs to be applied to identify trends in gene expression.

Wider implications of the findings: Understanding developmentally important pathways which contribute to human embryo health is critical for our understanding of potential risk factors to which human embryos are subject *in vitro*. Assessing the perturbation of such genetic pathways in response to ART is an essential part of a fully-informed risk assessment. This will ultimately lead to increased understanding of long term health outcomes for ART children and the consequent modification of risk factors will ultimately help to improve ART conditions and avoid unwanted impacts.

Study funding/competing interest(s): Funding by national/international organization(s) – This research is funded by the European Commission under the FP7 Health programme, as part of the EpiHealth consortium (co-ordinator Professor Andras Dinnyes).

Trial registration number: Embryos were donated with approval from Central Manchester Ethics Committee and the Human Fertilisation and Embryology Authority (HFEA licence R0026).

Keywords: Embryonic Genome Activation (EGA), Transcription Factors (TFs), Array Quality Metrics (AQM), Assisted Reproductive Technologies (ART)

O-199 Elective frozen-thawed vs fresh transfer of euploid embryos identified by comprehensive chromosome analysis using next generation sequencing – interim analysis of a randomized controlled trial

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Study question: Is the transfer of a frozen thawed blastocyst in an artificial cycle associated with higher implantation and ongoing pregnancy rates than fresh blastocyst transfer in a stimulated cycle?

Summary answer: Frozen thawed transfer (FET) of euploid blastocysts in an artificial cycle seems to yield significantly higher implantation, clinical and ongoing pregnancy rates than fresh transfer of euploid blastocysts in the stimulated cycle.

What is known already: some data suggesting FET is associated with better clinical outcome than fresh transfers. Endometrial receptivity is thought to be disrupted by high estradiol levels during stimulation. However the quality of evidence is low to moderate, and generalizability of the findings is questionable. There's a need for properly sized randomized controlled trials controlling for other factors including embryo aneuploidy.

Study design, size, duration: Planned interim analysis of a randomized controlled trial, including 76 women over one year of recruitment. The study is halfway at the time of submission. We expect to have results from over 100 women by the time of conference.

Participants/materials, setting, methods: Women < 42 without poor ovarian reserve or azospermic partner were randomized to fresh transfer or FET. Assisted hatching was on day 3, and trophoectoderm biopsy was done on day 5 or 6. Comprehensive chromosome screening was by next generation sequencing (Ion Torrent PGM). Only euploid blastocysts were transferred.

Main results and the role of chance: Fresh and FET groups included 37 and 39 women, respectively. 15 women in the fresh group had no fresh transfer (13 had no euploid embryos, 2 other reasons). 31 women in the FET group were available for analysis (8 awaiting FET presently). Five women in FET group had no euploid embryos. Baseline and stimulation cycle parameters including number of blastocysts transferred were similar. Ongoing pregnancy rates (OPR) were 14/37 (38%) vs 20/31 (65%), in the fresh and FET groups, respectively (p = 0.03). Per protocol analysis revealed OPR of 14/22 (64%) vs 21/27 (78%), in the fresh and FET groups, respectively (p = 0.23). Implantation rates were 67 vs 82%, difference was not statistically significant but was in favor of FET (p = 0.23).

Limitations, reason for caution: The observed differences can change when the trial is completed.
Wider implications of the findings: In this trial effect of fresh transfer on endometrial receptivity is almost isolated as embryonic aneuploidy is eliminated through CCS. If the difference in favor of FET is maintained, this finding can change practice worldwide. FET with CCS can enable single embryo transfer with excellent pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies) – Life, Technologies, Oregon Reproductive Medicine, Reprogenetics.
Trial registration number: NCT02000349.
Keywords: IVF, PGS, fresh, frozen, NGS

O-200 A new strategy in selecting oocytes using cumulus cells analysis of specific molecules of the apoptotic pathway, according to the ability to reach blastocyst stage

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Study question: The aim of the research was to investigate the apoptosis rate of individual cumulus cell–oocyte complexes (COC), associated to the level of *p*-Akt, to verify the difference between oocytes who produce embryos able to reach the blastocyst stage compared with embryos arrested during the *in vitro* culture.
Summary answer: It was demonstrated that DNA fragmentation in cumulus cells was remarkably lower in patients who achieved a pregnancy after ICSI cycles, related to the quality of oocytes and embryos. Akt pathway plays a critical role in the regulation of cell survival, and most growth factors activate this pathway.
What is known already: Studies on oocyte maturation have shown the importance of LH-induced activation of the epidermal growth factor (EGF)-like growth factors. This can be a potential mechanism for transducing the LH signal to oocyte. It is known that LH is an anti apoptotic agent. It is unknown the relationship between apoptosis and Akt in cumulus cell. Akt has been reported to coexist in a multimolecular complex containing also ribosomal S6 kinase 1 and phosphoinositide-dependent kinase 1
Study design, size, duration: The study had the duration of 24 month. 53 patients were involved after informed consent. In this observational prospective study, it has been measured the DNA fragmentation rate and the level of *p*-Akt in cumulus cells of single cumulus–oocyte complex (COC) for each follicle containing a mature oocyte
Participants/materials, setting, methods: Normo-responder patients have been selected. DNA fragmentation rate in cumulus cells has been examined with the use of a TUNEL assay *in situ*. *p*-Akt has been examined by immunological assay *in situ*. Statistic of molecule expression and DNA fragmentation was tested through the repeated measures ANOVA test of log-transformed variables
Main results and the role of chance: Out of 255 MII oocytes, 197 were fertilized and the derived embryos had the following evolution: 117 completed the development to blastocyst (day 5 or 6) and were transferred, 57 were vitrified at blastocyst stage and 23 were arrested during *in vitro* culture at different stages of cleavage. We found a statistical difference between the DNA fragmentation rate of cumulus cells between the arrested embryos compared to the transferred and vitrified blastocysts ($p = 0.004$), confirming that cumulus apoptotic rate of the cumulus cells could be considered as a marker of oocyte competence. Likewise we found a statistical significance between oocytes resulting in transferred blastocyst and arrested embryos in the ratio *p*-AKT/TUNEL ($p = 0.043$); Therefore, the ratio *p*-AKT/TUNEL could be considered also a marker of oocyte competence
Limitations, reason for caution: More studies are needed to confirm these data and to determine the how these molecular pathways are involved on the oocyte competence
Wider implications of the findings: We found that in the cumulus cells of the oocytes able to produce blastocysts apoptosis is significantly lower and the *p*-AKT/TUNEL ratio is higher than in cumulus cells of arrested embryos, indicating that DNA fragmentation is lower when *p*-AKT is higher. Data seems to demonstrate that DNA fragmentation rate and *p*-AKT value in cumulus cells could be considered a molecular marker of oocyte competence, to evaluated as a prognostic pattern of blastocyst formation
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Research awarded by "Grant for fertility innovation 2012".
Trial registration number: The trial is an observational study and no registration is needed.
Keywords: blastocyst formation, p AKT, apoptosis, cleavage arrest

O-201 Storage time does not modify the gene expression profile of cryopreserved human metaphase II oocytes

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Study question: Does storage time impact on transcriptome of slowly frozen cryopreserved human metaphase II (MII) oocytes?
Summary answer: For the first time, we demonstrate that the length of cryostorage has no effect on the gene expression profile of human metaphase II oocytes.
What is known already: Oocyte cryopreservation is a largely-used technique in IVF for storage of surplus oocytes, as well as for fertility preservation (i.e., women undergoing gonadotoxic therapies) and oocyte donation programs. Although it is known that cryopreservation negatively impacts on oocyte physiology and it is associated with decrease of transcripts, no experimental data about the effect of storage time on the oocyte molecular profile are available to date.
Study design, size, duration: This study included 20 women undergoing IVF treatment, ≤38 years aged, without any ovarian pathology. Surplus MII oocytes were donated after written informed consent. A total of 21 non-cryopreserved oocytes and 44 surviving slow-frozen/rapid-thawed oocytes (24 oocytes cryostored for 3 years and 20 cryostored for 6 years) were analyzed.
Participants/materials, setting, methods: Pools of ≈10 oocytes for each group were prepared. RNA was extracted by miRNeasy Micro Kit (Qiagen), analyzed by RNA 6000 Pico kit on 2100 Bioanalyzer (Agilent), amplified by Ovation Pico WTA System (Nugen), labeled and hybridized on 4x44K v2 microarrays (Agilent). Analyses were performed by GeneSpring software.
Main results and the role of chance: Analysis of variance (ANOVA) showed 22 probe sets (corresponding to 19 genes) differently expressed between fresh and cryopreserved oocytes (fold change >2, Benjamini-Hochberg false discovery rate adjusted *p*-value ≤0.05). Specifically, 10 genes were down- and 9 were up-regulated in cryopreserved oocytes respect to fresh oocytes. Gene Ontology analysis by DAVID bioinformatics resource disclosed that genes that were down-regulated in frozen oocytes mainly belong to ribosome function (i.e., *MRPS31*, *RPL5*, *RPL10*, *RPL23*) and intracellular protein transport (*YWHAG*, *LRP2*, *AP1G1*) pathways. Moreover, cryopreservation deregulates acetylation through up-regulation of *HNRNPUL1*, *AP1G1*, *PYGO2* genes. Intriguingly, comparison of gene expression profiles between surviving thawed oocytes after 3 and 6 years of storage in liquid nitrogen found no differently expressed genes.
Limitations, reason for caution: Ongoing experiments are aimed to validate our data in a larger cohort of samples, i.e., analyzing additional pools of oocytes for each group, including "older" frozen oocytes.
Wider implications of the findings: Our study demonstrates that specific transcript levels are altered by cryopreservation, in agreement with literature that reported injuries of morphology, physiology and molecular integrity of oocytes following freezing. No data about effect of long-term oocyte cryopreservation are available. For the first time our data suggest that the length of storage does not alter the gene expression profile of frozen oocytes. This finding is noteworthy for safety issue of long-term oocyte banking, i.e., oncofertility, gamete donation.

Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by a grant of the Italian Institute of Health (CCM 2012).
Trial registration number: NA.
Keywords: oocytes, cryopreservation, slow-freezing, microarrays

INVITED SESSION

SESSION 54: FERTILITY SPARING SURGERY IN BENIGN AND MALIGNANT CONDITIONS

Wednesday 17 June 2015 **08:30–09:30**

O-202 Fertility-sparing surgery (FSS) in severe endometriosis and adenomyosis: what is the gold standard approach?

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Several data are reporting on a possible negative impact of endometriosis and adenomyosis on reproductive performance, although a causal relation is not proven. It is hypothesized that endometriosis as adenomyosis are both a primary disease of the uterus, constituting a single entity with variable phenotypically expression. The disease is characterized by the grow of endometrial like tissue (glands and stroma) outside the uterine cavity. In his publication Larsen et al. (*Eur J obst. Gynecol, 2011*) found that endometriosis was associated with adenomyosis in 34% with an incidence of 42.8% in stage IV and with a disorder of the junctional zone in 39.9%. As endometriosis is a pleiotropic disease with increased inflammation and neoangiogenesis and with impact on the endometrium and ovarian function, it is utopic to believe that surgery will eradicate the disease. In the most optimal circumstances surgery will restore the normal anatomical relations. The advantages of surgery of ovarian endometriosis have to be balanced very carefully in each individual patient against the potential harm of damaging the ovarian reserve. This is upmost important as the presence of ovarian endometriosis without any previous surgery has as such already a negative impact upon the follicular reserve due to the smooth muscle metaplasia and fibrosis with diminished ovarian blood flow in the surrounding ovarian tissue *(Jun Jun Qui AOGS 2012, Kuroda M Obstet Gynecol 2012, Kitajima Fertil Syeril 2011*). Although the ESHRE guidelines recommend the performance of a cystectomy in case of ovarian endometrioma, based upon only one randomized controlled study, more data are now becoming available mentioning a lower impact upon ovarian reserve by an ablative surgical procedure. It can be questioned if cystectomy is the most adequate surgery in view of fertility preservation. Important factor in fertility preservation is the early detection and when indicated treatment of the disease in an early stage. In case of large endometriotic cysts a two-step operative procedure should be considered. If conservative surgery is not possible implantation of fresh normal ovarian tissue is an option. Where initially "adenomyosis uteri" was a diagnosis resulting from histologic examinations of hysterectomy specimens, it now changed in a clinical entity with the introduction of the Archimetra concept and the more performing indirect imaging techniques of MRI and 3-D ultrasound. A good classification is still lacking as adenomyosis can be seen under different forms like diffuse, focal, cystic, adenomyoma and polypoidal. Adenomyosis seems to have a negative impact on reproductive outcome in patients referred to IVF programs (*Vercellini P et al. Hum. Reprod. 2014;29:964–977*). It is obvious that in patients at reproductive age there is no place for hysterectomy. Hormonal suppression seems to have a positive effect on pregnancy rates after iVF. The conservative uterine sparing cytoreductive surgery is an alternative effective treatment for fertility preservation with a beneficial effect upon patient's wellbeing. Several surgical techniques are available. Non-excisional techniques using electrocoagulation are reported also to have a beneficial impact. Fertility sparing approaches for endometriosis and adenomyosis should be implemented wherever possible. It improves patient's fertility offering the possibility of spontaneous conceptions and increases patient's well being.
Keywords: endometriosis, adenomyosis, surgery, fertility sparing

O-203 Fertility-sparing surgery in cancer patients

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The issue of fertility preservation must be addressed early in the oncologic management of young patient. Conservative treatment can be offered in some well-defined situations; in other cases the data are more uncertain. A specialized multidisciplinary management (oncologist, surgeon, procreation specialist, and psychologist) had to be proposed. The physician must be able to explain that in some cases conservative treatment is not reasonable, and best oncological treatment prevails.
Keywords: fertility sparing surgery, gynecologic cancer

INVITED SESSION

SESSION 55: ICSI AND BEYOND

Wednesday 17 June 2015 **08:30–09:30**

O-204 Fertility in ICSI boys

M. Bonduelle
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O-205 RAMAN microspectroscopy: the new analytical tool for sperm, eggs and embryos

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Despite recent technological and analytical advances in the field of reproductive medicine, fertility clinics worldwide are still confronted with the large disparity between the high fertilization rates now achievable by modern artificial reproductive treatments and the concomitantly low pregnancy and/or take home baby rates. The introduction of many and varied, modern techniques has significantly increased the amount of information available to scientist and clinician alike, however the utility of most of these new methods is limited as the processes usually necessitate the modification and/or destruction of the cell. What is needed is a non-invasive, non-destructive means of obtaining accurate information, whilst not affecting the integrity of the cell and which allows for its selection and use. Such a method would radically transform the way clinical reproductive medicine is currently being conducted. Raman spectroscopy is based upon the inelastic scattering resulting from the interaction between light and matter. This produces a unique pattern of changes in the vibrational state of each affected molecule and its environment which constitute the sample's chemical "fingerprint." Discovered in 1928, the molecular deformations detected by Raman have been used to identify and classify a variety of different substances, when complemented by the three-dimensional spatial resolution afforded by confocal microscopy (i.e., Raman microspectoscopy) it can detect changes in and location of defined molecules within a cell. In medicine, these advances have led to its successful utilization in the discrimination, classification and diagnosis of pathological conditions such as different malignancies and tumors. Being non-invasive it has also been employed in the investigation and analysis of various living cells (e.g., living bacteria and stem cells) providing specific data without any adverse effects to the cells themselves. As such, Raman microspectoscopy appears to possess the attributes for the desired gamete assessment and selection technique. In reproductive medicine, the first Raman investigations were primarily oncological in nature; however, the past few years have seen an increase in its application in the assessment of both male and female gametes. More specifically, the characterisation and localisation of different structural and chemical elements identified by the technique have been associated with the vitality and maturity of oocytes, the integrity of the zona pellucida and the health and implantation potential of embryos. Furthermore, it has been suggested that the Raman identified chemical constituents of the medium used for culturing are indicative of the metabolism and hence health of ART produced embryos. In the male, studies have been conducted into the constituents of seminal plasma and the function of the testis and prostate and associations made

with different pathological states. The greatest progress thus far has been made in the identification, characterization and localization of sperm nuclear DNA damage, an area of increasing interest for ART. With the incorporation of recent technological advancements into the instrumentation of Raman spectroscopy, the capabilities and information obtainable by the method have grown quickly and significantly. Not surprisingly so has the enthusiasm surrounding the technique. However, as with all new techniques, if Raman microspectroscopy is to achieve its true potential it must undergo stringent validation and verification processes. This is the challenge that awaits.
Keywords: Raman microspectroscopy, gamete selection

INVITED SESSION

SESSION 56: OUTCOME AFTER ART; GENES, LIFE-STYLE OR TECHNOLOGY

Wednesday 17 June 2015 **08:30–09:30**

O-206 Towards comprehensive epigenomic profiling of the human ART embryo by genome-wide DNA methylation analysis

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The programming of epigenetic information is an essential requirement for normal development in mammals. DNA methylation is one particular epigenetic mark that is dynamically reprogrammed during gametogenesis and preimplantation development. In humans, these reprogramming events occur in the gametes and in preimplantation embryos at the time when ART is being performed. Inappropriate programming of epigenetic information, including DNA methylation, is associated with disease. Two genomic imprinting disorders may be associated with ART in humans although the precise cause remains to be identified. These cases are rare, however, it must be considered that ART cohorts may retain an epigenetic signature that reflects their exposure to the *in vitro* environment, and that this may in some cases have an adverse developmental legacy. The analysis of the epigenetic regulation of the human preimplantation embryo is of particular value for expanding our understanding of this critical period of development. If achievable at the single embryo level, the data may also reveal whether embryonic epigenetic programs are affected by assisted reproduction technologies (ARTs) and/or infertility or other parameters. Indeed, this information may eventually be useful for informing future approaches in ART. Whilst there have been numerous technology-driven advances in many fields of reproductive science (PGD/PGS for example), our progress in the understanding of epigenetic programming has until very recently been extremely limited in comparison, with experiments often limited to a small number of genes. Fortunately, the advent of next generation sequencing-based technologies such as Reduced Representation Bisulfite Sequencing (RRBS) and related techniques that allow the study of DNA methylation (5-methylcytosine) across the genome have now been applied successfully to the study of mammalian gametes and preimplantation embryos. Importantly, these techniques have been used to reveal very detailed information on the reprogramming of DNA methylation of human preimplantation development as reported recently by several research groups. These studies constitute a major leap in our comprehension of the biology of human gametogenesis and preimplantation development. In this presentation, these recent studies will be discussed in addition to the presentation of research from our laboratory which reveals that genome-wide DNA methylation analysis of single preimplantation embryos (human, bovine) by RRBS is indeed possible. Although much work remains to be done, such as mapping the reprogramming of the histone code and 5-hydroxymethylcytosine (5 hmC) in human gametogenesis and preimplantation development, collectively these advances will provide greatly enhanced understanding of normal human embryonic development and any developmental consequences that ART and/or infertility may have.
Keywords: epigenetics, assisted reproduction, DNA methylation, genomic imprinting, reprogramming

O-207 Increased morbidity and mortality after ART: related to the infertility or the reproductive technology?

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Since the birth of Louise Brown, more than 6 million babies have been born world-wide as a result of assisted reproductive technology. The scope of fertility treatment is rapidly growing in both western and less developed countries. Debates related to the safety aspects of the reproductive technology are ongoing. Several studies have focused on safety aspects of the reproductive technology. Most studies have investigated differences in pregnancy complications, perinatal outcomes, maternal risks, long term morbidity and development by comparing pregnancies following assisted fertilisation with pregnancies after spontaneous conception in the general population. For several years, attention to safety has been towards the multiplets with higher risk of both morbidity and mortality. During the last decade, along the implementation of single embryo transfer, focus on safety aspects has turned towards the outcome of singletons born after assisted fertilisation. Singletons born after ART have poorer perinatal outcomes compared to children born after natural conception. There are, however, major methodological problems in many of the studies that have evaluated effects of ART. In particular, comparisons of outcomes in pregnancies achieved by assisted fertilisation in subfertile women and outcomes following spontaneous conception in fertile women is challenging. Although, various approaches have been used to explain the gap between ART and spontaneously conceived pregnancies, the recurring objection has been the identification of relevant comparison groups to avoid confounding by indication. Recently, attention has been towards potential effects on birth weight of different culture media, days of culture and cryopreservation following the steady increase in the proportion of children born after cryopreservation or vitrification. Cryopreservation both maximizes the biological potential of each fertilized oocyte and reduces the strain for the couples by reducing the number of stimulated cycles. However, several studies have shown that the risk of being large-for-gestational age is higher in FET singletons compared to singletons born after fresh cycles and natural conception. This may indicate that the foetal growth potential may differ in children born after transfer of cryopreserved/thawed embryos. Whether the increase in birth weight is associated with epigenetic modification is not known.
Conclusion: The future of ART is crucially depending on the short and long term safety aspects for both children and mothers, and research and careful monitoring of outcomes are essential. New medications used for controlled ovarian hyper-stimulation, changes of media used for embryo culture and the rapid development of laboratory procedures of embryos are examples of the plethora of important factors that must be observed and continuously evaluated.
Keywords: safety, epidemiology, sibling study, perinatal outcome

INVITED SESSION

SESSION 57: CHINESE SOCIETY FOR REPRODUCTIVE MEDICINE EXCHANGE SESSION

Wednesday 17 June 2015 **08:30–09:30**

O-208 Genome and transcriptome analyses of single human oocytes and preimplantation embryos

Jie Qiao
Peking University Third Hospital, Medical Center for Human Reproduction, Dept. of OB/GYN, Beijing, China

O-209 First healthy baby born after PGD (preimplantation genetic diagnosis) – noninvasive haplotype screening in couples both carrying deafness gene GJB2 mutation

Z. Chen[1], Y. Gao[2], S. Huang[2], J. Yan[2], J. Li[2], M. Gao[2], Y. Zou[2], K. Wu[2], P. Xu[2], R. Kang[2]
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Introduction: Nonsyndromic hearing loss and deafness is characterized by congenital, non-progressive, mild-to-profound sensorineural hearing impairment. Despite extraordinary genetic heterogeneity, mutations in one gene, GJB2, which encodes the connexin 26 protein and is involved in inner ear homeostasis, are found in up to 50% of patients with autosomal recessive nonsyndromic hearing loss. In this study, a family with a hearing loss son was recruited and the proband has compound heterozygous mutations in GJB2 gene, c.235 del C from mother and c.299_300 del AT from father. In order to block the vertical transmission of causative mutations, PGD was performed and an embryo free of hearing problems was placed back in the uterus. A haplotyping based on noninvasive prenatal test with maternal plasma DNA for target mutations confirmation was constructed and aneuploidy screening was performed as well. Our study indicates that the haplotype-assisted target mutation testing and aneuploidy screening with maternal plasma for monogenic disease has potential application in pregnant women after PGD.
Material and methods: 12 STRs in chromosome 13 were used for linkage analysis in PGD, including D13S141, D13S175, D13S633, D13S1275, D13S250, D13S232, D13S1830, D13S292, GJB2-AT1, GJB2-AT2, GJB2-TG2, GJB2-AC1. The parental haplotype was constructed using a trio strategy through two different processes, namely, the parent-assisted haplotype phasing process and the proband-assisted haplotype phasing process. The fetal haplotype was deduced afterward based on both the maternal plasma sequencing data and the parental and the proband haplotyping. The causative mutations in GJB2 gene were final confirmed with amniotic fluid DNA by Sanger sequencing as well.
Results: After STRs linkage analysis of PGD, we got 5 carriers and 3 affected embryos, but no normal embryos. The couple agreed to transfer a carrier embryo into the uterus based on completely genetic counseling. Haplotype analysis and aneuploidy screening with maternal peripheral blood showed that the fetus carried a heterozygous mutation of c.235 del C in GJB2 gene and had no aneuploidy problem. Data from Sanger sequencing with amniotic fluid was also in accordance with PGD and non-invasive prenatal testing data. Finally, a baby boy without hearing problem was delivered.
Conclusions: It was proposed that haplotype-based mutation detection and aneuploidy screening combined with PGD may be extended to prenatal testing of most monogenic disease.
Keywords: congenital hearing loss, preimplantation genetic diagnosis, haplotyping screening, GJB2 Gene, aneuploidy screening

SELECTED ORAL COMMUNICATIONS

SESSION 58: ASSESSING BLASTOCYST DEVELOPMENT AND STEM CELL POTENCY

Wednesday 17 June 2015 **10:00–11:45**

O-210 Predicting pregnancy outcomes using a non-invasive analysis of secretome parameters in spent blastocyst culture media using MALDI-ToF MS in ART

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Study question: Can the non-invasive analysis of spent blastocyst culture media by MALDI ToF MS predict ART outcomes?
Summary answer: Algorithms exploiting mass/charge variability in the spectra generated from spent blastocyst culture media can accurately predict pregnancy outcomes.
What is known already: All available technologies to evaluate successful implantation, such as PGS, require embryo biopsy. MALDI-ToF MS is successfully exploited in microbiology, but hasn't been reported previously in the context of ART. Proteins in media from embryos have been detected using MALDI and were consistent with gene expression studies; however the findings were not correlated with pregnancy outcomes. This study represents the first to correlate the spectral analysis of peptides in the embryo secretome with pregnancy outcome.
Study design, size, duration: Prospective pilot study examining 75 samples of spent media from embryo cultures using MALDI ToF mass spectrometry and subsequent correlation with pregnancy outcomes. Samples were collected and analyzed between March and December 2014.
Participants/materials, setting, methods: Spent culture media from blastocysts in culture prior to embryo transfer were collected as part of routine ART cycles and stored at –20°C. The samples were shipped frozen to the analytical laboratory and subjected to matrix assisted laser desorption ionization (MALDI), time of flight (ToF) mass spectrometry (MS).
Main results and the role of chance: Data from spectra was collected from the region 12,000 to 50,000 m/z and normalized. Quantitative characteristics of the spectral data were used to compare four groups: pregnant ongoing ($N = 32$), pregnant spontaneous abortion (SAB) ($N = 11$), pregnant biochemical ($N = 9$), and not pregnant (negative pregnancy test) ($N = 23$) alongside media controls ($N = 5$). Algorithms exploiting the m/z variability were designed to predict each outcome and all classifications were assigned using a combination of <20 cut-off based criteria. All outcomes could be predicted with 95% accuracy with only 5% incorrectly classified (1fp biochemical, 1fp SAB, and 2fp pregnant).
Limitations, reason for caution: Relatively small number of cycles and samples.
Wider implications of the findings: Predicting pregnancy outcome by embryo viability has focused on identifying single or multiple markers of success/failure, in combination with assessments of embryo morphological quality by light microscope or time-lapse video. Despite these approaches and technological developments, pregnancy successes are largely unimproved. Unlike these approaches, MALDI ToF MS of the culture media prior to embryo transfer represents an accurate, rapid and non-invasive method of determining embryo quality and likelihood of pregnancy success based on their secretome.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by commercial/corporate company(ies) – Virginia Center for Reproductive Medicine. MAP Diagnostics.
Trial registration number: None.
Keywords: embryo selection, MALDI-ToF, blastocyst, ART

O-211 Blastocoel cavity contains miRNAs: novel potential biomarker of blastocyst quality

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Study question: Can we consistently profile and characterize miRNAs from blastocoel cavity?
Summary answer: MicroRNAs can be consistently profiled in human blastocoel cavity and the population of miRNAs detected within the blastocoel is mostly heterogeneous with respect to the cellular counterparts of the blastocyst, with some miRNAs significantly up-regulated or preferentially expressed in the former with respect to human ICM and TE.
What is known already: Only proteomic and metabolomic analyses have been published up to date characterizing human blastocoel, but no data have been ever reported profiling miRNAs within the inner cavity of a blastocyst. There are evidences of miRNAs secretion by donor cells in order to exert a gene regulatory effect upon recipient ones, highly suggesting the possibility of a miRNAs-mediated autocrine communication within the blastocyst via the blastocoel fluid.
Study design, size, duration: Five good quality expanded human blastocysts underwent blastocoel collection according to a previously published method between November and December 2014. Blastocoel samples were screened for miRNA content and compared to a reference database previously built on human ICM and TE miRNA expression profiles.
Participants/materials, setting, methods: MiRNA expression was evaluated using TLDA Cards A (Applied Biosystems) containing primer sets for 381 human miRNA sequences. Ct values at a level ≥37 cycles and miRNAs expressed in <60% biological replicates were excluded from the analysis. The mean expression level of expressed miRNAs was used for normalization.
Main results and the role of chance: Pearson's correlation of raw Ct values among blastocoel samples ranged between 0.69 and 0.78. It was instead comprised between 0.33 and 0.45 when comparing them to ICM, and between 0.29 and 0.39 to TE. Minimum and maximum number of miRNAs detected in

the blastocoel were 50 and 51. Thirty-three were expressed in at least 60% of samples run. Twenty-one out of them were also common to ICM (63.6%), 24 to TE (72.7%), and 19 to both (57.5%). Normalized Ct hierarchical clustering, built on complete linkage and Euclidean distance, highlighted a sharp division between ICM, TE and blastocoel. The volcano plot built on the parametric test Limma and Benjamini-Hochberg correction showed 6 and 7 miRNAs up-regulated ($p < 0.001$) in blastocoel versus ICM and TE, respectively. One and 2 were instead respectively down-regulated ($p < 0.001$).

Limitations, reason for caution: Since miRNAs secretion is a dynamic process throughout blastocyst expansion and only fresh good quality expanded blastocysts were included, we need data on blastocoels from thawed, bad quality and/or blastocysts of different expansion grades. Prospective studies are also needed to investigate correlations between blastocoel miRNA profiling and blastocyst implantation.

Wider implications of the findings: This is the first profiling of miRNAs from blastocoel. It can mirror important parameters such as blastocyst morphological quality, metabolic or degenerative processes, and reproductive competence. To this regard, we still lack methods to boost our predictive power upon blastocyst implantation potential beyond current limits, and blastocoel miRNA evaluation is an unexplored strategy in this scenario.

Study funding/competing interest(s): Funding by hospital/clinic(s) – G.EN.E.R.A. centres for reproductive medicine.

Trial registration number: None.

Keywords: blastocoel, miRNA, embryo selection

O-212 Association between blastocoel proteins abundance and embryo chromosomal status in humans

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Study question: Can protein contained in single human blastocoels be accurately measured? Could this assessment be employed to identify an embryo's developmental competence?

Summary answer: We succeeded in developing a sensitive Mass Spectrometry (MS) method allowing characterization of the proteome of blastocoel fluid in pooled samples. Further refinements now enable reliable quantification of selected proteins in blastocoel samples from individual embryos, revealing two proteins with abundance potentially associated with the ploidy status of the embryo.

What is known already: To develop an accurate embryo assessment methodology, several groups have been investigating embryonic secretions. However, the vast quantity of protein compounds already present in the culture media may mask much less abundant (yet clinically meaningful) proteins of embryonic origin, making biomarker discovery and subsequent measurement for clinical purposes extremely difficult. Blastocoel fluid retrieval via microsuction (Blastocentesis) provides a sample of almost exclusive embryonic origin, minimizing exogenous compound contamination and thus allowing more reliable protein identification.

Study design, size, duration: Prospective study. Tandem MS was performed on 80 samples of human blastocoel fluid. Subsequently, gene expression analysis was performed on 27 human blastocysts to confirm embryonic expression of detected proteins. Parallel Targeted MS and comprehensive cytogenetic analysis were performed on 14 human blastocysts. This study was performed over 3 years.

Participants/materials, setting, methods: The blastocoel proteome was characterized using Tandem MS. Support for embryonic origin of identified proteins was obtained via transcriptomic analysis (microarrays and quantitative PCR) of whole blastocysts. Nine Selected Reaction Monitoring Assays for quantification of confirmed protein targets were developed and validated on single blastocoels using labeled synthetic peptides.

Main results and the role of chance: Tandem MS identified 288 proteins that exist within the human blastocoel (false discovery rate <1%). Comparison of transcriptomic and proteomic data suggests that >80% of proteins found within blastocoel fluid are derived from genes actively transcribed at the blastocyst stage. Using Targeted MS, we succeeded in measuring the concentration of specific proteins of interest in single blastocoels. Interestingly, we observed that cytoplasmic proteins with a role in metabolism show an apparent increase in abundance in aneuploid embryos compared to those that are chromosomally normal. Although non-significant in this small sample ($P = 0.089$) the apparent difference warrants further investigation. Nuclear markers detected within the blastocoel, involved in chromatin stabilization, also displayed a tendency towards altered abundance in embryos with an abnormal karyotype (Fisher's Exact $P = 0.087$).

Limitations, reason for caution: Although a trend in protein abundance can already be appreciated in the cohort of samples investigated, population size should be increased to evaluate statistical significance.

Wider implications of the findings: This study offers valuable information on the transcriptome and secretome of the human blastocyst. More importantly, we demonstrate that the detection and quantification of multiple embryo-derived proteins from single human blastocoels is feasible despite the minute size of the sample analyzed. Future expansion of this study may allow confirmation of a relationship between abundance of specific blastocoel proteins and embryo developmental competence, potentially introducing a novel embryo assessment methodology based on functional proteomics.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Oxford Fertility Unit.

Trial registration number: None.

Keywords: blastocoel, proteomics, targeted mass spectrometry, embryo chromosomal status

O-213 Balancing between totipotency and differentiation in the human embryo

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Study question: This study aimed to investigate the relationship between unique characteristics of the cell cycle and the developmental capacity of undifferentiated early blastomeres and embryonic stem cells (hESC) from human origin. We analyzed the role of cell cycle related protein Cyclin E1 (CCNE1) in totipotency and early differentiation of hESC.

Summary answer: We described a new CCNE1-dependent intermediate embryonic cell state balancing between totipotency and differentiation. Cells in this high developmental potential state are capable to develop into trophectoderm (TE) and three distinct inner cell mass (ICM) populations: pluripotent epiblast, hypoblast and a population with a visceral endoderm (VE)-like phenotype.

What is known already: We have chosen to study CCNE1 because it is abundantly expressed during human preimplantation development and because its activity is changing depending on the developmental capacity of the embryonic cells. The periodicity of CCNE1 expression is lost in pluripotent cells as compared to somatic cells. Therefore, constitutive expression of CCNE1 was suggested to be associated with the undifferentiated state in human embryonic cells.

Study design, size, duration: First, we analyzed the CCNE1 protein expression pattern in human preimplantation embryos and hESC and correlated it with the distribution of already known pluripotency and differentiation markers. Then, CCNE1 function was examined in hESC and extrapolated to human embryos using CCNE1 overexpression and downregulation studies.

Participants/materials, setting, methods: The project was approved by the Local and the Federal Ethical Committees for research on human embryos *in vitro*. Human embryos were obtained from patients at our IVF Centre after written informed consent. HESC lines were derived at our VUB research group. Samples were analyzed by qRT-PCR and immunocytochemistry.

Main results and the role of chance: CCNE1 was ubiquitously expressed in human embryos from embryonic genome activation onwards. It was downregulated in some of the ICM cells and all the TE cells during blastocyst expansion. On day 6, CCNE1-positive cells were found exclusively in a cluster of ICM cells negative for pluripotency (epiblast) and differentiation markers (TE and hypoblast). CCNE1 co-localized with the VE marker transthyretin (TTR) in embryos, including the early cleavage stages, and in spontaneously differentiating hESC. *CCNE1* overexpression induced a VE–like phenotype in hESC. TE cells from outgrowths of plated expanded blastocysts regained CCNE1 expression

and consequently NANOG expression and eventually were converted into a pluripotent state. Upon *CCNE1* siRNA addition, blastocyst outgrowths degenerated, proving that CCNE1 is critical for the growth of undifferentiated cells.
Limitations, reason for caution: These results are based on a limited number of good-quality human embryos donated for research.
Wider implications of the findings: Our study sheds light on the processes underlying the high developmental potential of early human embryonic cells. This unique characteristic allows embryos to recover after fragmentation, cryodamage or (single cell) biopsy on day 3 for preimplantation genetic diagnosis. Knowledge on the expression and function of genes responsible for this flexibility will help us to better understand the undifferentiated state in stem cell biology and may allow us to improve techniques in assisted reproduction.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Our research is supported by grants from the Fund for Scientific Research – Flanders (FWO-Vlaanderen), the Methusalem (METH) of the VUB and Scientific Research Fond Willy Gepts of UZ Brussel.
Trial registration number: None.
Keywords: human preimplantation embryo, totipotency, cell cycle

O-214 Comparative analysis of different culture conditions inducing naïve pluripotency in human embryonic stem cells

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Study question: What are the differences and similarities between naïve human embryonic stem cells (hESCs) produced via different conversion protocols, specifically in their signaling mechanisms and lineage-specific differentiation potential?
Summary answer: Converted naïve hESCs exhibit unbiased lineage-specific differentiation. Unlike other naïve media, our Naïve Conversion Medium (NCM) generated naïve hESCs with significantly increased *KLF2* and *PRDM14* expression, known to induce robust naïve pluripotency in mouse ESCs. Furthermore, NCM revealed the role of PI3K/AKT pathway in inducing naïve pluripotency in hESCs.
What is known already: Until recently, hESCs were shown to exist in a state of primed pluripotency. Mouse stem cells display a naïve or primed state of pluripotency, depending on the embryo stage from which they are harvested and the used culture conditions. Naïve mESCs can undergo single cell bulk culture and are more homogenous leading to unbiased and efficient directed differentiation. Several culture conditions have been reported inducing conversion of primed hESC towards a more naïve state.
Study design, size, duration: UG11 (XY, primed state) hESC line was subjected to naïve conversion on mouse embryonic fibroblasts in low oxygen conditions in (i) Naïve Human Stem Cell Medium, NHSM, (ii) Reverse toggling protocol, RT and iii) our novel medium NCM containing PD0325901, CHIR99021, Forskolin, Ascorbic acid and basic fibroblast growth factor.
Participants/materials, setting, methods: The following outcome parameters were compared in the converted line induced by the 3 different culture conditions: doubling time, single cell clonogenicity, karyotype, spontaneous differentiation, pluripotency and directed differentiation towards ectoderm. Converted naïve hESCs were also assessed for signaling mechanisms via qRT-PCR.
Main results and the role of chance: Domed hESC colonies emerged within 5–7 days in NCM and NHSM conditions, whereas this occurred after 2 weeks using RT. NCM cells manifested a faster doubling time (16 h) compared to NHSM (19 h), RT (24 h) and the primed counterpart (48 h). Single cell clonogenicity was similar between NCM and NHSM (95%) but reduced in RT (77%). Converted hESCs were positive for *OCT4/NANOG* and spontaneously differentiated into all germ layers. NCM-hESCs showed significant upregulation of *KLF2* compared to other conditions whereas in NHSM-hESCs, *KLF4* was significantly upregulated. Differentiation towards ectoderm demonstrated upregulation of ectoderm markers in NCM and NHSM-hESCs whereas primed and RT-hESCs co-expressed mesodermal and endodermal markers. The role of PI3K/AKT pathway in naïve pluripotency was shown by upregulation of *PRDM14* and *TCL1B* ($p < 0.001$) in NCM-hESCs.
Limitations, reason for caution: To further validate these results, the number of hESC lines need to be increased including both male and female cell lines. Also, directed differentiation potential towards mesoderm and endoderm needs to be assessed.
Wider implications of the findings: Our results reveal subtle differences in signaling mechanisms controlling pluripotency, and more importantly, reveal a functional variety in naïve hESCs produced in different culture conditions. The unique properties of naïve hESCs facilitating unbiased lineage-specific differentiation and their ability to undergo single cell bulk culture makes them highly clinically relevant.
Study funding/competing interest(s): Funding by University(ies) – This research is supported by the Concerted Research Actions funding from Bijzonder Onderzoeksfonds University Ghent (BOF GOA 01G01112).
Trial registration number: NA.
Keywords: hESC, primed pluripotency, naïve pluripotency, signaling pathway

O-215 Effect of cryopreservation on mitochondrial DNA of human embryonic stem cells

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Study question: To establish if routinely applied protocol of cryopreservation causes any changes in mitochondrial DNA (mtDNA) sequence.
Summary answer: This preliminary data suggests that cryopreservation can cause an increase in mtDNA mutation/variants in human embryonic stem cells.
What is known already: Cryopreservation is a routine technique used in assisted reproduction for storage of embryos, sperm and oocytes. The negative impact of freezing has been extensively studied on the cellular level; however, much less attention has been paid to the potential effect of freezing on the integrity of genome. No studies have ever been done to assess direct effect of freezing on the DNA of human embryos or stem cells.
Study design, size, duration: MtDNA was sequenced in 4 lines of embryonic stem cells derived from embryos with no known genetic abnormalities (KCL), carrying Huntington disease (HD), Von Hippel-Lindau (VHL) or Myotonic dystrophy (MD) before cryopreservation, immediately and 72 h after cryopreservation (number = 135). Four different regions of mtDNA (CYT-b, ATPase6, ND-4 and loop) were analysed.
Participants/materials, setting, methods: Embryonic stem cells were derived from the blastocysts. Cells were vitrified using ethylene glycol and dimethylsulfoxide containing cryoprotectant media and straws. After multiple displacement amplification of mtDNA, the loci of interests were amplified and sequenced before and after cryopreservation. Multi-factor ANOVA was used to examine the differences in mutation frequency.
Main results and the role of chance: The analysis of mtDNA before and immediately after vitrification showed that the rate of mutation increased significantly to 0.03 per 100 bps ($p = 0.038$). The significant change in mutation rate persisted at the rate of 0.05 after cryopreserved-thawed cells were incubated for further 72 h. There was also a significant difference in the mutation rate between different cell lines ($p < 0.001$, ANOVA). For example, VHL-4 line had more prominent increase in mutation rate to 0.1, whereas HD and KCL lines had 0.013 and 0.039 respectively. The rate of mutations was significantly ($p < 0.034$) higher in Loop region (0.12) in comparison with CYT-B (0.18) or ND-4 (0.12). The majority of detected mutations was mostly substitutions ($n = 16$). There were also single base-pair deletions ($n = 9$) and insertions ($n = 2$).

Limitations, reason for caution: The above findings might be related to the individual cell lines or protocols used in this experience and cannot be generalised to a wider population. However, this study warns the need for further investigations if different protocols of cryopreservation may affect mtDNA in embryonic stem cells and human embryos.
Wider implications of the findings: Our results demonstrate that vitrification can cause an increase in mtDNA variations in human embryonic stem cells. However, this effect is not equally seen between different cell lines. It is necessary to elucidate further if these changes have any immediate or delayed effect on cell function. It is also important to establish, if similar effect is present in human embryos during cryopreservation.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Guys and St Thomas Hospital, King's College London.
Trial registration number: NA.
Keywords: vitrification, mitochondrial DNA, stem cells

O-216 The copy number of mitochondrial DNA detected by next generation sequencing (NGS) can effectively predict the genetic stability of human blastocysts

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Study question: Is the copy number of mitochondrial DNA (mtDNA) correlated to the genetic stability and developmental potentiality of blastocysts?
Summary answer: Significant difference of the copy number of mtDNA can be found in blastocysts with both euploid and aneuploid genome. Blastocysts from young women contained significantly lower copy number of mtDNA compared to aged women. Meanwhile, developing faster and good quality blastocysts contained lower copy number of mtDNA.
What is known already: Mitochondria are important organelles providing energy for ovular activation and embryo development. Change of the copy number of mitochondria may lead to inadequate mitochondrial activities and result in abnormal development or even pregnancy failure. Whole genome amplification of single-cell and next generation sequencing (NGS) can be used to analyze embryo genome and mtDNA.
Study design, size, duration: A retrospective study was performed involving 440 couples with indications to *in vitro* fertilization treatment, 1528 blastocysts were biopsied and frozen embryo transplant was carried out using embryos with balanced genome. Embryos were subjected to preimplantation genetic diagnosis/screening using next generation sequencing (NGS-PGD/PGS) between October 2011 and September 2014.
Participants/materials, setting, methods: The study was performed at the Reproductive and Genetic Hospital of CITIC-Xiangya, and BGI-Health, China. The ratio between the mean sequencing depth of the mtDNA and the genome of each blastocyst was calculated, which represented the relative copy number of mtDNA. Mann-Whitney-Wilcoxon (MWW) was used for statistical analysis.
Main results and the role of chance: The sequencing data covered 5.5 ± 1.2% of the whole human genome and 98.7 ± 3.1% of mtDNA. The copy number of mtDNA in euploid blastocysts was significantly lower than that of the chromosomally abnormal blastocysts (291.46 vs. 317.39, $P < 0.001$). Significantly reduced copy number of mtDNA was also found in blastocysts from young women (age ≤35 years) comparing to blastocysts from the aged women (300.07 vs. 322.07, $P = 0.002$). Similarly, The copy number of mtDNA also correlated to the development rate of the embryo and the blastocyst quality, blastocysts with higher ranking contained considerably fewer copies of mtDNA than blastocysts with lower ranking and poor development ($P < 0.001$).
Limitations, reason for caution: Complete pregnancy outcomes could not be obtained as some blastocysts were not transferred yet. Future data collection is warranted.
Wider implications of the findings: Preimplantation genetic diagnosis/screening using NGS-based whole genome sequencing is able to achieve high coverage of mtDNA. It is possible to evaluate the potential of embryonic development by embryonic mitochondrial analysis. MtDNA copy number variation can be used as a potential marker to predict pregnancy rate and live birth rate.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by a grant from the Major State Basic Research Development Program of China (No. 2012CB944901) and National Science Foundation of China (No. 81222007). The authors have no competing interests to declare.
Trial registration number: NA.
Keywords: mitochondrial DNA, next generation sequencing, human blastocysts, preimplantation genetic diagnosis, genetic stability

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SELECTED ORAL COMMUNICATIONS

SESSION 59: CHALLENGES IN OVARIAN STIMULATION

Wednesday 17 June 2015 **10:00–11:45**

O-217 Pregnancy rates and risk of ovarian hyperstimulation syndrome (OHSS) in a fixed GnRH-antagonist versus GnRH-agonist protocol: randomized controlled trial including 1099 first IVF/ICSI cycles

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Study question: Are ongoing pregnancy rate (OPR) and risk of OHSS similar in a GnRH-antagonist vs. agonist protocol in first cycle patients <40 years of age including both low and high responders? What is the risk of hospital admission and ascites puncture due to OHSS in each treatment group?
Summary answer: Similar OPR (23.8 vs. 22.8%) ($P = 0.240$) and risk of OHSS (6.0 vs. 5.7%) ($P = 0.282$) were observed in the GnRH-antagonist vs. agonist group with a mean number of 1.1 embryos transferred. However 1.6 vs. 3.3% ($P = 0.055$) were admitted to hospital and none vs. 1.8% had ascites puncture in the GnRH-antagonist vs. agonist group.
What is known already: Previous studies on GnRH-antagonist vs. agonist protocol show similar pregnancy rates, but most studies are smaller and heterogeneous including selected groups. Most studies show reduced risk of OHSS with GnRH-antagonist protocol however the OHSS criteria differ. A meta-analysis reported no significant difference in OPR when GnRH-antagonist and GnRH-agonist was compared (OR = 0.88, = 0.05). And found a significant difference in OHSS favouring GnRH-antagonist (RD = -0.03, $p < 0.00001$). The largest RCT is from 2000 and included 730 infertile randomized 2:1.
Study design, size, duration: A prospective RCT including 1099 subjects randomized to GnRH-antagonist vs. agonist in a ratio 1:1 and enrolled over a period of 5 years. A non-inferiority study designed to detect a 2.5% difference in moderate/severe OHSS between the two groups, stratified for age, IVF-centre and IVF/ICSI. Women were <40 years.
Participants/materials, setting, methods: All first IVF/ICSI cycles ($n = 1099$) including women with irregular cycles were given fixed rFSH dose of 150 IU or 225 IU according to age <=36 years or >36 years, with dose-adjustment at stimulation day-6. OHSS parameters were obtained day 3- and 14 post-transfer. Ongoing pregnancy was determined by transvaginal ultrasound in gestational week 8–9.
Main results and the role of chance: OPR was 23.8 vs. 22.8% in the GnRH-antagonist vs. agonist group ($P = 0.240$) with a mean number of 1.1 embryos transferred. Mean age (32.1 vs. 32.0 years) and mean BMI (23.1 vs. 22.7) were similar in the groups. Furthermore both groups included low-responders and 12.7% women with irregular cycles. The overall incidence of mild OHSS was 29.6 vs. 32.2%, moderate OHSS 3.8 vs. 3.1% and severe OHSS 2.2 vs. 2.6% in the GnRH-antagonist vs. agonist group with no significant differences. In the GnRH-antagonist vs. agonist groups 4.2 vs. 7.5% were seen by a physician due to OHSS ($P = 0.009$) and 1.6 vs. 3.3% were admitted to hospital due to OHSS ($P = 0.055$). None had ascites-puncture in the GnRH-antagonist group vs. 1.8% ($n = 10$) in the GnRH-agonist group.
Limitations, reason for caution: Groups were equal with regard to age, BMI and women with irregular cycles, however a small difference in duration of infertility was observed. Ultrasonic measurements were performed by different

physicians and inter-observer bias may be present. Finally, the physicians were not blinded to GnRH-treatment group.
Wider implications of the findings: Both GnRH-antagonist and agonist protocol can be applied to first cycle patients <40 years including low and high responders as long as the fixed dose of rFSH does not exceed 150–225 IU. However caution should be taken as 1.8% in the GnRH-agonist group had ascites puncture. Future treatment should not be restricted to either of the two protocols, but can be individualised according to patient ovarian reserve and patient perceptions towards the two treatment approaches.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Research grants are funded by Merck Sharp & Dohme (MSD). The funders had no influence on the data collection, analyses or conclusions of the study. No conflict of interests to declare.
Trial registration number: EudraCT #: 2008-005452-24. ClinicalTrial.gov: NCT00756028.
Keywords: GnRH antagonist, GnRH agonist, IVF, ovarian hyperstimulation syndrome, pregnancy rates

O-218 Transdermal testosterone pre-treatment in poor responders undergoing ICSI: a randomized clinical trial

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[3]*University of Thessalia Medical School, Department of Obstetrics and Gynecology, Larissa, Greece*

Study question: Does pre-treatment with transdermal testosterone increase the number of cumulus-oocyte complexes retrieved (COCs) in poor responders, undergoing intracytoplasmic sperm injection (ICSI) using recombinant follicle stimulating hormone (FSH) and gonadotropin releasing hormone agonists (GnRHa)?
Summary answer: Transdermal testosterone pre-treatment (10 mg for 21 days) does not seem to increase the number of COCs retrieved in poor responders undergoing ICSI.
What is known already: Androgens have been suggested to play an important role in early follicular development by enhancing ovarian sensitivity to FSH. In a recent meta-analysis, testosterone pretreatment has been shown to result in an increase of 1.5 COCs as compared to no pretreatment. However this effect was based on the analysis of two randomized controlled trials (RCTs) including 163 patients. Consequently, there is a need for more RCTs to confirm or deny these results.
Study design, size, duration: The present RCT has been designed to detect a difference of 1.5 COCs (sample size required = 42 patients). From 02/2014 until 12/2014, 39 poor responders fulfilling the Bologna criteria have been randomized (using a randomization list) to either testosterone pre-treatment for 21 days (n = 22) or no pre-treatment (n = 17).
Participants/materials, setting, methods: All patients underwent a long follicular GnRHa protocol. Recombinant FSH stimulation was started on day 22 following GnRHa initiation. In the testosterone pre-treatment group, a daily dose of 10 mg of testosterone gel was applied transdermally for 21 days starting from GnRHa initiation. Results are expressed as median (interquartile range).
Main results and the role of chance: No differences in baseline characteristics were observed between the two groups compared. Testosterone levels were significantly higher in the testosterone pre-treatment on the day of initiation of FSH stimulation [114 (181) ng/dL vs. 20 (26) ng/dL, respectively, p = 0.001]. Duration of FSH stimulation was comparable between the groups compared [13 (3) days vs. 12 (3.5) days, respectively, p = 0.39]. The number of COCs retrieved was not different between the testosterone pre-treatment and the no pre-treatment groups [3 (5) vs. 3 (2), 95% CI for the median: 1.26–4 vs. 3–4, respectively]. Similarly no differences were observed regarding fertilization rates [66.7% (27.8) vs. 66.7% (41.2), respectively, p = 0.71] and ongoing pregnancy rates per randomized patient (9.1 vs. 11.8%, respectively, rate difference: -2.7%, 95% CI: -23.0 to +17.7).
Limitations, reason for caution: An improvement in IVF outcome using a larger dose of testosterone or a longer pre-treatment period cannot be excluded. Moreover, due to sample size restrictions, no conclusions can be drawn regarding the probability of pregnancy.
Wider implications of the findings: The results of this randomized clinical trial, suggesting that pre-treatment with 10 mg of transdermal testosterone for 21 days cannot improve ovarian response by 1.5 oocytes or more, could be used to more accurately consult patients with poor ovarian response.
Study funding/competing interest(s): Funding by national/international organization(s) – This study has been partially funded by a Scholarship awarded to the principal investigator from the Academy of Athens.
Trial registration number: NCT01961336.
Keywords: transdermal testosterone pre-treatment, poor ovarian response, androgens

O-219 The optimal dose of triptorelin for triggering final oocyte maturation in patients at high-risk for OHSS: a randomized clinical trial

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Study question: Does the dose of triptorelin, used for triggering final oocyte maturation, affect the maturity of the oocytes retrieved in patients at high risk for ovarian hyperstimulation syndrome (OHSS) undergoing ovarian stimulation for IVF using GnRH antagonists and recombinant follicle stimulating hormone (recFSH)?
Summary answer: The dose of triptorelin for triggering final oocyte maturation does not seem to affect the maturity of the oocytes retrieved in high risk for OHSS patients undergoing ovarian stimulation for IVF.
What is known already: Triptorelin is widely used, as a substitute to human chorionic gonadotropin, to eliminate the risk of OHSS in patients undergoing ovarian stimulation for IVF. However, no data is currently available regarding its optimal dose used to trigger final oocyte maturation.
Study design, size, duration: Currently 50 patients at high-risk for OHSS have completed the study. Patients were randomized to receive 0.1 mg [0.1 mg group (n = 17)], 0.2 [0.2 mg group (n = 18)] mg or 0.4 mg [0.4 mg group (n = 15)] of triptorelin to trigger final oocyte maturation.
Participants/materials, setting, methods: Ovarian stimulation was performed with recFSH and GnRH antagonists. Hormonal evaluation included assessment of FSH, LH, estradiol (E2) and progesterone (P) on the day of triggering, 8, 36 h as well as on days 3, 4, 7, 10 following triptorelin administration. 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 0.1 mg group, the 0.2 mg group and the 0.4 mg group. The number of COCs retrieved was not different among groups compared [25 (18) vs. 26 (19) vs. 27 (20) COCs, respectively, p = 0.86]. Regarding the primary outcome measure, the percentage of mature oocytes retrieved, no differences were observed among the groups compared [81.8 (18.7)% vs. 80.4 (25.2)% vs. 83.8 (12.6)%, respectively, p = 0.91]. Duration of luteal phase was also not significantly different among groups [7 (2) vs. 7 (3) vs. 7 (2) days, respectively, p = 0.47]. No significant differences were present in serum levels of LH, FSH, E2, P following triggering of final oocyte maturation among the groups compared.
Limitations, reason for caution: The results obtained refer to the comparison of various doses of the commonly used GnRH agonist triptorelin, according to the published literature. Whether these results are also valid for different types of GnRH agonist used for triggering final oocyte maturation cannot be deducted from this study.
Wider implications of the findings: Based on the results of the current study it appears that high doses (0.2 mg, 0.4 mg) of the GnRH agonist triptorelin as compared to a low (0.1 mg) dose, used to trigger final oocyte maturation, do not confer any apparent benefit.
Study funding/competing interest(s): Funding by University(ies) – Aristotle University of Thessaloniki, Greece.
Trial registration number: NCT01973842.

Keywords: agonist trigger, triptorelin dose, high risk for OHSS, oocyte maturity, OHSS prevention

O-220 A randomized clinical trial comparing two cycles of minimal stimulation IVF with single embryo transfer to one cycle of conventional IVF with double embryo transfer

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Study question: Are there differences in clinical outcomes between 2 cycles of minimal stimulation *in vitro* fertilization (IVF) with single embryo transfer versus one cycle of conventional IVF with double embryo transfer?
Summary answer: Although minimal stimulation IVF resulted in relatively lower live birth rates, it prevented ovarian hyperstimulation syndrome (OHSS), lowered multiple pregnancy rates, and decreased gonadotropin use.
What is known already: Minimal stimulation IVF with single embryo transfer is re-emerging in many centers worldwide as an alternative for conventional IVF.
Study design, size, duration: A randomized controlled trial was completed among 564 infertile women between 2009 and 2013 at a single fertility center.
Participants/materials, setting, methods: Women under the age of 38 who underwent their first IVF cycle were randomly allocated to either one cycle of conventional IVF (Group 1) or to 2 cycles of minimal stimulation IVF (Group 2). Women in Group 1 received high doses of gonadotropins following long GnRH agonist suppression protocol then fresh transfer of 2 blastocysts. Women in Group 2 received extended clomiphene citrate regimen and low-dose gonadotropins followed by freeze-all embryo strategy. Then, a single thawed blastocyst transfer was performed in 2 consecutive cycles.
Main results and the role of chance: 279 women were included in Group 1 and 285 women in Group 2. The number of oocytes retrieved (4.3 ± 3.2 vs. 12.8 ± 8.0; $p < 0.0001$), MII oocytes (3.1 ± 2.4 vs. 8.3 ± 5.8; $p < 0.0001$) and blastocysts formed (2.6 ± 1.9 vs. 5.9 ± 4.3; $p < 0.0001$) were significantly lower in Group 2 compared to Group 1. Group 1 had implantation rate of 56%, cumulative ongoing pregnancy rate of 74%, and live birth rate of 62%. Group 2, first cycle, had implantation rate of 47%, cumulative ongoing pregnancy rate of 47%, and live birth rate of 39%. Group 2, second cycle, had implantation rate of 48%, cumulative ongoing pregnancy rate of 48%, and live birth rate of 41% (RR = 0.73; CI: 0.62–0.86; $p = 0.0001$ compared to Group 1). There were 16 cases of moderate to severe OHSS cases in Group 1 but no cases of OHSS in Group 2. There were 34% multiple pregnancy rates in Group 1 compared to 9.3% in Group 2 ($p < 0.05$). Gonadotropin consumption per cycle was significantly higher in Group 1 compared to Group 2 (2079 ± 389 vs. 459 ± 131 IU, $p < 0.0001$).
Limitations, reason for caution: This trial compared two completely different protocols, thus it is unable to disentangle the effects of various components of minimal versus conventional IVF on the outcomes of the study.
Wider implications of the findings: This study was performed at a single center, thus hampering generalisability to other fertility centers.
Study funding/competing interest(s): Funding by hospital/clinic(s) – New Hope Fertility Center.
Trial registration number: The trial was registered before its start at clinicaltrials.gov: NCT00799929.
Keywords: IVF, oocyte, minimal stimulation, OHSS, multiple pregnancy

O-221 Double blind cross-over randomized controlled trial comparing letrozole versus clomiphene citrate for ovulation induction in women with polycystic ovarian syndrome

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Study question: Would letrozole as a primary ovulation induction agent generate better pregnancy rates than clomiphene citrate (CC) in subfertile women with anovulatory polycystic ovarian syndrome?
Summary answer: Based on blind analysis, ovulation induction with treatment B produced a significantly ($p = 0.026$) higher pregnancy rate (61%) compared to that (43%) produced by treatment A in anovulatory women with PCOS.
What is known already: Theoretically, letrozole is superior to CC due its short half-life and absence of peripheral anti-oestrogenic effects. According to the most recent Cochrane systematic review (2014), Letrozole appears to improve live birth and pregnancy rates in anovulatory women with PCOS, compared to clomiphene citrate. However, the authors of this review stated that the quality of evidence in this review was rated low due to poor reporting of study methods and possible publication bias.
Study design, size, duration: This double blind RCT included 159 participants between April 2007 and June 2014. Subjects were randomly allocated to either Treatment A ($n = 79$) or B ($n = 80$) in a 1:1 ratio. Both drugs were encapsulated to look identical. Randomization was performed in mixed blocks and stratified by patients' BMI (<30 and 30–35 kg/m^2).
Participants/materials, setting, methods: Treatment started with one tablet (CC 50 mg, letrozole 2.5 mg) increasing to two in non-responders and continuing until pregnancy or for up to 7 cycles. Non-responders were crossed over to the other treatment after a 6-week break. Cycles were initially monitored with ultrasound follicle tracking then mid-luteal serum progesterone measurement.
Main results and the role of chance: Amongst the 159 participants included in the intention-to-treat analysis, four women conceived before treatment and four were lost-to-follow-up. The remaining 151 participants (74 on A and 77 on B) completed at least first treatment. Women receiving treatment B achieved a significantly ($p = 0.026$) higher pregnancy rate (61%) than those on treatment A (43%). Livebirth (LB) rates were not statistically different between the two groups, although there was a trend towards higher rates on treatment B (49 vs. 35%). Amongst the 76 women crossing over, pregnancy and LB rates on B (29 and 24%) were not statistically ($P = 0.604$ and $P = 0.781$) different from A (23 and 19%).
Limitations, reason for caution: This study was powered at 80% as a parallel head-to-head RCT to detect 20% difference in pregnancy rates (before the cross-over) at 5% significance level. An interim power calculation confirmed that at least 75 patients per arm were needed to achieve 80% power.
Wider implications of the findings: Although, the treatment code has not been broken at the time of writing this abstract, all the details will be presented in the meeting. Treatment B appears significantly more effective than A and should be considered a first choice in anovulatory PCOS women. We believe that these results are generalisable and will have an impact on clinical practice worldwide as our PCOS cohort has been selected according to the widely accepted Rotterdam criteria.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Derby Hospitals NHS Foundation Trust R&D funding Scheme. University of Nottingham Medical School at Derby. No conflict of interest to declare.
Trial registration number: www.Clinicaltrials.gov – NCT00478504.
PCOS, ovulation induction, letrozole, clomiphene citrate

O-222 A novel model of luteal phase ovarian stimulation in follicular phase is an alternative in women with polycystic ovarian syndrome: a pilot study

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Study question: To clarify the effect of ovarian stimulation using hMG and medroxyprogesterone acetate (MPA) in terms of ovarian response and the subsequently pregnancy outcome in frozen-thawed embryo transfer (FET) cycles in women with polycystic ovarian syndrome (PCOS).
Summary answer: The study shows that hMG and MPA protocol lead to similar efficacy compared to short protocol in inducing oocyte maturation without adversely affecting the pregnancy outcome meanwhile eliminating the risk of OHSS.

What is known already: Our previous studies showed that no cases experienced a premature LH surge or moderate/severe ovarian hyperstimulation syndrome in PCOS patients during the luteal-phase ovarian stimulation cycles. Furthermore, luteal-phase ovarian stimulation is feasible for producing competent oocytes/embryos in women undergoing IVF/ICSI treatments, with optimal pregnancy outcomes in FET cycles. But it is difficult to perform ovarian stimulation during the luteal phase of the menstrual cycle since some PCOS cases have irregular menstrual cycles or anovulation.
Study design, size, duration: In the prospective controlled study, 120 PCOS patients undergoing IVF/ICSI were recruited between January 2014 and October 2014 and classified into two groups according to the ovarian stimulation protocols: hMG and MPA (group A, $n = 60$) or short protocol (group B, $n = 60$).
Participants/materials, setting, methods: In the study group, hMG (225 IU) and MPA (10 mg/day) were administered simultaneously beginning on cycle day 3. Ovulation was co-triggered by a GnRH agonist (0.1 mg) and hCG (1000 IU) when dominant follicles matured. A short protocol was used as a control. Viable embryos were cryopreserved for later transfer in both protocols.
Main results and the role of chance: No evidence of statistically difference was found in the proportion of mature oocytes retrieved (86.72 vs. 89.2%) and fertilization rate (76.82 vs. 68.78%) between the two groups. Doses of hMG administrated and serum E_2 on day of hCG in group A are significantly higher than group B ($P < 0.05$). Clinical pregnancy rates per transfer (45.65 vs. 44.15%), ongoing pregnancy rates (41.98 vs. 41.56%), implantation rates (33.08 vs. 31%) and cumulative pregnancy rates per patient (56.67 vs. 53.33%) were comparable between the two groups. The incidence of OHSS was low between the two groups (0 vs. 1.67%), with no significant difference.
Limitations, reason for caution: Current evidence about the safety of MPA used in COH is scarce. MPA was used during the follicle phase and was not further exposed after oocyte retrieval in this trial. No direct evidence demonstrated the role of MPA on oocyte development potential in previous reports.
Wider implications of the findings: MPA has the advantages of an oral administration route, easy access, more control over LH levels and eliminating the risk of OHSS, which will help to establish a new regimen of ovarian stimulation in PCOS patients in combination with embryo cryopreservation.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by grants from Shanghai Natural Science Foundation of China (14ZR1423900 to YW), National Natural Science Foundation of China (NSFC) the 81470064 (to YW).
Trial registration number: ChiCTR-OCH-14004424.
Keywords: PCOS, medroxyprogesterone acetate, luteal phase ovarian stimulation

O-223 Endometrial scratching for women with previous IVF failure undergoing IVF treatment

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Study question: Does endometrial scratching in the luteal phase of the preceding cycle improve live birth rate after IVF treatment in women with, at least one, previous IVF failure?
Summary answer: Endometrial scratching does not improve live birth rate in women undergoing IVF treatment with previous one IVF failure. Nevertheless, it may mprove live birth in women with two or more previous IVF failures
What is known already: A Cochrane review examining the effect of this intervention had found some evidence that endometrial scratching may improve IVF outcome.
Study design, size, duration: A parallel two arms multicentre RCT. The study was conducted between 2010 and 2014. We estimated that our intervention may increase clinical pregnancy rate from 30% in control group to 45% in the intervention group, hence, a total of 350 women is needed for trial with 80% power and 5% significance level.
Participants/materials, setting, methods: 387 Participants with at least one previous failed IVF cycle. Three participating Units; one university unite and two private units. Randomization through a computer generated tables of random numbers. Opaque sealed envelope to conceal allocation. Women were blinded to their allocation while physicians were not. Women in the intervention group were subjected to endometrial scratching twice in the luteal phase of the preceding IVF cycle while women in the control group were subjected to a placebo procedure (introduction of a sound intracervically).
Main results and the role of chance: Our results showed no difference in live birth rate between the two groups of women (47.2 versus 38.1%, $p = 0.08$). However, regression analysis revealed that endometrial scratching was an independent predictor of live birth in the subgroup of women with two or more previous failure after control of other independent predictors ((OR) 3.4, $p = 0.005$).
Limitations, reason for caution: We acknowledge that this study has two important potential sources of bias. The first is the use of intra-cervical manipulation in the control group and the second is the fact that 12/194 (6.2%) women in the control group had a hysteroscopy. Although regression analysis showed a potential benefit of this intervention in women with two or more previous failures, the number of women is underpowered to give firm evidence.
Wider implications of the findings: Endometrial scratching should not be performed in women without at least two previous IVF failures. Larger trial with adequate power is warranted to scrutinize the effect of this procedure and to clarify the real population that may benefit from this intervention.
Study funding/competing interest(s): Funding by University(ies) – Mansoura University, Egypt.
Trial registration number: ClinicalTrials.gov under identifier: NCT01245309.
Keywords: IVF, endometrial scratch

SELECTED ORAL COMMUNICATIONS

SESSION 60: FEMALE FERTILITY PRESERVATION

Wednesday 17 June 2015 **10:00–11:45**

O-224 Orthotopic ovarian tissue transplantation – results in relation to experience of the transplanting centers, overnight tissue transportation and transplantation into the peritoneum

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Study question: Have the experience of transplanting centers and the overnight transportation of ovarian tissue before cryopreservation an impact on the transplantation success? What is the success rate of transplantation into the pelvic peritoneum?
Summary answer: The success rate was higher in experienced transplantation centers and the overnight transportation of ovarian tissue did not have a negative impact on the transplantation result. Transplantation into the pelvic peritoneum resulted in high success rates.

What is known already: Cryopreservation of ovarian tissue before cytotoxic therapies and transplantation is increasingly performed. Centralized tissue banks and transplantations in specialized centers have been suggested to optimize these procedures. Ovarian tissue should be transplanted orthotopically. However, the impact of centers experience in tissue transplantation, the overnight transportation of tissue to centralized tissue banks as well as the transplantation success in the pelvic peritoneum has not been analysed.
Study design, size, duration: Retrospective analysis of 72 transplantations performed in the network Ferti*PROTEKT* (www.fertiprotekt.com) from 2007 till 2014. Transplantations performed within <12 month of data analysis, transplantations non-orthotopically, repeated transplantations and loss of follow up before 12 month after transplantation resulted in exclusion of cases, resulting in 37 analysed transplantations (one/woman) in 8 centers.
Participants/materials, setting, methods: Exclusions were due to: transplantation <12 month of data analysis (n = 17), transplantation non-orthotopically (n = 1), repeated transplantations (n = 14) and loss of follow up (n = 3). Success rate was defined as tissue activity (menstrual cycles) after 1 year and as achieved pregnancies and deliveries. Experienced centers were those who had performed ≥3 transplantations.
Main results and the role of chance: The first analysis compared the success rates in relation to the experience of the centers. Tissue activity in centers with low (n = 10) vs. high (n = 27) experience was 30 vs. 89% (p < 0.001). Pregnancy and delivery rates were 10 vs. 67% and 10 vs. 22%. Due to these differences, the following analysis included only transplantations in experienced centers. Tissue activity following no overnight transportation (n = 13) vs. overnight transportation (n = 14) was 77 vs. 100% (p = 0.05), pregnancy and delivery rates were 23 vs. 50% and 15 vs. 29%. Transplantation into the peritoneum (n = 22) resulted in tissue activity in 91% of cases, pregnancies in 36% and deliveries in 23% of cases. 86% (n = 5) of the 6 women following transplantation into the pelvic peritoneum conceived spontaneously.
Limitations, reason for caution: The data are drawn from a retrospective analysis and statistical analysis is based on low case numbers. Since 2008 the cryopreservation and transplantation techniques have been modified over the years.
Wider implications of the findings: The data suggest that centralizing ovarian tissue storage, requiring overnight transportation, is possible. Centralization of tissue allows transplantations in few experienced centers. Furthermore, centralization of these activity will improve the follow up of patients. As transplantation into the peritoneum result in high success rate, this approach might be an alternative to transplantation into the ovary. However, a randomized study is required to define the best transplantation site.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – No competing interests.
Trial registration number: N/A.
Keywords: fertility preservation, ovarian tissue, transplantation, ovary, peritoneum

O-225 Experience with 26 cryopreserved ovarian tissue grafts in a single centre: are there factors for predicting success?

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Study question: Cryopreservation of ovarian tissue followed by transplantation has a unique role for fertility preservation however experience in humans is limited and there are uncertainties regarding its clinical efficacy, optimal grafting site, tissue volumes and factors which may predict success.
Summary answer: Clinical pregnancy occurred in 2/11 (18%) of patients who underwent stimulated cycles. Mature oocytes were largely harvested from non-ovarian graft sites and longer duration of ovarian activity was correlated with increased tissue volume and follicular density. All pregnancies occurred in women under 25 years at the time of tissue cryopreservation.
What is known already: While efficiency of this technique may have been overestimated in the literature, the main cause of follicular loss after grafting appears to be initial ischaemic injury during angiogenesis. Frozen-thawed ovarian tissue can be transplanted to the ovary, another pelvic site and outside the pelvis. While there are significant advantages of using heterotopic sites (i.e., not in or adjacent to the ovary), the clinical value of this approach has been questioned due to inferior pregnancy rates.
Study design, size, duration: We conducted a retrospective analysis of reproductive outcomes following ovarian tissue transplantations at our centre to date (2006–2014). This included 26 transplant procedures in 19 patients, following a diagnosis of premature ovarian failure.
Participants/materials, setting, methods: Ovarian tissue was harvested from 19 patients with malignant (14/19.74%) and benign (5/19.26%) diseases, and cryopreserved. Three of 19 patients had prior cytotoxic treatment. Following a diagnosis of premature ovarian failure ovarian tissue was grafted to pelvic and extra-pelvic sites, on average 5 years following extraction.
Main results and the role of chance: Ovarian activity resumed in 17/19 patients (19/26 grafts) after an average of 5.3 months. Seven patients underwent a second transplantation procedure 1–2 years after the first. Stimulated cycles in 11 patients with low dose rFSH, yielded 52 eggs (largely from non-ovarian grafts). MII oocytes were retrieved from mean follicular size 13.5 mm (range 6–20). The overall 2PN fertilisation rate was 64%. A total of 22 fresh and frozen embryos were transferred in 11 women, resulting in 2 clinical pregnancies (18%). The delivery of healthy twin girls has been reported and there is currently an ongoing 16 week pregnancy. All pregnancies occurred in patients under 25 years at cryopreservation. Follicular density and tissue volume grafted appear to be the strongest predictors of ovarian activity duration over 12 months.
Limitations, reason for caution: Trends alone can be observed from this data as the numbers are small.
Wider implications of the findings: These results add to our knowledge of worldwide experience with this technique and demonstrate the need for ongoing research to improve efficiency, especially by reducing the follicular loss which occurs prior to the establishment of neovascularisation. There is encouragement for continuing investigation into non-ovarian graft sites and optimal volumes of tissue to graft. These results support offering this technique to young women who are undergoing fertility threatening treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Royal Women's Hospital and Melbourne IVF.
Trial registration number: NA.
Keywords: female fertility preservation, ovarian tissue cryopreservation, ovarian tissue grafting

O-226 mTOR inhibitor can protect the primordial follicle pool in bovine ovarian tissue treated with cyclophosphamide after xenotransplantation

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Study question: Can administration of rapamycin or resveratrol with spermidine protect ovarian follicles from gonadotoxicity of cyclophosphamide (Cp)?
Summary answer: Coadministration of rapamycin or resveratrol with spermidine protected ovarian follicle reserve from chemotoxicity of Cp in xenografted bovine ovarian tissue.
What is known already: Cp exposure was found to induce developing follicle atresia and aberrant primordial follicle activation via the stimulation of phosphatidylinositol 3-kinase (PI3K)/Akt and mammalian target of rapamycin (mTOR) signaling pathways. Rapamycin inhibits the kinase activity of mTOR, which has been shown to result in apoptosis or autophagy depending on cell type studied. Two plant-derived agents with antioxidant properties, resveratrol (a polyphenol) and spermidine (a polyamine), enhance autophagy by modulation of S6 kinase 1 activity.
Study design, size, duration: Prospective experimental study. Fresh bovine ovarian tissue was transplanted to 6-week-old SCID mice. A total of 18 mice were randomly divided into four groups according to treated agents; group 1 PBS (control), group 2 Cp (75 mg/kg/weekly), group 3 Cp plus rapamycin (7.5 mg/kg 3 times a week), group 4 Cp plus resveratrol and spermidine (50 mg/kg 3 times a week, respectively).
Participants/materials, setting, methods: Two weeks after transplantation, these agents were injected for 2 weeks. Seven days after the last injection, ovarian grafts were recovered and processed for ovarian histology and apoptosis were analyzed by TUNEL assay. Phosphorylation of mTOR and ribosomal protein S6 (rpS6) and protein expression of LC3B were determined by Western blotting.
Main results and the role of chance: The percentage of primordial follicles was significantly higher in the group 1 and 3 (34.4 ± 17.2, 35.7 ± 17.1,

respectively) than the group 2 and 4 (20.6 ± 11.0, 20.7 ± 18.4). The percentage of apoptotic cells were similar irrespective of whether rapamycin or resveratrol was coadministered. Phosphorylation of mTOR and rpS6 were inhibited by rapamycin or resveratrol. Increased LC3B expression was noticed in the group 3 and 4 compared to in the group 2.

Limitations, reason for caution: We used a single dose of rapamycin and resveratrol with a fixed exposure time. Possible protective effects of various doses of rapamycin and resveratrol against Cp should be evaluated.

Wider implications of the findings: Rapamycin or resveratrol with spermidine may be applicable for fertility preservation in young cancer patients treated with cyclophosphamide containing regimens.

Study funding/competing interest(s): Funding by national/international organization(s) – The Korea Health Care Technology R&D Project, Ministry of Health and Welfare, Republic of Korea.

Trial registration number: NA.

Keywords: rapamycin, resveratrol, spermidine, cyclophosphamide, fertility preservation

O-227 Molecular evidence against the preventive actions of GnRH agonists in chemotherapy induced damage in human ovary and granulosa cells

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Study question: Is there any molecular evidence for-or-against the protective effect of GnRH agonists in chemotherapy induced damage in human ovary?

Summary answer: Yes, this study provides the first evidence against the protective or attenuative effects of GnRH agonists in chemotherapy induced ovarian damage by using a novel experimental methodology utilizing real-time and quantitative transcriptional and cellular cytotoxicity indices, ovarian histomorphometry, and the markers of DNA damage, apoptosis and hormone production.

What is known already: There is an ongoing debate over the effects of GnRH agonists in the prevention of ovarian failure in women exposed to cytotoxic chemotherapy regimens since randomized controlled trials launched to answer this question yielded conflicting results. The question remained unanswered as to whether GnRH administration during chemotherapy is protective of ovarian function. We therefore aimed to investigate GnRH acting via its cognate receptors protects against cyclophosphamide induced DNA damage and cell death in human ovary.

Study design, size, duration: An in-vitro model of human ovary and granulosa cells.

Participants/materials, setting, methods: Ovarian cortical pieces (n = 10, age 14–37), nonproliferating human luteinized granulosa cells (HLGC) and proliferating nonluteinizing human granulosa cells (COV434) were cultured for 24 hrs with cyclophosphamide, paclitaxel, cisplatin, TAC regimen (docetaxel, adriamycin and cyclophosphamide) and ionizing radiation (RT, 2 Gy = LD_{50} of oocytes) with and without GnRH agonist Leuprolide acetate (12.5–25.50 ng/mL).

Main results and the role of chance: The expression of GnRH receptor in the samples were validated with qRT-PCR. E2, P and AMH productions and follicle counts in ovarian tissue samples exposed to chemotherapy and RT were significantly reduced compared to controls. The addition of GnRH did not cause any notable differences in hormone levels and follicle counts compared to chemotherapy and RT subgroups and controls (Fig-1). Similarly HLGC exposed to cytotoxic chemotherapy agents underwent apoptosis and their E2 and P productions were significantly reduced (Fig-2). COV434 cells treated with chemotherapy exhibited growth arrest and apoptosis in a dose dependent manner (Fig-3). GnRH did not prevent DNA damage or apoptosis in both cell types.

Limitations, reason for caution: These results do not rule out the possibility that GnRH may offer protection in-vivo by some mechanisms other than the interaction of GnRH with its cognate receptors in the ovary.

Wider implications of the findings: These results provide for the first time a solid molecular evidence against the protective effects of GnRH in cyclophosphamide induced damage in human ovary and granulosa cells. Therefore any patients who opt only to receive GnRH for gonadal protection should be warned about it.

Study funding/competing interest(s): Funding by University(ies) – Koc University School of Medicine and the Graduate School of Health Sciences, Istanbul Turkey.

Trial registration number: NA.

Keywords: GnRH, cyclophosphamide, ovary, apoptosis, AMH

O-228 Effect of ovarian reserve on undesirable estradiol elevation despite of letrozole co-treatment during controlled ovarian hyperstimulation for oocyte vitrification in breast cancer patients

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Study question: The aim of this study is to assess ovarian reserve effect on serum estradiol elevation above 500 pg/mL during controlled ovarian hyperstimulation (COH) with letrozole co-treatment in breast cancer patients undergoing oocyte preservation.

Summary answer: In letrozole-exposed controlled ovarian hyperstimulation cycles for oocyte vitrification, final serum estradiol correlates positively with basal antral follicular count (AFC), but it is not significantly associated and can not predict final estradiol elevation ≥500 pg/mL.

What is known already: Letrozole co-treatment is added to FSH activity under GnRH-antagonist protocol for COH in breast cancer patients who undergo fertility preservation, in order to avoid associated estradiol increase in positive estrogen-receptor tumours. Although security threshold in estradiol elevation in these cycles has not been established, estradiol production reaches conventional levels in some cases, in spite of letrozole administration. High ovarian reserve could be a risk factor for letrozole-resistant estradiol increase.

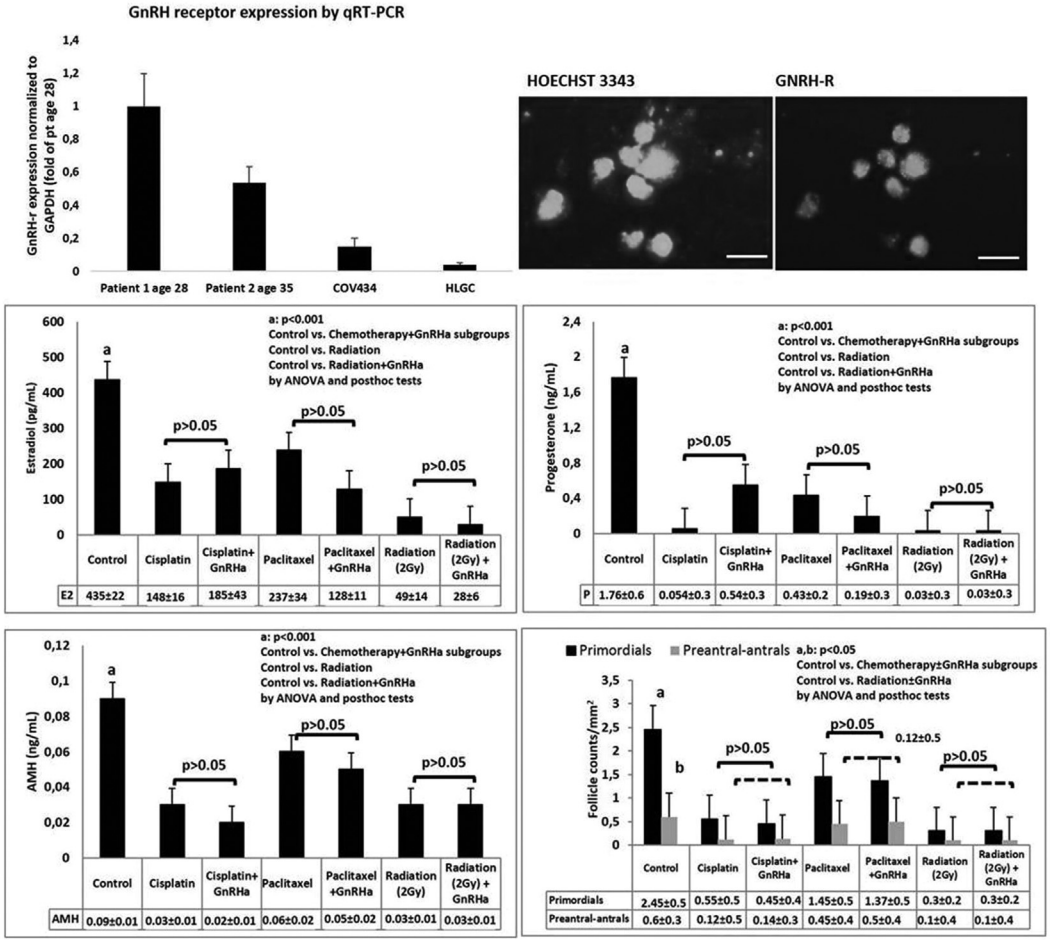
Study design, size, duration: Cohort-nested retrospective case-control study of an opportunistic sample of breast cancer patients treated between 2010 and 2014:16 cycles of COH for oocyte vitrification with final serum estradiol >500 pg/mL (cases) were compared with 71 cycles in which that level was not reached (controls) to estimate odds ratio for high AFC.

Participants/materials, setting, methods: During study period, 96 patients underwent COH with letrozole cotreatment for oocyte cryopreservation, and 88 achieved oocyte retrieval. We analyze the association between AFC and cycle characteristics with final estradiol increase over 500 pg/mL. Appropriate statistical tests have been applied; odds ratios have been estimated as effect size measure.

Main results and the role of chance: Final estradiol exceeded 500 pg/mL in 18.2% of letrozole-exposed COH cycles. Amongst pre-treatment variables, only AFC was significantly correlated with final serum estradiol (Rho: 0.20; p = 0.003). Nevertheless, AFC failed to predict estradiol elevation >500 pg/mL (AUC: 0.59; 95% CI: 0.45: 0.72; p = 0.72). An interval analysis of case frequency by AFC strata showed a considerable but not significant increase of cases from 15 to 19 follicles or more. However, this effect was not significant again (OR AFC > 15: 1.8; 95% CI: 0.6; 5.6; p = 0.73; OR AFC > 19: 2.24; 95% CI: 0.7; 7.15; p = 0.16).

Limitations, reason for caution: Applied cohort-nested case-control design could be appropriate for bias control. Assuming observed exposure differences between cases and controls for AFC >15 and >19, sample size determined low statistical powers (18 and 28% respectively).

Wider implications of the findings: Letrozole-resistant estradiol increase >500 pg/mL seems to be present in almost 1/5 breast cancer patients undergoing OCH for oocyte vitrification. Although estradiol levels at the end of ovarian stimulation correlates with ovarian reserve, our study has not



identified a critical level in AFC useful to predict a significant overexposure to estradiol.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital General Universitario Gregorio Marañón.

Trial registration number: Cohort-nested retrospective case-control study.

Keywords: oocyte preservation, letrozol, estradiol, security, AFC

O-229 What threshold values of antral follicle count and anti-Müllerian hormone levels should be considered in cancer patients, candidates for oocyte vitrification following *in vitro* maturation?

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Study question: What threshold values of ultrasonographic antral follicle count (AFC) and serum anti-Müllerian hormone (AMH) levels should be considered for ensuring the vitrification of different number of *in vitro* matured (IVM) oocytes, in cancer patients seeking fertility preservation (FP)?

Summary answer: The present investigation shows that AFC and serum AMH values above 21 follicles and 3.8 ng/mL, respectively, are required for obtaining at least 10 IVM oocytes vitrified. Given these relatively high values, the combination of IVM and ovarian tissue cryopreservation should systematically be discussed for optimizing the FP strategy.

What is known already: IVM of cumulo-oocyte-complexes (COCs) followed by oocyte vitrification has emerged recently as an option for urgent FP. Recent data have reported that, in healthy patients, 8–20 vitrified oocytes after ovarian stimulation would be required to obtain a live birth. Although both AFC and AMH have been reported as predictive factors of IVM success in polycystic ovary syndrome, there is a dramatic lack of data regarding the values of these parameters in oncologic patients.

Study design, size, duration: From July 2013 to December 2014, we prospectively studied 327 cancer patients, aged 18–40 years, candidates for oocyte vitrification following IVM.

Participants/materials, setting, methods: All patients had AFC and AMH measurements, 48–72 h before oocyte retrieval, whatever the phase of the cycle. COCs were recovered under ultrasound guidance 36 h after hCG priming. Logistic regression allowed determining threshold values of AFC and AMH, for obtaining at least 8, 10 or 15 vitrified IVM oocytes.

Main results and the role of chance: Among the 327 cancer patients included, 261 were diagnosed with breast cancers, 26 had a hematological malignancies and 43 underwent this procedure for others indications. Overall, the mean age of the population was 31.7 ± 0.26 years. Mean AFC and serum AMH levels were 21.2 ± 0.7 follicles and 4.7 ± 0.3 ng/mL respectively. IVM was performed in follicular phase for 46.5% of patients and during luteal phase for 36.4%. The statistical analysis shows that AFC and AMH values above 28 follicles and 3.9 ng/mL, 21 follicles and 3.8 ng/mL and 19 follicles and 3.3 ng/mL, respectively, are required for obtaining at least 15, 10 or 8 IVM vitrified oocytes.

Limitations, reason for caution: The potential of IVM oocytes vitrified in cancer patients is unknown. However, data obtained from infertile women showed a dramatically reduced competence of IVM oocytes when compared to oocytes recovered after ovarian stimulation. As a consequence, the optimal number of IVM oocytes vitrified in candidates for FP is currently unpredictable.

Wider implications of the findings: Vitrification of oocytes after IVM should be considered in the FP strategy when ovarian stimulation is unfeasible, in particular when markers of the follicular ovarian status are relatively high. Further investigation will be needed to objectively assess the real potential of these IVM oocytes after vitrification. Therefore, even when a good COCs yield is expected, we should systematically encourage IVM in combination with ovarian tissue cryopreservation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Jean Verdier Hospital.
Trial registration number: NA.
Keywords: fertility preservation, cancer, *in vitro* maturation, AMH, antral follicle count

O-230 Mesenchymal stem cells facilitate *in vitro* development of human preantral follicle

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Study question: Whether mesenchymal stem cells (MSCs) could help to reconstruct a microenvironment to facilitate the human *in vitro* follicle development?
Summary answer: Human MSCs could effectively promote *in vitro* preantral follicle development in a dose-dependent manner.
What is known already: Biological folliculogenesis is a lengthy and complicated process and follicle growth microenvironment is poorly understood. The inaccessibility of rebuilding follicle growth niche is the main obstacle for the *in vitro* follicle development.
Study design, size, duration: Ovarian tissues were obtained from 7 women who underwent laparoscopic surgery. Ninety one morphologically intact preantral follicles, characterized as primary and secondary with a similar diameter ranging from 45 to 65 mm, were encapsulated and randomly assigned to be co-cultured with MSCs at different density for 8 days.
Participants/materials, setting, methods: During the *in vitro* culture (IVC) period, the morphology of each follicle was recorded and the diameter was measured. Follicle viability was assessed by Calcein-AM and Ethidium homodimer-I. The gene expression in IVC follicles was analyzed using single-cell RNA analysis. BMP4, TGF-b1 and estradiol were measured by ELISA and radioimmunoassay.
Main results and the role of chance: Human MSCs significantly promote the survival, increase the growth and improve the viability of preantral follicles in a dose dependent manner in a three-dimensional culture system. Further analyses revealed that GDF9 and BMP15 in oocytes and INHBA and TGF-b1 in granulosa cells within the follicles co-cultured with MSCs express notably higher than those in the follicles cultured without MSCs.
Limitations, reason for caution: Since follicle development is a lengthy and complicated process and different stages require different supporting gradients, the present study has limited ability to reveal the long-term effect of MSCs on follicles development.
Wider implications of the findings: Our findings demonstrate MSCs could promote preantral follicle development and provide a useful strategy to optimize fertility preservation and restoration by facilitating *in vitro* follicle growth.
Study funding/competing interest(s): Funding by national/international organization(s) – National Basic Research Program of China (2011CB944500), the National Science Foundation (No. 31230047, No. 81471508).
Trial registration number: 2009005.
Keywords: mesenchymal stem cell, fertility preservation, preantral follicle development, *in vitro* follicle growth

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SELECTED ORAL COMMUNICATIONS

SESSION 61: (EPI)GENETICS OF MALE INFERTILITY

Wednesday 17 June 2015 **10:00–11:45**

O-231 Risk level of intracytoplasmic sperm/spermatid injection for 115 non-mosaic Klinefelter syndrome (KS) patients

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Study question: Is the sex chromosome abnormality incidence for ICSI children increased when KS patient's sperm/spermatid is used?
Summary answer: Our data indicates the risk level of KS patients' intracytoplasmic sperm or spermatid injection into oocytes is much lower than previously expected.
What is known already: It has been reported that the sex chromosome abnormality incidence rate for ICSI children increased when KS patient sperm had been used. The thorough informed consent about the risk of this treatment should be carried out.
Study design, size, duration: We have performed a retrospective analysis of clinical outcome of ICSI in KS men using testicular sperm/spermatid during 2007 ~ 2012. In addition, we performed chromosomal analysis of 21 new born babies and FISH analysis of 500 round spermatids of KS patients.
Participants/materials, setting, methods: In a clinical IVF setting, this study dealt with 215 men who had previously been diagnosed as having non-mosaic KS. ICSI was performed using testicular sperm and spermatids were injected into electrically activated oocytes. We performed FISH analysis for remaining spermatids to confirm they are haploid cells.
Main results and the role of chance: 1. Out of 215 patients, slowly motile testicular sperms in 22 patients [A: 10.2% (22/215)], early stages of spermatid in 94 patients [B: 43.7% (94/215)] and no spermatogenic cells in 99 patients [C: 46.0% (99/215)] were found. Pregnancy rates per treatment cycles miscarriage rates and delivery rates in group A, B, were [A: 22.9% (8/35), 12.5% (1/8), 20.0% (7/35)], [B: 16.0% (36/225), 22.2% (8/36), 12.4% (28/225)] respectively. 37 healthy babies were born (male:female = 15:22) with 21 normal karyotype. No major congenital abnormalities were observed. 2. FISH analysis for early stage spermatids showed 100% monosomic, no disomic cells (X = 224/485, Y = 261/485, XX, YY, XY = 0/485).
Limitations, reason for caution: Total number of new born babies from KS patients so far are about 150 (including our data) and about only 35 (including our data) babies are proved to be chromosomaly normal. It might be premature to conclude the conception using KS gametes are not genetically safe.
Wider implications of the findings: This treatment is believed to indicate the risk level of KS patients' intracytoplasmic sperm or spermatid injection into oocytes is much lower than previously expected considering the facts the karyoplast of new born babies using KS men's gamete are 100% normal and FISH analysis for 500 spermatids from KS men were haploid.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Saint Mother Obstetrics and Gynecology Clinic and Institute for ART.
Trial registration number: None.
Keywords: klinefelter syndrome, ICSI, MD-TESE, chromosome

O-232 Increased risk of sperm chromosomal abnormalities and decreased recombination frequency in non-obstructive azoospermic males

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Study question: The aim of this study was to assess meiotic recombination in primary spermatocytes, synaptonemal complex length and the correlation with chromosomal abnormalities in testicular spermatozoa from infertile men with idiopathic non-obstructive azoospermia (NOA).

Summary answer: Our study shows an increased sperm aneuploidy rates and decrease in recombination levels and in NOA patients compared to fertile controls. These findings would corroborate the correlation between both parameters and the higher aneuploidy risk for the offspring of NOA patients.

What is known already: During the first meiotic division in spermatogenesis there are two critical events. First, synapsis between homologous chromosomes and formation of the synaptonemal complex (SC), which regulates sister chromatid cohesion and provides the template for localization of recombination machinery proteins. Secondly, recombination between homologous chromosomes, which is essential for the correct segregation. Errors during these two processes may induce incorrect segregation of chromosomes and are a major cause of gamete aneuploidy.

Study design, size, duration: Prospective cohort study from January 2008 to December 2014. Meiotic progression, total length of SC, frequency of recombination and sperm aneuploidy were evaluated in samples obtained from testicular biopsies ($n = 13$) from NOA patients. The study group was compared with a control group ($n = 18$) from post-vasectomized (OA) patients.

Participants/materials, setting, methods: Immunocytogenetics with SCYP3, CREST and MLH1 antibodies for meiotic progression, SC length, and recombination. Fluorescence *in situ* Hybridization (FISH) for chromosomes 1, 4, 6, 13, 16, 18, 21, 22 in primary spermatocytes and chromosomes 13, 18, 21, *X*, *Y* on sperm. MicroMeasure 3.3 program was used for SC length.

Main results and the role of chance: The overall mean number of MLH1 foci per cell showed a significant decrease in NOA patients compared to the control group (45.2 vs. 48.7; $p < 0.0001$). Comparing mean number of MLH1 foci per chromosome we observed a significant decrease for chromosome 4 (2.5 vs. 3.0, $p < 0.0001$) chromosome 16 (1.7 vs. 2.0; $p < 0.0001$), chromosome 18 (1.8 vs. 2.6; $p < 0.0001$) and chromosome 22 (1.0 vs. 1.2; $p = 0.0394$) compared to the control group. Regarding the total length of the SC, no significant differences were observed between the groups (276.8 vs. 276.7 mm; $p = 0.9526$). FISH analysis in testicular spermatozoa showed an increase of chromosomal abnormalities in the NOA patients compared to the study group (0.4 vs. 0.2; $p < 0.0001$).

Limitations, reason for caution: The major limitation is the limitation of the sample size due to the difficulty in their identification and obtention, this is the main reason for caution.

Wider implications of the findings: These studies are crucial to understand the origin of aneuploidy and to better characterize different phenotypes that may contribute to higher spermaneuploidy risk.

Study funding/competing interest(s): Funding by national/international organization(s) – Fondo de Investigación Sanitaria Grant (FIS Grant). Code: PS09/01725.

Trial registration number: None.

Keywords: meiotic recombination, chromosomal abnormalities in sperm

O-233 De novo point mutations in the male gamete are directly associated with paternal aging and reduced fecundity

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Study question: Is there an increase of de novo point mutations in the gametes of an individual male in association with paternal aging and reduced fecundity?

Summary answer: Sperm exome sequencing detected the accumulation of significant de novo point mutations in the spermatozoa coding sequence of individual males as they aged. These de novo point mutations resulted in amino acid changes, which are predicted to affect protein/enzyme function in key biochemical pathways that may impact spermatogenesis.

What is known already: Increasing evidence supports an association between paternal aging and reduced reproductive function that includes significant declines in many semen traits. As an aging reproductive population in the developed world, a greater focus and recognition of the impact of parental age and the underlying mechanisms may lead to better patient outcomes during infertility treatment. Additionally, advances in sequencing technology have allowed for investigations of genome-wide mutations rates revealing 75% originating in the paternal lineage.

Study design, size, duration: Young outbred male mice ($n = 13$) with proven fecundity underwent surgery to remove a single testicle. Males were then routinely mated to monitor fecundity until 15 months of age, when the second testicle was surgically removed.

Participants/materials, setting, methods: Motile epididymal sperm was subjected to somatic cell lysis. cDNA fragment libraries were generated for exome capture, hybridization and sequencing on the ION PI v2 chip. DNA reads were aligned to mouse reference genome UCSC 10 mm and single nucleotide polymorphisms (SNPs) compared to UCSC dbSNP 137 with Avadis NGS platform.

Main results and the role of chance: All males experienced significantly reduced fecundity by 15 months of age. Sperm exome sequencing generated 75 million reads, high coverage and 99.5% accuracy in single base nucleotide calls. A total of 3,343 significant SNP effects passed QC, with 625 de novo point mutations (18.7%) observed only in sperm of individual old vs. young males, all resulting in amino acid changes that are predicted to affect protein/enzyme function ($P < 0.05$). Gene ontology and pathway analysis of these affected proteins/enzymes identified enriched biochemical pathways that may impact spermatogenesis including: cell surface receptor linked signaling pathway, signal transducer activity, transmembrane receptor activity and G-protein coupled receptor signaling ($P < 0.05$).

Limitations, reason for caution: This study was performed using a murine model, which was a limitation, however also a strength since the same male individuals were examined over their natural lifetimes. Additionally, exome sequencing does not identify de novo point mutations in the regulatory and intronic regions of the genome.

Wider implications of the findings: This novel study allowed for the direct association between paternal aging and the generation of de novo point mutations in an individual males' gametes. Characterization of these de novo point mutations revealed amino acid changes predicted to be responsible for protein/enzyme dysfunction that may provide the framework for perturbed spermatogenesis resulting in the observed decline in reproductive fecundity.

Study funding/competing interest(s): Funding by hospital/clinic(s) – National Foundation for Fertility Research.

Trial registration number: None.

Keywords: sperm, exome, de novo mutation, paternal age, infertility

O-234 Investigation of sperm telomere length as a potential marker of paternal genome integrity and semen quality

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Study question: Is measurement of sperm telomere length (STL) potentially useful for assessing semen quality?

Summary answer: Reduced STL is associated with chromosomal abnormality, especially meiotic failure producing diploid sperm, and may lead to increased apoptosis during spermatogenesis. Given the importance of aneuploidy and apoptosis, it is likely that measurement of STL can convey clinically relevant information.

What is known already: Telomeres are essential for maintaining chromosome integrity. Recent studies have shown that elongation of telomeres occurs during human spermatogenesis. Shorter STL has been reported in samples from men with idiopathic infertility, and one study has demonstrated a correlation with sperm count in young men. The biological reasons for different STL and its correlation with clinical features are still poorly understood. No data has been reported on STL in relation to chromosome abnormality in human sperm.

Study design, size, duration: For this prospective study, semen samples were collected from 73 infertile men.

Participants/materials, setting, methods: STL was assessed using quantitative polymerase chain reaction in combination with an assessment of sperm DNA fragmentation index (SDF), employing the Sperm Chromatin Dispersion Test. Chromosome abnormalities were evaluated in ~2000 sperm per sample using multi-colour fluorescence in situ hybridization (chromosomes 13, 18, 21, *X* and *Y*).

Main results and the role of chance: A significant correlation between STL and diploidy was found (r = -0.29, p = 0.02). The association between SDF and aneuploidy, previously reported, was confirmed (r = 0.31, p = 0.009). STL, the aneuploidy rate and SDF were each associated with sperm count (r = 0.34, p = 0.004; r = -0.27, P = 0.025; r = -0.49, p < 0.0001). Interestingly, samples with isolated oligozoospermia showed shorter STL (p = 0.002), higher aneuploidy rate (P = 0.048) and SDF (p = 0.0005) compared to normozoospermic men.

Limitations, reason for caution: A technical limitation of this study is the inability to assess the entire chromosome complement in individual sperm. Consequently, aneuploidy rates are likely to be underestimated and any potential correlations between SDF, STL and abnormalities affecting specific chromosomes cannot be determined.

Wider implications of the findings: STL has potential as a fast and inexpensive form of sperm quality assessment, which could be considered before undertaking DNA fragmentation and aneuploidy analysis. Combined together, the three tests represent a useful genetic assessment, providing information independent of traditional sperm parameters, assisting in the identification of men who might benefit from preimplantation genetic screening of their embryos due to elevated diploidy/aneuploidy risk. The finding of samples with atypical STL also raises interesting biological questions.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Reprogenetics UK.

Trial registration number: NA.

Keywords: telomere length, sperm DNA fragmentation, aneuploidy, male infertility, human sperm

O-235 Clinical outcomes of preimplantation genetic screening cycle are not related to male characteristics: observational longitudinal cohort study involving 734 consecutive cycles

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Study question: Is male factor associated with laboratory and clinical outcomes of blastocyst stage aneuploidy screening cycles?

Summary answer: Secretory azoospermia is associated with lower blastocyst rate in PGS cycles. However, when blastocysts form, male factor does not impact on aneuploidy rate and on implantation potential of euploid blastocysts

What is known already: Chronological and biological female age is highly related to aneuploidy rate in blastocysts. It's thus generally recognize that the main indication for PGS are *advanced maternal age*, *recurrent pregnancy loss* and/or *recurrent implantation failure*. However, little is known about *severe male factor* incidence on embryo developmental competence and aneuploidies.

Study design, size, duration: This is an observational longitudinal cohort study involving 734 consecutive blastocyst stage PGS cycles from January 2012 to December 2014. Primary outcome measures were aneuploidy rate and implantation rate of euploid blastocysts in single cryopreserved embryo transfer cycles. Logistic regression analysis was used to exclude any possible confounding factor.

Participants/materials, setting, methods: Basal and cycle characteristics were recorded (female and male age, FSH, infertility factor, duration of infertility, previous miscarriages and IVF failures, number of mature eggs). Patients were clustered according to semen analysis following the parameters defined by WHO 2010. Blastocysts underwent trophectoderm biopsy with 24-chromosome screening analysis

Main results and the role of chance: From the 734 egg retrieval cycles, 4992 MII were injected resulting in 1640 biopsied blastocysts. Cycles with no blastocysts were 173 (23.6%, 95% CI = 20.5–26.8). 1640 blastocysts were biopsied and 656 were euploid (40.0%, 95% CI = 37.6–42.4). 249 were already transferred resulting in 134 ongoing implantations (52%, 95% CI = 46.2–58.9). As expected, female age and number of MII were associated with aneuploidy rate (OR = 0.68, 95% CI = 0.60–0.76 and OR = 1.83 95% CI = 1.56–2.15, respectively, p < 0.01). Male factors, male age, BMI, smoking habits and sperm parameters were all not related with blastocyst development and aneuploidy rate (OR = 1.03, 95% CI = 0.99–1.08; OR = 0.94, 95% CI = 0.85–1.04; OR = 1.02 95% CI = 0.98–1.07; OR = 1.18 95% CI = 0.39–3.6, respectively). Testicular sperm extraction was associated with a lower blastocyst rate (OR = 0.19, 95% CI = 0.4–0.81). None of the covariates was associated with implantation of euploid blastocysts, including female age and all male characteristics (intercept p-value = 0.24).

Limitations, reason for caution: Longitudinal cohort study from a single centre. Most of the couples included underwent PGS cycle for advanced female age. Overall, few azoospermic patients were included in this dataset, limiting the conclusion for this category. Furthermore, no additional functional semen analyses, such as DNA fragmentation, have been performed.

Wider implications of the findings: At present, it is still unclear whether severe male factor could be considered a valid indication for PGS. Our study on a large population of patients failed to identify a correlation between male characteristics and blastocyst chromosomal constitution and implantation potential. If the results are confirmed, PGS should not be proposed in these cases.

Study funding/competing interest(s): Funding by hospital/clinic(s).

Trial registration number: None.

Keywords: male factor, preimplantation genetic screening

O-236 Altered FISH in sperm, would you be indicated preimplantation genetic diagnosis (PGD)?

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Study question: Is there a correlation between sperm aneuploidy and transmission of chromosomal abnormalities to their offspring?

Summary answer: The analysis of our results indicates that probability of finding a chromosomally altered embryo is significantly higher in those men with altered sperm FISH to at least one of the chromosomes analyzed respect for those men with normal FISH specially in couples with young women.

What is known already: The development of more sophisticated techniques such as ICSI, revolutionized the management of male infertility, allowing the use of sperm from men with severely compromised semen parameters. Nevertheless, approximately 20% of pregnancies end in miscarriage. Aneuploidy has a negative effect on reproductive outcomes and represents one of the main causes of implantation failure and miscarriage. PGD has been applied for the selection of euploid embryos aiming to improve clinical outcomes.

Study design, size, duration: We performed a retrospective observational study. Overall, 834 embryos (140 cycles) were biopsied and diagnosed. Of these, 634 embryos were analysed by FISH after biopsy on day 3 and 200 embryos were analyzed using a-CGH after biopsy on day 5. Study was performed from January 2007 to April.

Participants/materials, setting, methods: PGD (SGP or a-CGH) was performed to couples who attended the Instituto Bernabeu with recurrent miscarriage, implantation failure, severe male factor or altered karyotypes. We analyzed 7 chromosomes in spermatozoa and 9 in the blastomeres by FISH (SGP). For a-CGH, trophectoderm genome was amplified and performed Agilent SurePrintG3 8x60K.

Main results and the role of chance: Globally, 412 out of 834 biopsied embryos were aneuploid (49.4%). Significant differences were reported in terms of embryonic aneuploidy among those embryos from men with altered sperm FISH (64.5%) and normal (49.4%) (p < 0.05). For embryos diagnosed by a-CGH, we also found significant differences in the rate of embryonic aneuploidy depending on whether the man had altered sperm FISH (68.6%) or normal (39.6%) (p < 0.05). However, not significant differences were found (p = 0.196) when analyzing the embryos by SGP. We also considered other parameters such as semen quality, DNA fragmentation and implantation rate in relation to embryonic aneuploidy. We only obtained significant differences for semen quality (p < 0.05, p = 0.736 and p = 0.26 respectively).

Limitations, reason for caution: One limitation of the study is the use of FISH to determine aneuploidy in sperm and embryos biopsied on day 3, since in this way we only study a certain number of chromosomes. This could be the reason why no significant differences in SGP were found.

Wider implications of the findings: This study shows that altered sperm FISH could be an indication in itself for the performance of a PGD by a-CGH to reduce implantation failure and recurrent miscarriage.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu Biotech.
Trial registration number: No trial registration number.
Keywords: male factor, sperm FISH, PGD, CCS, a-CGH

O-237 Epigenetic heterogeneity in sperm of infertile men

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Study question: Are the aberrant sperm DNA methylation levels detected in infertile males caused by widespread errors affecting all sperm produced by an individual or by the concomitant existence of populations of sperm with different methylation patterns?
Summary answer: In a distinct group of men with severe oligozoospermia, but not in normozoospermic individuals, sperm is composed of discrete populations of spermatozoa with opposing DNA methylation patterns at imprinted regions resulting in epigenetic mosaicism.
What is known already: Imprinted genes are silenced by methylation either in the oocyte (maternally imprinted) or the sperm (paternally imprinted). This parental-specific methylation pattern is maintained in the somatic lineages of the offspring but requires erasure and reprogramming in the germline. Recent studies have associated aberrant methylation of imprinted genes in sperm DNA with abnormal sperm parameters and male infertility. Moreover, a higher prevalence of imprinting disorders in children born after assisted reproductive techniques (ART) has been suggested
Study design, size, duration: Swim-up purified sperm was obtained from 42 men with normal (n = 19) and abnormal (n = 26) sperm parameters (according to the World Health Organization criteria). DNA methylation levels of imprinted genes were analysed by pyrosequencing-based Oligo-Sperm Methylation Assay (OSMA) and by Deep Bisulfite Sequencing (DBS).
Participants/materials, setting, methods: DNA was isolated and bisulfite-treated from pools containing 10 spermatozoa or the whole swim-up fraction. DNA methylation of *KCNQ1OT1* was measured in fractions of 10 spermatozoa by OSMA. DNA methylation of maternally (*KCNQ1OT1* and *MEST*) and paternally (*H19* and *MEG3*) imprinted genes was analysed by DBS at single cell level.
Main results and the role of chance: An increased variation in the DNA methylation values of the maternally methylated gene *KCNQ1OT1* was found by OSMA in samples with abnormal sperm parameters (such as those with a combination of oligo-, astheno- and teratozoospermia) but not in normozoospermic samples. DBS showed that normozoospermic samples have a homogenous pattern of DNA methylation (*KCNQ1OT1*: 3.78 ± 5.29; *MEST*: 0.76 ± 0.27; *H19*: 93.40 ± 2.49; *MEG3*: 0.88 ± 0.19), while oligoasthenoteratozoospermic samples show discrete populations with either normal or abnormal methylated patterns, resulting in aberrant average methylation values (*KCNQ1OT1*: 29.38 ± 7.49; *MEST*: 26.18 ± 4.65; *H19*: 2448 ± 6.71; *MEG3*: 69.50 ± 10.13). Aberrant methylation of *H19* appears to occur preferentially in the maternally inherited allele.
Limitations, reason for caution: OSMA could only be used for analysing one imprinted gene, *KCNQ1OT1*, due to the difficulty in working with such limited amounts of DNA. Analysis by DBS was only performed on a limited number of severely oligozoospermic samples and should be extended in the future to samples with milder defects.
Wider implications of the findings: The results presented herein demonstrate for the first time the existence of sperm populations with different imprinting status, ranging from normal to aberrant DNA methylation levels, coexisting in the semen of oligoasthenoteratozoospermic men, therefore providing an explanation for the findings of previous studies. These men produce portions of normally methylated sperm which, in the future, might be targeted and selectively used to improve ART outcome.
Study funding/competing interest(s): Funding by national/international organization(s) – Deutsche Forschungsgemeinschaft (DFG FOR1041/2). Bundesministerium für Bildung und Forschung (Network Imprinted Diseases, 01GM1114A).
Trial registration number: NA.
Keywords: epigenetic heterogeneity, DNA methylation, imprinting, sperm, male infertility

SELECTED ORAL COMMUNICATIONS

SESSION 62: BASIC ASPECTS OF ENDOMETRIOSIS

O-238 Endometrial mesenchymal stem cells present with high regeneration and migration potential suggesting their involvement in endometrial monthly regeneration and development of endometrial disorders

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Study question: As endometrium beholds vast regeneration potential during menstrual cycle and also ability to establish endometriosis, we aimed to study how endometrial mesenchymal stem cells (eMSCs) differ as for their regeneration and migration potential from their presumable progenitors, bone marrow mesenchymal stem cells (bmMSCs), and their progeny, endometrial stromal fibroblasts (eSF).
Summary answer: In terms of differentiation, wound healing/regeneration and migration potential, the eMSCs were similar to bmMSCs, whereas the eSFs showed lower regeneration and migration capacity. The findings may give important insight to endometrial eMSCs-related regeneration and may underline the role of eMSCs in ectopic lesion formation in endometriosis.
What is known already: Recently, eMSCs were identified in the endometrium in perivascular space and their gene profile was established indicating eSFs being their progeny. Furthermore, according to previous studies in mice and humans, it has been suggested that eMSCs originate from bmMSCs. Although eMSCs have been characterized for their differentiation and proliferative potential, the role of eMSCs in endometrial tissue regeneration and migration (in response to cytokine/hormonal triggers) compared to bmMSCs or eSFs has not been well established.
Study design, size, duration: Prospective, university-based, case-control, *in vitro* study using endometrial biopsies (n = 8) and bone marrow aspirates (n = 6) obtained from fertile aged women undergoing surgery for benign gynaecological reasons or scoliosis. The study was designed to compare the regeneration (wound healing, WH) and migration properties of eMSCs to bmMSCs or eSFs.
Participants/materials, setting, methods: The eMSCs and eSFs were FACS-isolated (eMSC: $CD146^+/PDGF\beta^+$; eSF: $CD146^-/PDGF\beta^+$) and bmMSCs were obtained through culturing. All cell types were cultured and tested (passage 2–4) for stem cell regeneration (2% serum) and migration [serum (2 vs. 10%, estradiol (E_2, 10 nM) vs. progesterone (P_4, 1 μM) vs. E_2P_4 (10 nM/1 μM, IL-1b (10 ng/ml)] properties.
Main results and the role of chance: The eMSCs and bmMSCs were able to differentiate and as expected, the eSFs were not. Furthermore, the eMCs displayed high clonogenic potential and stem cell surface marker profile. The regeneration capacity of eMSCs was comparable to bmMSCs (although faster healing rate in WH assay) and significantly higher than in eSFs ($p < 0.03$). Furthermore, the eMSCs showed higher migration response to serum (2 vs. 10%, up till 96 h) compared to bmMSCs and eSFs ($p < 0.001$). In contrast, the hormonal trigger with E_2 or P_4 or E_2P_4 did not result into any migratory activity

in any of the three cells types. Interestingly, the IL-1b was shown to be the highest migration trigger for eMSCs ($p < 0.001$), whereas the IL-1b migration response of bmMSCs and eSFs was comparable to serum triggering.
Limitations, reason for caution: This is a pilot *in vitro* study with small sample size. The obtained results should be confirmed in a larger data set with synchronized endometrial samples and applied in *in vivo* models. For practical reasons, it was impossible to isolate all cell types from the same patient.
Wider implications of the findings: According to the present data, the eMSCs possess high regeneration potential and therefore most likely play an important role in monthly endometrium regeneration where cytokine-trigger seems to provoke more robust migration than hormones. Furthermore, these findings may indicate a key role of eMSCs in ectopic endometrium lesion formation in establishing endometriosis. The vast proliferative and migratory nature of eMSCs may also indicate involvement of these cells in other endometrium-related pathologies like adenomyosis and cancer.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s). Funding by national/international organization(s) – Sigrid Juselius Foundation, Academy of Finland, Finnish Medical Foundation, Orion-Farmos Research Foundation.
Trial registration number: NA.
Keywords: endometrium, endometrial mesenchymal stem cells (eMSCs), Bone marrow mesenchymal stem cells (bmMSCs), migration, cytokine

O-239 Activating transcriptional factor 3 facilitates decidual prolactin expression in human endometrial stromal cells

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Study question: To explore whether Activating Transcriptional Factor 3 (ATF3) participates in the process of regulating human endometrial decidualization.
Summary answer: ATF3 is responsive to the stimulation of sexual hormone and sequentially elevates the secretion of prolactin (PRL), a specific decidual factor, in human endometrial stromal cells (hESCs).
What is known already: Decidualization is tightly controlled by multiple genes that regulate the production of proteins, one of which is prolactin (PRL) that plays a key role during early pregnancy including the regulation of uterine epithelial cell differentiation, trophoblast growth, and the modulation of the immune response. Stress response gene ATF3, a member of the ATF/CREB family, is strongly associated with some specific physiological procedures such as inflammatory response, cytothesis and cellular anti-apoptosis.
Study design, size, duration: Human endometrial stromal cells were infected with Ad-CTL or Ad-ATF3 adenovirus, otherwise stimulated with 1 μM medroxyprogesterone acetate (MPA) and 0.5 mM 8-Br-cAMP after knockdown ATF3, all treated hESCs were applied for 3 and 6 days.
Participants/materials, setting, methods: Human endometrial stromal cells isolated from endometrial biopsy were treated with 8-Br-cAMP and MPA to induce decidualization. The expression of ATF3 was analyzed by the Q-PCR and western blot. ELFA was performed to measure secretion of prolactin.
Main results and the role of chance: Western Blot showed ATF3 was notably lower expressed in endometrial tissue of 13 repeated implantation failure (RIF) patients compared with 12 successfully concepted women with once embryo transplantation, all patients undergoing IVF treatment in Drum Tower Hospital. ATF3 mRNA expression in hESCs was significantly increased ($P < 0.001$) after decidualization stimulated by 8-Br-cAMP and MPA in early time. Adenovirus-mediated overexpression of ATF3 in hESCs markedly increased PRL mRNA expression and PRL secretion ($P < 0.01$) in a concentration-dependent manner, simultaneously making hESCs demonstrate a morphological transformation from fibroblast-like to decidual cell-like. On the contrary, knockdown of ATF3 in hESCs observably decreased decidual PRL mRNA expression and PRL secretion ($P < 0.01$) induced by 8-Br-cAMP and MPA.
Limitations, reason for caution: This study was a phenomenal investigation to illustrate ATF3 is involved in process of decidualization and further research is required to explain its concrete mechanism.
Wider implications of the findings: We provided convinced evidences that ATF3 is a relevant factor in decidualization due to its function in regulating the secretion of PRL in hESCs. Extra experiments have showed ATF3 can promote embryo adhesion with in-vitro attachment model of BeWo spheroids adhering to Ishikawa cells, indicating that ATF3 may also modulate endometrial capacity. Considering its immunological properties, ATF3 may take part in embryo implanting via adjusting uterine immune microenvironment.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by The National Natural Science Foundation of China Grant 81170570 and The National Natural Science Foundation of China Grant 81370683.
Trial registration number: None.
Keywords: embryo implanting, decidualization, decidual prolactin, activating transcriptional factor 3

O-240 Regulatory mechanism of endometrial mesenchymal-like stem cells during menstruation

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Study question: The human endometrium is highly regenerative in a woman's reproductive life. Endometrial stem cells have been shown to contribute to this remarkable process [1]. We hypothesized that endometrial niche cells during menstruation regulated the behavior of endometrial mesenchymal-like stem cells (eMSCs) and thereby regeneration of the endometrium.
Summary answer: The eMSC population (co-expression of $CD140b^+CD146^+$ cells) was enriched during menstruation. Soluble factors secreted by the endometrial niche (epithelial or stromal) cells from menstrual phase significantly increased the eMSCs' proliferative activity and phenotypic expression.
What is known already: Regeneration of the endometrial mucosa takes several days after menstruation [2]. A cascade of growth factors, cytokines and chemokines are present at the onset of menstruation [3].
Study design, size, duration: Sequential beading with magnetic beads coated with anti-CD140b and anti-CD146 antibodies was used to isolate eMSCs from menstrual ($n = 6$), proliferative ($n = 4$) and secretory ($n = 3$) phase. The eMSCs were seeded at low density, and indirectly co-cultured at a ratio of 1:30 with endometrial niche (epithelial or stromal) cells from menstrual ($n = 9$) and secretory phase ($n = 2$) for 15 days. The cloning efficiency and proportion of cells expressing the eMSC markers was evaluated.
Participants/materials, setting, methods: Menstrual phase samples are obtained from women aged 36.1 ± 4.1 years undergoing IVF treatment on their second day of menstruation. Proliferative and secretory phase samples are obtained from women aged 44.6 ± 2.8 years undergoing total abdominal hysterectomy. Endometrial cells are isolated enzymatically and the percentage of eMSCs was analyzed by flow-cytometry.
Main results and the role of chance: Overall, a significantly higher proportion of eMSCs was detected in endometria from the menstrual (4.02%) and proliferative phase (4.26%) than the secretory phase (1.46%). Menstrual phase eMSCs underwent more self-renewal passages (6 rounds) than those from other phases (4 rounds, $P < 0.05$). Indirect co-culture of menstrual phase eMSCs with menstrual phase niche cells significantly increased the cloning efficiency (control: 1%, epithelial: 14.77%, $P < 0.05$; stromal 8.09%, $P < 0.01$) and the proportion of eMSC markers expressing cells (control: 1%, epithelial: 1.67%, $P < 0.05$; stromal 1.83%, $P < 0.01$). Similar effect was observed for secretory phase eMSCs (cloning efficiency: control: 1%, epithelial: 11.79%, stromal: 3.32%, $P < 0.05$; phenotype: control: 1%, epithelial 1.49%, stromal 1.38%, $P < 0.05$). Interestingly, secretory phase niche cells did not affect the proportion of eMSC markers expressing cells.
Limitations, reason for caution: The behavior to the cells may be altered during *in vitro* culturing. The functionality and multipotency of the eMSCs after coculture needs to be determined.
Wider implications of the findings: Our findings provide preliminary evidence that at menses, specific regulatory factors contribute to maintenance of eMSCs. The identification of candidate proteins from menstrual phase niche cells will provide a better understanding of eMSC biology and may subsequently unveil causes of gynecological complications such as dysmenorrhea and Asherman's syndrome.
Study funding/competing interest(s): Funding by University(ies) – The University of Hong Kong.
Trial registration number: NA.
Keywords: stem cell, niche, endometrium, menstruation, regeneration

O-241 1,25-Dihydroxy vitamin D3 modulates endometriosis-related features of human endometriotic stromal cells

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Study question: Vitamin D3 has various immunomodulatory effects on many biological processes which are closely related to endometriosis development. Yet, vitamin D3 deficiency is the most common nutritional deficiency worldwide. In this study, we examined whether vitamin D3 modulates endometriosis-related features of human endometriotic stromal cells.
Summary answer: We showed for the first time that vitamin D3 significantly increased adhesion and apoptosis, while reduced invasiveness, proliferation, pro-inflammatory cytokine production and angiogenesis potential of endometrial stromal cells (ESCs) from women with endometriosis.
What is known already: The results of recent studies show that vitamin D3 lowers endometriotic lesion score, inhibits implantation and development of transplanted endometrial tissue and decreases peritoneal inflammation in animal models of endometriosis.
Study design, size, duration: The effects of vitamin D3 on the eutopic (EuESCs), ectopic (EESCs) and control (CESCs) stromal cells from 25 women with and 20 women without endometriosis at proliferative phase of the menstrual cycle were investigated *in vitro*.
Participants/materials, setting, methods: The effects of vitamin D3 on adhesion, invasion, proliferation, apoptosis and angiogenesis potential of ESCs from endometriotic patients and control women were examined with cell adhesion assay, matrigel invasion, XTT and real-time PCR, respectively. Concentration of IL-6, IL-8, IL-17, TGF-b, TNF-a and IFN-g in culture supernatants were determined using ELISA.
Main results and the role of chance: In all groups, vitamin D3 significantly increased cell adhesion ($p < 0.05$), while decreased invasion ($p < 0.05$) and proliferation ($p < 0.01$) of EuESCs and EESCs. Such treatment also resulted in a significant decrease in IL-6 production by EESCs ($p < 0.05$), but had no significant effect on the IL-8 production. Stromal cells from all groups, showed no detectable secretion of other cytokines. This vitamin also caused significant decrease in Bcl-2 gene expression by EuESCs ($p < 0.05$) and Bcl-xL by EESCs ($p < 0.05$), but exerted no significant effects on Bax and caspase-3 genes expression. In addition, vitamin D3 treatment reduced VEGF-A gene expression by EESCs ($p < 0.01$).
Limitations, reason for caution: The effect of vitamin D3 was investigated in patients with stage III-IV endometriosis.
Wider implications of the findings: With regard to the increased adhesion and apoptosis and also reduced invasiveness, proliferation and angiogenesis potential of endometrial stromal cells in the presence of vitamin D3 and having considered the worldwide pattern of vitamin D3 deficiency especially in women at reproductive age, it seems that this hormone can be viewed as the first and an effective therapeutic modality in patients with endometriosis.
Study funding/competing interest(s): Funding by University(ies) – Avicenna Research Institute and Mashhad University of Medical Sciences.
Trial registration number: NA.
Keywords: endometriosis, endometriotic stromal cells, invasion, proliferation, vitamin D3

O-242 The role of microRNAs in mediating a state of enhanced endometrial receptivity in response to trophoblast cells: an integrative analysis

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Study question: Is there any role for microRNAs in mediating the early stages of the embryonic – endometrial communication prior to implantation?
Summary answer: The endometrium robustly alters its microRNA and transcriptomic profiles in response to very brief non – contact exposure to trophoblastic cells. MicroRNA – targeted differentially altered transcripts regulate subcellular pathways of known relevance to early implantation.
What is known already: *Background:* Besides synchronizing the adaptive cellular and sub-cellular changes that are necessary for implantation, early embryonic – endometrial communication may be a decision-making step for the maternal investment in the approaching embryo. MicroRNAs are large scale regulators of translation and an effective intercellular communication tool, and, as such, uniquely positioned to mediate both functions. In vitro studies on human endometrial stromal cell lines suggest that the process of decidualization is associated with an increased capacity for production of microRNAs coupled with some restriction in microRNA mediated silencing. Their role in the early stages of the embryo – endometrial dialogue is largely undefined.
Study design, size, duration: design: Experimental, *in vitro.*
Participants/materials, setting, methods: *Methods*: Human immortalised secretory endometrial epithelium monolayers were incubated with human trophoblast spheres for 4 h in a non – direct contact setting. Total RNA was extracted from the endometrial cells and profiled at the microRNA and transcriptomic levels using Affymetrix GeneChip microarrays. The results were validated using quantitative PCR. Target prediction followed by an integrated analysis to highlight microRNA – targeted transcript differentially expressed transcripts, and pathway analysis performed to highlight KEGG pathways enriched for microRNA regulation.
Main results and the role of chance: *Results*: The endometrium responded significantly to the brief non – direct contact co-incubation with trophoblast spheres. The response was present at the microRNA (65/3,391 human probes, $P < 0.01$) and transcriptomic (2023/39,000 human transcripts, $P < 0.05$) levels. 36 unique conserved microRNAs had a fold change of 1.5 times or more. They targeted 28% (444/2023) of all differentially expressed mRNAs in the same samples. Pathway enrichment analysis predicted the microRNA mediation of focal adhesion, regulation of the actin cytoskeleton and endocytosis. These pathways regulate plasma membrane transformation, water and ion channel regulation and the expression of cell adhesion molecules, all of which are well recognized epithelial adaptations involved in early implantation.
Limitations, reason for caution: *In vitro* experimental setting.
Wider implications of the findings: *Conclusion:* We demonstrated an early and important response of the secretory endometrium to approaching trophoblast cells prior to the establishment of physical contact. MicroRNA – targeted differentially regulated transcripts cluster into several subcellular pathways of known relevance to early implantation. MicroRNAs may therefore play an important role in mediating an early trophoblast – induced state of enhanced endometrial receptivity prior to embryo attachment. They have future diagnostic and therapeutic potentials in the management of patients with recurrent implantation failure.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). University of Sheffield and Sheffield teaching Hospitals NHS FT.
Trial registration number: NA.
Keywords: microRNA, implantation, embryo – endometrial communication

O-243 Non-invasive and real-time assessment of peritoneal lesions generated from human endometrial cell transplants locally accumulated by magnetic force in immunodeficient mice

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Study question: Can we make a mouse model of peritoneal endometriosis in which peritoneal lesions derived from human endometrial cell transplants can be assessed in a non-invasive, real-time, and quantitative manner?
Summary answer: Intraperitoneal accumulation and local placement of human endometrial cells by magnetic force enabled the formation of peritoneal lesions assessable with bioluminescence imaging (BLI) in a non-invasive, real-time, and quantitative manner in immunodeficient mice.
What is known already: Peritoneal endometriosis is the most common among various types of endometriosis. Several animal models of endometriosis have

been made through transplantation of human endometrial tissues, primary endometrial cells, or endometrial cell lines at various ectopic sites including the peritoneal cavity in immunodeficient mice. There has been, however, no mouse model of peritoneal endometriosis whose lesions can be assessed in a non-invasive, real-time, and quantitative manner.

Study design, size, duration: Immortalized human endometrial cells stably expressing a bioluminescent protein were labeled with magnetic beads and intraperitoneally transplanted into immunodeficient mice. A neodymium magnet was subcutaneously placed at the abdomen of each mouse to accumulate the labeled cell transplants onto the ventral peritoneum. These mice were then subjected to BLI.

Participants/materials, setting, methods: A stable cell line expressing enhanced green fluorescent protein (EGFP), a luciferase variant (CBR), and non-functioning nerve growth factor receptor (dNGFR) was generated from immortalized human endometrial cells using lentivirus and then labeled with magnetic-beads attached to anti-NGFR antibody.

Main results and the role of chance: We successfully constructed lentivirus harboring EGFP, CBR and dNGFR genes. Immortalized human endometrial cells were able to express EGFP, CBR and dNGFR simultaneously upon infection with the lentivirus. Furthermore, a stable cell line expressing EGFP, CBR and dNGFR was established from the infected immortalized cells through EGFP-positive cell sorting. BLI revealed that localized bioluminescent signals were detectable and assessable in a non-invasive, real-time and quantitative manner in mice transplanted with beaded cells, but no or little signals in mice transplanted with non-beaded cells, 4–10 weeks after cell transplantation and abdominal placement of a neodymium magnet.

Limitations, reason for caution: It remains to be elucidated whether peritoneal lesions generated from human endometrial cell transplants reflect and resemble endometriotic lesions.

Wider implications of the findings: Magnet force-driven engraftment of human endometrial or endometriotic cells at ectopic sites will be a possible novel strategy to develop *in vivo* models of various types of endometriosis in mice or other animals.

Study funding/competing interest(s): Funding by University(ies). Funding by national/international organization(s) – Funding by commercial/corporate company(ies). Japan Society for the Promotion of Science, Keio University Sakaguchi-Memorial Medical Science Fund, the Japan Medical Association, the Uehara Memorial Foundation, the Kanzawa Medical Research Foundation, the Cell Science Research Foundation, and the Takeda Science Foundation.

Trial registration number: NA.

Keywords: endometriosis, animal model

O-244 MAPK and VEGFR inhibition by sorafenib controls the progression of endometriosis

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Study question: can Sorafenib, a multi-kinase inhibitor targeting serine/threonine kinases RAF (RAF-1 and B-RAF) and receptor tyrosine kinases (VEGFR-1, -2), controls the growth of endometriosis?

Summary answer: Our data suggest that Sorafenib is an orally active, dual action, RAF kinase and VEGFR inhibitor that targets endometriotic cell proliferation and angiogenesis. Sorafenib controls the growth of endometriosis *in vitro* and *in vivo*.

What is known already: Endometriosis is complex multistep process. Mitogen Activated Protein Kinases (MAPKs) and VEGFR are both involved in the proliferation and survival of endometriotic lesions. Sorafenib is a strong multikinase inhibitor targeting two different pathways of endometriosis pathogenesis: RAF kinase and VEGFR.

Study design, size, duration: We conducted a prospective laboratory study in a tertiary-care university hospital between January to September 2012. After complete surgical exploration of the abdominopelvic cavity, 10 histologically proven endometriotic women were allocated to the endometriosis group and 10 women without any evidence of endometriosis were allocated to the control group.

Participants/materials, setting, methods: Ex-vivo stromal primary cells were extracted from endometrial and endometriotic biopsies from patients with and without endometriosis. Proliferation, apoptosis and MAPKs and VEGFR-2 autophosphorylation were explored with and without Sorafenib treatment. Human endometriotic lesions were implanted in 30 nude mice randomized in two different groups according to Sorafenib or Placebo treatment.

Main results and the role of chance: Performed ex vivo on stromal primary cell lines extracted from endometrial biopsies of 20 patients with and without endometriosis, this study highlights the role of Sorafenib in the pathogenesis of endometriosis. Sorafenib exerts cytostatic effects in endometriotic stromal cells by decreased MAP kinase ERK1/2 activity. Treating endometriotic cells with Sorafenib abrogated the phosphorylation of ERK with significant reduced pERK/ERK ratio in stromal cells of endometriotic women as compared to controls. In addition, this study highlights the antiangiogenic role of Sorafenib in endometriotic tissues. Treatment with Sorafenib decreased VEGFR-2 autophosphorylation highlighted by decreased pVEGFR-2/VEGFR-2 ratio in endometriosis. Using our mice model of endometriosis, we confirmed that Sorafenib regulate the endometriosis activity *in vivo*, possibly targeting proliferation and endometriosis-related angiogenesis.

Limitations, reason for caution: The study was conducted in a referral center specialized in endometriosis surgery and therefore women operated in our center might have particularly severe forms of endometriosis. This referral bias might have amplified the difference in MAPK levels between endometriosis and controls women.

Wider implications of the findings: This study opens the doors to future, more mechanistic studies to determine the exact role of Sorafenib, and its safety, in the pathogenesis of endometriosis. This novel model (*in vitro* and *in vivo*) of non-hormonal treatment of endometriosis opens new avenue in the targeted non hormonal treatment of endometriosis.

Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique – Hôpitaux de Paris (AP-HP), Groupe Hospitalier Universitaire (GHU) Ouest, Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, 75679 Paris, France.

Trial registration number: None.

Keywords: endometriosis, sorafenib, MAPK, RAF kinase, VEGFR2

SELECTED ORAL COMMUNICATIONS

SESSION 63: LESSONS FROM GENETIC SCREENING AND LARGE DATABASES

Wednesday 17 June 2015 **10:00–11:30**

O-245 Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening – an ESHG/ASHG position statement

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Study question: In the past few years, several professional societies have issued position statements on the role of non-invasive testing (NIPT) in prenatal screening for common autosomal aneuploidies. Ethical aspects have not yet been a main focus of those statements. This ESHG/ASHG position statement aims to fill this lacuna.

Summary answer: Responsible innovation in this field requires guarantees of the quality of the screening process as a whole (including non-laboratory aspects such as information and counseling), education of professionals, systematic evaluation in the light of the aim of the practice, accountability to all stakeholders and promotion of equity of access.

What is known already: The relevant normative framework consists of a context-specific articulation of more general principles for population screening, as initially formulated by Wilson and Jungner and further developed in the past decades. In order to avoid ethical pitfalls related to (selective) abortion, relevant policy documents stress that prenatal screening for fetal abnormalities is aimed at enabling autonomous reproductive choices by pregnant women and their partners rather than at preventing the birth of children with the relevant disorders.
Study design, size, duration: This document is the result of a unique collaboration between the Public and Professional Policy Committee of the European Society of Human Genetics (ESHG), and the Social Issues Committee of the American Society of Human Genetics (ASHG). The final version was approved by the Boards of both Societies (December 2014).
Participants/materials, setting, methods: The first draft was written by the first author and discussed by members of both committees and external experts. After adaptation, the text was emailed and posted on the ESHG website for membership consultation, and sent to the ASHG and ESHG Boards to elicit further comments.
Main results and the role of chance: NIPT has the potential of helping the practice of prenatal screening better achieve its aim of facilitating autonomous reproductive choices, provided adequate pretest-information and non-directive counseling are available. With improving technologies and decreasing costs, it will become possible in the near future to significantly expand the scope of prenatal screening beyond common autosomal aneuploidies. As the widely endorsed 'autonomy paradigm' does not provide a ready answer as to what the scope of prenatal screening for fetal abnormalities should be, a qualification of this account of the aim of prenatal screening is necessary. This position statement argues for a cautious expansion to serious congenital and childhood disorders, only following sound validation studies and a comprehensive evaluation of all relevant aspects.
Limitations, reason for caution: This document is based on expert opinion and consensus. Its recommendations pertain to a highly dynamic field and new developments may require reconsideration, adaptation or fine-tuning.
Wider implications of the findings: Whereas the introduction of NIPT into clinical practice has until now been largely left to commercial laboratories, a core message of the statement is that in countries where prenatal screening is offered as a public health programme, governments and public health authorities should adopt a more active role to ensure the responsible innovation of prenatal screening on the basis of ethical principles.
Study funding/competing interest(s): Funding by University(ies) – Maastricht University, NL.
Trial registration number: NA.
Keywords: ethics, prenatal screening, non-invasive test, position-statement, responsible innovation

O-246 Time to reconsider the time limit for human embryo-research?

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Study question: As new developments may well enable human embryo-research to move beyond the internationally accepted 'fourteen-day limit', the question whether this limit blocks promising medical research (e.g., exploring causes of miscarriage) is no longer hypothetical. What are the ethical and legal arguments behind the fourteen-day limit and how convincing are they?
Summary answer: The existing consensus reflects the will of policy-makers to draw a line somewhere rather than a shared understanding of why this should be at fourteen days instead of another developmental stage, for instance early brain-activity. None of the reasons given for a limit at fourteen days are argumentatively convincing.
What is known already: The reason why there has been no debate about human embryo research beyond the fourteen day limit is that until now, this has been regarded as hypothetical. This may change now that British researchers have developed a culture system that allows studying mouse post-implantation embryos, while others are considering research using (manipulated) human embryonic stem cells (hESC) that yields structures very similar to the post-implantation embryo.
Study design, size, duration: In this explorative study, we have 1. charted the arguments behind the fourteen day limit as presented in policy documents and in the relevant bioethical and legal literature; 2. determined their validity in terms of internal and external consistency; 3. proposed arguments and criteria for a possible alternative time limit.
Participants/materials, setting, methods: The study consists of interviews with scientists involved in human embryo/ESC research, a review of the bioethical and legal arguments behind the fourteen day limit (or alternatives), followed by a theoretical reflection and recommendations for further debate. The method is the 'wide reflective equilibrium', widely used in biomedical ethics.
Main results and the role of chance: Extended human embryo research *in vitro* may lead to important knowledge about early post-implantation development. Such research, however, would violate the widely accepted 14 day limit. An often proposed argument for this limit is that of 'ontological individuality': the moment after which twinning is no longer possible should be regarded as an ethically decisive transition point. Still, the question remains whether this and other arguments provide sufficient normative backing for a limit at 14 days. Those contesting this have pointed at other transition points that would be ethically more important, such as the beginning of brain-activity. However, as brain-related criteria are highly diverse and contested themselves, ethical scrutiny and societal debate of these alternative criteria for embryonic moral status are urgently needed.
Limitations, reason for caution: This is an explorative study, that does not itself answer the question regarding the time limit. However, it sets the agenda for a necessary new debate about what the limit should be, by confronting new developments in research with a review of arguments pro and con several different time limits.
Wider implications of the findings: Our conclusions make clear that an accepted element of the normative framework for human embryo research is less firmly rooted than many think. Instead of uncritically using the widely accepted limit to curb important scientific developments, policy makers should accept the need for fine-tuning the balance between embryo protection and reaping the benefits of human embryo research. This will require further ethical reflection and societal debate along the lines proposed in this study.
Study funding/competing interest(s): Funding by University(ies) – Maastricht University, The Netherlands.
Trial registration number: NA.
Keywords: embryo research, time limit, ethics

O-247 Infertility, fertility treatment and mammographic density in a large Swedish cohort

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Study question: is mammographic density influenced by hormonal fertility treatment?
Summary answer: Hormonal fertility treatment does not seem to cause any long term changes in mammographic density, with the possible exception of women who remain nulliparous after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
What is known already: Hormonal fertility drugs are used for ovarian stimulation in women with anovulation, and for controlled hyperstimulation as part of IVF or ICSI treatments. The drugs increase estrogen and progesterone levels and have therefore been suspected to influence breast cancer risk. Mammographic density is a useful marker for breast cancer risk which is also related to reproductive characteristics, such as parity and age at first birth.
Study design, size, duration: This cross-sectional study included 47,266 women recruited to the KARolinska MAmmography project between 2010 and 2013. Extensive background information, including fertility problems and treatment, was collected in a web-based questionnaire at mammography screening. Relative and absolute mammographic density were obtained from digital mammograms using the automated volumetric method Volpara™.
Participants/materials, setting, methods: Among the women who reported a history of fertility problems, 1,689 had gone through IVF or ICSI, 1,540 had had hormonal stimulation without IVF/ICSI and 6,528 had received no hormonal fertility treatment. The associations with mammographic density were calculated using linear regression, estimating mean ratios stratified by parity.

Main results and the role of chance: We found no differences in mammographic density between women with untreated or hormonally treated infertility compared to women without fertility problems. However, nulliparous women treated with IVF/ICSI had a small but significant increase in absolute dense volume (Mean ratio = 1.11, 95% CI = 1.06–1.17).
Limitations, reason for caution: Limitations of this study include inability to obtain information on cause of infertility and number of treatments. Information on treatment date was only available for a limited number of women.
Wider implications of the findings: While these results are reassuring, continued monitoring of cancer risk following IVF/ICSI is warranted since most women who have gone through treatment are still below the age at which breast cancer is usually diagnosed. The increased absolute density in nulliparous women may indicate that IVF/ICSI treatments have long term effects that are counteracted by having children. The association could also be the result of residual confounding by the underlying infertility.
Study funding/competing interest(s): Funding by University(ies). Funding by national/international organization(s) – 7th framework EU funding (the IDEAL project), the Swedish Research Council and Karolinska Institutet (strategic research grant in epidemiology).
Trial registration number: NA.
Keywords: infertility, fertility drugs, IVF, ICSI, mammographic density

O-248 Poor response in IVF treatment and the risk of a trisomic pregnancy

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Study question: Is there an association between ovarian response in IVF treatment and the risk of a trisomic pregnancy resulting from that treatment?
Summary answer: Subfertile women with a poor response in IVF treatment are not at a higher risk of a trisomic pregnancy resulting from this IVF treatment.
What is known already: Poor response is mostly the result of low number of follicles available in ageing women, representing decreased ovarian reserve. The limited pool hypothesis states that decreased ovarian reserve may result in selection of low-quality oocytes prone for non-disjunction during meiosis, increasing the risk of aneuploidy. Supporting this hypothesis, we showed, in a previous study, an association between low ovarian response (≤4 oocytes) and trisomic pregnancy, independent of maternal age (Haadsma et al., 2010).
Study design, size, duration: This is a matched case-control study. Cases (n = 110) are women with a confirmed trisomic pregnancy from 1983 till 2011 resulting from IVF, regardless of pregnancy outcome (live birth, termination or stillbirth). Controls (n = 455) are women with a live born child without a trisomy, resulting from IVF treatment.
Participants/materials, setting, methods: Data were obtained from Danish and Dutch medical registries. Matching criteria were age and year of ART treatment. Number of controls matched per case ranged from 1 to 5. Analyses were performed for Danish, Dutch and combined cohorts using Generalized Estimating Equations and Spline Regression.
Main results and the role of chance: Results shown are from analyses of the combined Danish and Dutch cohorts. Cases and controls were compared by number of oocytes retrieved. Mean maternal age was 36 years. The mean number of oocytes retrieved for cases was 9.3 and for controls 9.4. Thirty three women had poor response (≤3 oocytes, 423 women had normal response and 109 women had unknown response. Poor response was detected in 9.1% of cases (8/88) and 6.8% of controls (25/368). Poor response was not associated with a higher risk of having a trisomic pregnancy (OR 1.2, 95% CI [0.77–1.89]).
Limitations, reason for caution: We were not able to adjust the analyses for dose of gonadotropin stimulation, cycle number, BMI and smoking due to limited database information.
Wider implications of the findings: In our study, there was no association between poor response in ART and aneuploidy of the resulting pregnancy. These results do not support the hypothesis that decreased oocyte quantity, reflected by poor response, is associated with decreased oocyte quality, reflected by aneuploidy, which is postulated in the limited pool hypothesis. However, confirmation of our findings in a larger population is warranted.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Gratama Stichting, University of Groningen and the University Medical Center Groningen, The Netherlands.
Trial registration number: NA.
Keywords: IVF, poor response, trisomy, oocyte pool, ovarian reserve

O-249 Cumulative live birth rates following *in vitro* fertilisation in the United Kingdom: analysis of data from 107,347 women

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Study question: What is the cumulative live birth rate following multiple *in vitro* fertilisation (IVF) treatment cycles in the UK for different patient and diagnostic subgroups?
Summary answer: Couples undergoing IVF in the UK have a cumulative chance of live birth of up to 50% (for term singleton live birth up to 25%) over 2 years.
What is known already: Live birth rates following IVF have traditionally been reported per cycle. However, many couples undergo multiple cycles with every subsequent cycle increasing their cumulative chances of success. Cumulative live birth rates (CLBR) have not been published in the UK and have only recently been reported in the USA. Also, cumulative rates for term singleton live birth, deemed to be the most favourable pregnancy outcome, have never been reported.
Study design, size, duration: In this retrospective cohort study, the cumulative live birth rates of 107,347 women who had their first in-vitro fertilisation treatment attempt in the UK from 1999 to 2007 were calculated. All cycles were included up to 2 years since the first attempt or first live birth occurrence, whichever came first.
Participants/materials, setting, methods: A database containing all UK IVF treatments was obtained from the Human Fertilisation and Embryology Authority (HFEA). Every treatment cycle, defined as a fresh treatment attempt or frozen embryo transfer attempt, was linked to the woman. Cumulative live birth and term (>37 weeks) singleton live birth rates were calculated.
Main results and the role of chance: A total of 107,347 women had 186,512 cycles over 2 years from 1999 to 2009. The most frequent cycle patterns were one fresh cycle (53.9%), two fresh cycles (22.8%), three fresh cycles (8.2%), and one fresh followed by one frozen cycle (4.5%). After the third cycle, the CLBR for women aged <31, 31–35, 36–40 and >40 years were 49.7, 48.3, 33.2 and 9.8% respectively. For term singleton live birth the corresponding rates were 27.4, 27.7, 21.4 and 7.3%. In couples with unexplained, tubal, anovulatory, or male factor infertility the CLBRs after three cycles were 39.9, 36.7, 37.3, 43.0% respectively (term singleton live birth: 24.0, 21.2, 21.1, 26.0%). In fresh cycles only, the CLBR for women who cryopreserved embryos was 44.2 versus 30.1% who could not.
Limitations, reason for caution: We could not account for treatment-independent live birth outcome due to the nature of the HFEA database. Information on IVF treatments in 2010 and 2011 were available from the HFEA. However, from 2010 patients had to opt for their data to be used for research purposes, lowering the numbers substantially.
Wider implications of the findings: The UK cumulative rates could be used in the counselling of patients commencing IVF treatment. The results could also be used by policy-makers and researchers to determine the provision of an optimum number of IVF cycles per patient based on their characteristics. The next stage will be to develop a clinical prediction model that can predict live birth after n number of cycles.
Study funding/competing interest(s): Funding by national/international organization(s) – Chief Scientist Office.
Trial registration number: PDF/12/06.
Keywords: IVF, cumulative live birth rate, term singleton live birth

O-250 Cost per life birth after IVF/ICSI in France: a comparison of two gonadotropins based on published data from randomized trials and data from French practice

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Study question: What is the cost of a live birth (cost/LB) after an IVF/ICSI in France with two different gonadotropins (HP-hMG or rFSH) using the Markov model applied to two effectiveness scenarios (data from clinical trials and from French practice)? Which factors can influence the cost/LB and the incremental cost-effectiveness ratio (ICER)?
Summary answer: Using effectiveness data from clinical trials, cost/LB was €11,870 with HP-HMG and €13,928 with rFSH. Using data from French practice, these values were respectively €15,805 and €18,705. HP-hMG was more cost-effective. Expected effectiveness (LB rate – LBR) is the main contributing factor of final cost/LB, followed by gonadotropins total doses.
What is known already: Given that, in France, up to 4 IVF/ICSI attempts are fully reimbursed by the public health insurance system, cost of treatments is often and improperly neglected by patients and physicians. Whereas drugs used for controlled ovarian stimulation (COS) have different prices, data on costs/LB after IVF/ICSI still is lacking and should play a determining role in the choice of the most cost-effective COS protocol.
Study design, size, duration: A cost-effectiveness analysis using Markov model was performed. LB probabilities after IVF/ICSI, when HP-hMG or rFSH were used, were based on published randomized trials (Merit and Megaset). A correction factor was subsequently applied to accommodate published data to French practice, as reported by the national ABM registry (30% lower).
Participants/materials, setting, methods: Transition probabilities were applied to a virtual cohort of 30,000 IVF/ICSI candidates undergoing up to 4 COS cycles, including outcome possibilities as LB, cancellation/drops-outs, frozen/thawed embryos transfer... Costs encompassed all steps of infertility treatment and were based on French insurance system values. French experts validated all steps of the model.
Main results and the role of chance: In this decision analytic modelling where the Markov model was applied to a virtual cohort of 30,000 IVF/ICSI candidates, cost/LB with HP-HMG was €11,870 considering the published data and €15,805 considering the recalculated French registry data. These costs were lower than those observed with rFSH (€13,928 and €18,705, respectively). Further, in the first model, the ICER was estimated at €-11870 for any additional birth by using HP-HMG instead of rFSH. In the model using data from the French registry, estimated ICER was €-14,125. In both models expected effectiveness (LB rate) and total gonadotropin doses were the factors that influenced the most the ICER. Sensitivity analyses (one-way and probabilistic), performed to evaluate inaccuracies, corroborate these findings and attest their robustness.
Limitations, reason for caution: Markov model is a theoretic model of cost-effectiveness applied to a virtual cohort and limited to pre-defined transition probabilities. As treatment strategies are extremely variable, we needed to set some transition probabilities based on experts' opinion that reflect as far as possible French practice, but may not be universally applicable.
Wider implications of the findings: The present results indicate that HP-hMG is more cost-effective that rFSH for IVF/ICSI in France. They corroborate data from previous published data from international studies and should raise awareness for French health authorities on cost-effectiveness ratio of different gonadotropins for COS in the setting of their health policy on IVF/ICSI reimbursements. Also, potential economy related to the use of a less expensive gonadotropin is higher as the expected effectiveness increases.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Ferring SAS, Gentilly, France.
Trial registration number: NA.
Keywords: gonadotropins, medico-economics model, Markov model, cost-effectiveness, ART

INVITED SESSION

SESSION 64: STEM CELLS: OUTSTANDING QUESTIONS FINALLY ANSWERED

Wednesday 17 June 2015 **12:00–13:00**

O-251 Human therapeutic cloning: advances and technical challenges

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Cytoplasmic factors present in mature, metaphase II (MII)-arrested oocytes have a unique ability to reset the epigenetic identity of transplanted nuclei to the oocyte state. Using somatic cell nuclear transfer (SCNT), we demonstrated successful reprogramming of human skin fibroblasts into embryonic stem cells (NT-ESCs). A battery of genetic, epigenetic and transcriptional analyses performed on human NT-ESCs confirmed their close similarities to genuine IVF-derived ESCs than the traditional induced pluripotent stem cells.
Keywords: embryonic stem cells, somatic cell nuclear transfer, reprogramming

O-252 Naïve vs. primed pluripotency: a step further in regenerative medicine

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The mammalian embryo's ability to orchestrate the production of specific tissues in the correct time and place depends upon specification and control of a pool of pluripotent cells. We are interested in how these cells are segregated and the process by which they can be captured to produce naïve pluripotent embryonic stem cell lines. Using the mouse as an experimental system, we have generated transcriptional and functional data demonstrating that embryonic cells acquire the properties of naïve pluripotency (the capacity to self-renew from a single cell and contribute to all tissues of the adult organism, including the germline) during blastocyst expansion. Furthermore, we showed that the closest molecular counterpart to ES cells in the embryo is the epiblast just before blastocyst implantation. Because the pluripotent stem cell lines conventionally derived from human embryos have many divergent properties compared with mouse ES cells, we have used immunohistochemistry to establish whether markers of naïve pluripotency in the mouse are also present in human blastocysts. Reassured by the detection of Klf4 and Tfcp2l1 specifically in the inner cell mass, we have utilised a recently described culture regime to derive naïve pluripotent stem cell lines from human embryos by single cell explantation with high efficiency. Preliminary characterisation and expansion of these lines is consistent with their identity as naïve pluripotent ES cells. These will provide a useful resource for biomedical research and modelling human differentiation.
Keywords: epiblast, embryonic stem cell, naive pluripotency

INVITED SESSION

SESSION 65: ASRM/SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY (SART) EXCHANGE SYMPOSIUM: "INCORPORATING NEW TECHNOLOGICAL APPROACHES INTO ART PRACTICE: CLINICAL AND LABORATORY COORDINATION"

Wednesday 17 June 2015 **12:00–13:00**

O-253 Why should I transfer 1 versus 2 embryos?

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[2]*Michigan State University, Department of Ob/Gyn and Reproductive Biology, East Lansing, MI, U.S.A.*

[3]*Seattle Reproductive Medicine, Department of Obstetrics and Gynecology, Seattle, WA, U.S.A.*

The strong desire of patients dealing with infertility is having a healthy child – balancing cost versus increased pregnancy rate. Expectations by patients have increased as we have been more successful in creating the best embryo. However, there has been little linked National data to help provide an information benchmark regarding success and the individual parameters that surround the decision to transfer more than 1 embryo. Our hope is to provide a model for patients and physicians that can be used to clarify this choice.

Materials and Methods: Society for Reproductive Medicine (SART) collects data each year under a government mandate with the express purpose of informing patients. Data was studied from 2006 to 2012 utilizing a previously validated model. Cycles up to and including the first live birth were used.

Objective: To compare estimates of live birth rates (LBR) and multiple live birth rates (MBR) using autologous oocytes in one cycle with a double embryo transfer (DET) versus two successive cycles with single embryo transfer (SET) using either a fresh or frozen embryo in the subsequent cycle. Furthermore, other variables such as: age, body mass index (BMI), number of prior full term births, number of infertility diagnoses and infertility diagnoses were studied. From a previous validation paper, morphology and day of transfer and their role are discussed.

Results: Using logistic regression modeling a backward stepping algorithm was used eliminating variables until those remaining were $P < 0.05$. Models predicted LBR and MBR accounting for woman's age, BMI, prior births, and diagnosis, as well as number of embryos transferred. From over 320,000 patients and 500,000 cycles, the model used 33,065 for the first cycle SET and 126,921 for first cycle DET. In the subsequent cycle, SET included 8682 fresh cycles and 6747 thawed cycles. Results were calibrated to year 2012. An example of a 20 year old, BMI-40, and male factor might be used: her chance of pregnancy would be improved with 2 SET transfers (fresh or thawed) and her chance of twins reduced 18 fold. The LBR was found to be SET- 40.4%, 2 SET- 56%, 1SET/1FET- 60.5%, DET- 47.7%. Morphology and day of transfer may additionally alter these results; further refinement of the prediction model is planned.

Discussion and Conclusions: This predictive model utilized national linked data to predict live birth outcome. The prediction model included aspects of: age, BMI, full term births, diagnosis and number of embryos transferred. Limitations include the potential of errors of data entry (although validated annually) and that SET cycles may not be as representative of all cycles. Future plans include incorporation of day of transfer and morphology, as well as factors from prior cycles (successful and unsuccessful) to create a more in-depth model that physicians may use to counsel patients to establish expectations and thus strive to reduce the MBR.

Keywords: multiple birth rate, SART database, optimize IVF outcome, increase single embryo transfer

O-254 Coordination of new technology with laboratory resources

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Introduction: Changing IVF treatment options have challenged the abilities of laboratories to provide the desired level of service to patients. These challenges have primarily been sourced in oocyte cryopreservation, embryo biopsy for preimplantation genetic screening (PGS), and cryopreservation of all suitable embryos prior to embryo transfer. Each of these technologies requires a unique laboratory technician skill set, equipment availability, and space for additional personnel and equipment. All these technologies, sometimes quite complex, also bring additional risks as the likelihood of an error increases due to the additional work load. In order to meet these challenges, it is imperative that clinics evaluate their experience and practice pattern trajectories in order to supply the required resources to meet these challenges.

Objective: The objective of this study was to evaluate the impact of overall cycle volume increase as well as three specific cycle types: oocyte cryopreservation, embryo biopsy, and "freeze all" cycles (where all embryos are cryopreserved with no intent to transfer embryos during the active treatment cycle) on laboratory workload. In turn, this may help laboratories determine which skill level and how many technicians may need training and how many, if any, technicians are required to meet clinic needs.

Materials and methods: The dataset available through the Society for Assisted Reproductive Technologies (SART) was evaluated for estimation of increased workload for US ART laboratories. Cycle volumes changes as well as cycle type change were evaluated from 2009 through 2013.

Results: There are clear US trends in ART cycle volumes and type which reflect both increased overall utilization of ART in the US as well as a shift toward more complicated cycles. Overall IVF volume increased 23% (+32,721 cycles) while egg banking cycles increased 749% (+4,269 cycles). Embryo banking cycles increased 359% (+14,881 cycles). Frozen embryo transfer cycles increased 76% (+18,659 cycles). PGD/PGS cycle numbers can only be estimated due to limited data requirements prior to 2014 cycle initiation. However, as example, the presenting author's ART program experienced an increase of 1,040% in PGD/PGS utilization

Conclusion and Discussion: Changing ART practice patterns have created resource challenges for the laboratory. These challenges are manifest in overall ART cycle volume and the complexity of each cycle. With each stage of complexity, a unique and increased chance for potential error is exposed. Especially with PGS, an error has significant impact on patients and increased liability for clinics. It is therefore imperative that laboratories anticipate practice changes and assess resources accordingly. These data reflect the nature of practice pattern changes in the US. Both the cycle volume and complexity of cycles present challenges to the laboratory in order to meet patient expectations as well as to avoid errors. Several strategies can be employed to meet these challenges including additional personnel, additional equipment, and space to house both. Additionally, coordination with clinical personnel to manage daily cycle volumes and types may maximize efficiency of staffing and equipment utilization.

Keywords: laboratory, technology

INVITED SESSION

SESSION 66: PARAMEDICAL INVITED DEBATE SESSION: THE INTERNET DOES MORE HARM THAN GOOD

Wednesday 17 June 2015 **12:00–13:00**

O-255 Pro

S. Gameiro[1]
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This talk aims to provide evidence that the Internet can cause harm. In this context harm was defined as adverse events or bad outcomes, either physical or emotional, that occur after the use of materials, medications, and/or information obtained from a web site.

The literature regarding the use of the internet for assessing information, social-media (e.g., patient-foruns networking) and psychosocial-support (e.g., patient advocacy groups, psychosocial interventions) by infertile and medically assisted reproduction (MAR) patients was reviewed. The evidence collected supports the claims that the overall quality of infertility and MAR information available online is low, that infertility forums can have detrimental consequences and that internet-based interventions are not more effective than standard one-to-one psychosocial interventions.

First, there is considerable evidence showing that the overall quality of the information presented in infertility web sites is low and does not comply with existing guidelines. Infertility information in the Internet can also be overwhelming, distressful and subject to no content and quality control and regulatory mechanisms. There are examples in the health literature of events when specific online inaccurate information resulted in clinically significant emotional distress and physical harm, leading up to death. Other patient-reported negative effects of information consumption on the Internet are unnecessary visits to the physician, requiring that the physician take specific course of actions, taking up the physician's time and interfering with the patient-physician relationship.

Second, studies focusing on the disadvantages of using online infertility related forums show that up to 58% of patients report negative experiences such as

unjustified worry and anxiety, being exposed to the pregnancy of other people and inaccurate information, and addiction problems. Other less common experiences are aggressions and bullying, misunderstandings in communication and anxiety due to the time-lag of asynchronous communication.
Third, there is an overall agreement that peer-reviewed literature provides a gross underestimate of harm associated with health-related information and interventions because authors tend to concentrate on efficacy and effectiveness and rarely assess harm or other possible secondary effects. But even when this positive outlook is taken, evidence about the therapeutic benefits of internet-based interventions in not compelling. Overall the results seem to reproduce what is already known about infertility psychosocial interventions that are delivered in standard format: educational interventions increase knowledge and decrease anxiety but complex therapeutic or counselling interventions do not seem to increase patient quality-of-life during treatment. In the case of internet-based interventions, the causes for the lack of success become harder to disentangle. It may be that the psychosocial active components of the interventions are not effective, but it can also be that it is difficult to translate these from a one-to-one therapeutic context to an internet-based one. Overall the data suggests that treatments that include guidance from a mental health professional lead to better outcomes than unguided treatments.
In summary, while empirical evidence on the benefits of internet-based educational and therapeutic interventions within the infertility field is limited, the few studies that investigated the Internet harm potential produced worrisome results. Considering that the Internet has penetrated all areas of human life and numerous professional operations and that it is here to stay, more emphasis should be made to put in place the necessary regulatory mechanisms to avoid detrimental outcomes for infertile patients.
Keywords: Internet, evidence-based-medicine, information provision, social-media, psychosocial support

O-256 Con

Y. Khalaf[1]
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The proposal is a one-sided view that focuses on the negative aspects of this phenomenal invention, which has changed lives of the inhabitants of planet earth for good!
Like all important discoveries, there is a method failure and there is use failure. Nobody can put forward a persuasive argument that the internet has failed as a method. However, any use failure is relatively limited and should not blind us to the incontestable fact that life can not be the same without the internet, be it for medical information, or any other type of knowledge.
I will be arguing against the motion and will elaborate further on the day!
Keywords: internet, harm than good, con

SELECTED ORAL COMMUNICATIONS

SESSION 67: IMPACT OF CRYOPRESERVATION TECHNIQUES

Wednesday 17 June 2015 **14:00–15:15**

O-257 Effects of vitrification of 8-cell mouse embryos on blastocyst and postnatal development

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Study question: Does vitrification of murine embryos at the 8-cell stage alter blastocyst formation and postnatal growth, blood pressure or blood glucose homeostasis?
Summary answer: Our results suggest that while vitrification alters blastocyst development it does not change postnatal body weight, blood pressure and glucose tolerance in mice.
What is known already: Animal models have shown that vitrification can impair ultrastructure and developmental ability of oocytes, embryo survival rates, pregnancy rates and result in a low birth weight but any long term effects on offspring is still unknown.
Study design, size, duration: Three different treatment groups were studied (10 litters in each, 1) controls (non-ART treatment) 2) embryos cultured from 8-cell to blastocyst and subsequently embryo transferred (ET) to pseudo-pregnant mothers and 3) vitrified 8-cell embryos, reanimated and cultured to blastocyst and ET. From each group developed blastocysts were also analysed for cell number and allocation to trophectoderm or ICM.
Participants/materials, setting, methods: Embryos were vitrified at the 8-cell stage and held in LN_2. Embryos were warmed and cultured until they formed blastocyst before embryo-transfer. Offspring were weighed weekly, systolic blood pressure (SBP) taken at weeks 9, 15, 21 and glucose tolerance tests (GTT) carried out prior to cull for organ collection at week 27.
Main results and the role of chance: Offspring body weight for both sexes showed a significant increase ($P < 0.05$) for both non-vitrified ET ($n = 54$) and vitrified-ET ($n = 32$) groups when compared to controls ($n = 81$). Analysis of organ weight showed that kidneys and liver were significantly heavier in both treatment groups for both male and female. Males demonstrated a significant increase in SBP and a significantly impaired GTT[n1]. However there were no significant differences between the vitrified and non-vitrified ET groups in terms of all the above parameters. Differential labelling of blastocysts showed vitrification reduced both trophectoderm and inner cell mass (ICM) cell number significantly when compared to controls ($n = 81$–95 blastocysts per treatment). Our results suggest that while vitrification alters blastocyst development, it does not *per se* change postnatal body weight, blood pressure and glucose tolerance in mice, rather *in vitro* culture may alter these parameters.
Limitations, reason for caution: Extrapolation of these results to other species requires caution as the culture protocols, and stage of embryos may differ between species.
Wider implications of the findings: In the authors' knowledge, this is the first report on the effect of vitrification on postnatal health in mice. Further studies with gene expressions from stem cell derived from vitrified embryos in mice would be useful to further explain the link between vitrification with the low ICM cell number and any effect on postnatal health.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the European Research Council (FP7-EPIHEALTH) and MARA, Malaysia.
Trial registration number: None.
Keywords: cryopreservation, vitrification, postnatal health, mice, ART

O-258 Elective frozen embryo transfer does not improve reproductive outcome in normo-responder patients

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Study question: Does freeze all and differed embryo transfer improve reproductive outcome in normo-responder patients compared to fresh embryo transfer?
Summary answer: Freezing all embryos and differing transfer to a subsequent endometrial preparation cycle does not improve cycle outcome in terms of ongoing pregnancy and take home baby rates when compared to fresh embryo transfer.
What is known already: Several studies suggest that endometrial receptivity could be diminished during controlled ovarian stimulation (COS) due to morphological, biochemical and genetic changes. Some authors have suggested the solution by transferring the embryo in a delayed cycle in which the endometrium has not been exposed to the supra-physiological levels of gonadotropins. These findings support a freezing all approach.
Study design, size, duration: It is a single center retrospective observational cohort study. We include 882 patients aged 20–44 undergoing their first or second IVF/ICSI cycle between January 1st 2013 and December 31st, 2013, in which a normal oocyte yield (4–20) was obtained and embryo transfer was performed.
Participants/materials, setting, methods: The study was performed in the Instituto Valenciano de Infertilidad, Spain. Included 882 patients of which 364

(41.3%) had a fresh embryo transfer and 518 (58.7%) were submitted to freeze all according to doctor's preference. Ongoing pregnancy and take home baby rates are compared by multinomial logistic regression.
Main results and the role of chance: No differences were observed between both patients groups in terms of demographic characteristics and ovarian stimulation parameters. The raw analysis showed that ongoing pregnancy (33.2 vs. 32.9%, $p = 0.94$) and take home baby (36.2 vs. 33.8%, $p = 0.51$) rates for freeze-all vs. fresh cycles respectively were similar.
A multinomial logistic regression analysis that included all variables related to the cycle outcome (i.e: patients' age, serum E2, endometrial thickness, oocyte yield, fertilization rate, PGS, number of embryos transferred, and embryo stage at transfer) was performed. No impact of freezing-all could be observed, with an Odds Ratio (95% CI) of 0.90 (0.66–1.47) for ongoing pregnancy rate and 1.01 (0.73–1.40) for take home baby rate.
Limitations, reason for caution: The main limitation is that this is a retrospective study. Furthermore, the decision to transfer fresh embryos or freezing all was taken personally by each doctor.
Wider implications of the findings: These findings do not support a change in IVF practice moving to a freeze-all strategy in normo-responder patients. These may benefit patients since the treatment cost and the time to pregnancy is shorter when fresh embryos are transferred Only if well designed randomized controlled trials show different findings this change should be considered.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Valenciano de Infertilidad (IVI), Valencia, Spain.
Trial registration number: NA.
Keywords: frozen embryo transfer, fresh embryo transfer, ongoing pregnancy, freeze-all, IVF/ICSI

O-259 Laser-assisted zona pellucida thinning may negatively effect pregnancy outcome of frozen-thawed embryos

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Study question: Can laser-assisted hatching of the zona pellucida (ZP) positively effect pregnancy outcome of frozen-thawed cleavage stage embryos?
Summary answer: Performing assisted hatching (AH) on frozen-thawed cleavage stage embryos prior to ET in women above 35 years was shown to reduce clinical pregnancy rate, to increase chemical pregnancy and missed abortion rates. No significant effect of AH was shown regarding those parameters in women below 35 years.
What is known already: Hatching of the embryo through the zona pellucida is a necessary step for successful implantation. AH, a laser opening in the ZP has been proposed as a method for improving embryo implantation. Controversy exists regarding the effect of AH on frozen-thawed embryos on clinical pregnancy, implantation and delivery rates. The most relevant conclusion obtained so far is that AH in fresh cycles has a beneficial effect in women with repeated embryo implantation failures.
Study design, size, duration: A retrospective study included 334 patients that underwent frozen-thawed embryo transfer between 2012 and 2013. AH was performed on embryos of 170 patients in 2012. We compared IVF outcomes: chemical and clinical pregnancy rates, implantation, abortion and delivery rates according to age groups above/below 35 years.
Participants/materials, setting, methods: The data were divided into 4 groups according to women's age and laser-AH performance; group 1 –below 35 years (245) and group 2 over 35 (89). AH performance: group 1a- below 35 (118) and group 2a- over 35 years (52). Statistical analysis was carried out using *t*-test and Chi-Square Tests.
Main results and the role of chance: No differences were found between AH/no AH groups regarding clinical pregnancy rate, chemical pregnancy rate, implantation rate, missed abortion rate and delivery rate in women below age 35 (mean age 28.5 + 3.41). Surprisingly, performing AH in women above 35 years (mean age 38.6 + 2.6) was shown to reduce clinical pregnancy rate (9.6 vs. 23.3%), $P < 0.05$, to increase chemical pregnancies (11.5 vs. 0%), $P < 0.05$ and to increase missed abortion rate (0 vs. 44%), $P < 0.05$. No differences were found between the groups concerning other confounding variables which could effect implantation and pregnancy rate. There were no differences in implantation and clinical pregnancy rates in the fresh cycles between the years 2012–2013.
Limitations, reason for caution: This study was carried out retrospectively. Patients were compared between cases and controls that were recruited in consecutive years (cases 2012 control 2013). To exclude bias, only cycles of frozen-thawed cleavage embryos (48–72 h) were included. Blastocysts were excluded from the study because of the thinner zona pellucida.
Wider implications of the findings: Performance of AH was suggested to improve the outcome of frozen thawed embryo transfer because of ZP hardening during the freezing and thawing process. However, our data shown that in cycle of frozen-thawed cleavage stage embryo transfer, AH in women over 35 years, could decrease IVF outcomes. In frozen-thawed cycles we would recommend to transfer the embryos with no further involvement. The potential value of AH for frozen-thawed embryos has to be weighed carefully.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Soroka University Medical Center.
Trial registration number: None.
Keywords: laser-AH, frozen-thawed embryos, clinical pregnancy rate

O-260 The impact of cryopreservation on the oocyte transcriptome: vitrification versus slow freezing

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Study question: Although clinical data exists concerning the survival of oocytes cryopreserved using different methods, there is little information on the biological impact of different technologies. This study evaluated the impact of cryopreservation on oocyte mRNA transcripts and considered whether any differences observed might have implications for clinical efficacy and safety.
Summary answer: Microarray data revealed low-level alterations in the expression profiles of cryopreserved human oocytes, with larger number of genes affected in vitrified samples. However, follow-up real-time PCR analysis, demonstrated that most alterations were not significant, suggesting that slow-freezing and vitrification are likely to be safe and suitable for clinical use.
What is known already: Oocyte cryopreservation is increasingly widely used. The main indications are related to fertility preservation preceding cancer treatment and for social reasons. Additionally, the use of cryopreservation in conjunction with oocyte donation is becoming more common. In the last decade, vitrification has become the dominant method of oocyte cryopreservation, however recent protocol improvements have led to some resurgence in the use of slow-freezing. Both methods have given numerous live births, but molecular studies remain limited.
Study design, size, duration: This study involved women undergoing IVF treatment for either tubal or male infertility factors (i.e., without any detectable ovarian pathology). All patients were under 36 years (range: 28–35; average: 33.4) and had given informed consent.
Participants/materials, setting, methods: A total of 25 metaphase II oocytes (8 fresh, 8 slow-frozen and 9 vitrified) were analyzed. Processing of samples was carried out rapidly to minimise RNA degradation. Gene expression real-time PCR analysis utilised validated TaqMan assays, targeting genes with potential alterations in gene expression, highlighted by previous microarray analyses.
Main results and the role of chance: A small reduction in the quantity of mRNA transcripts was detected for some genes in slow-frozen oocytes (fold-changes from 0.6 to 0.8; $p < 0.05$). The genes affected by loss of transcripts were *HSP90AA1*, *HSPD1* and *EIF2C2* (compared with fresh oocytes) and *EFCAB11*, *EIF2C2* and *PDPR* (compared with vitrified oocytes). No significant alterations in mRNA levels were detected in vitrified oocytes for any of the genes investigated. Results from multiple oocytes derived from different women were combined together in order to minimize the effect of any patient-specific variability in gene expression. The average female age was similar for the three groups of oocyte analysed (33 years for fresh and slow frozen and 34

for vitrified eggs). Samples were run in triplicate to avoid potential experimental errors.
Limitations, reason for caution: The number of potentially altered genes, highlighted by microarray analysis, which could be followed up, was limited due to the finite amount of RNA obtained from each sample. Analysis of microRNAs would be valuable for understanding mRNA utilization in the oocyte but could not be assessed during this study.
Wider implications of the findings: The growth in cryopreservation has led to an urgent need for improved understanding of the impact of different methodologies on the oocyte. Techniques in current use are associated with high clinical pregnancy rates, but there are few molecular studies to support one particular technique over another. This study provided reassurance that vitrification is not associated with significant changes to the oocyte transcriptome. Alterations seen for slow-frozen oocytes were modest and of questionable clinical significance.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by a grant to Casa di Cura Città di Udine from the Italian Institute of Health.
Trial registration number: NA.
Keywords: human oocyte cryopreservation, transcriptome, vitrification, slow freezing

O-261 Fresh vs. aseptically vitrified sibling oocytes in an oocyte donation program. A prospective, observational, comparative study

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Study question: The aim of this study was to compare the embryological and clinical outcome of sibling fresh versus vitrified (in a closed system) oocytes, in an oocyte donation program.
Summary answer: We found a difference in the embryological outcome between the compared groups but we found no differences in the clinical outcome. Although blastocyst rate is significantly lower in the vitrification group, this does not affect the clinical and the ongoing pregnancy rates which are similar compared to the fresh group.
What is known already: There are studies which show very promising results after vitrification of oocytes. Open vitrification systems are more popular and more frequently reported in literature. A debate on whether open vitrification is considered aseptic and a directive from EU, for aseptic manipulation of cryopreserved cells and tissue, led to the need of a valid closed system for vitrification. There are not many studies which compare directly fresh with vitrified in a closed system oocytes.
Study design, size, duration: A prospective, observational, comparative study was performed from January to November 2014. The retrieved oocytes of a donor were randomly assigned into group I (fresh oocytes) or group II (vitrified oocytes). During the pickup, oocytes were given sequential numbers. Odd numbers were assigned into group I and even numbers into group II.
Participants/materials, setting, methods: 75 cases were included in each group. In group I, fresh oocytes were fertilized, developed and transferred on day 5. In group II, retrieved oocytes were vitrified using a closed system vitrification carrier device. When the recipient couple was ready and properly prepared, oocytes were warmed, fertilized, developed and transferred on day 5.
Main results and the role of chance: Fertilization rate is similar between the compared groups (77.2 vs. 72.1%) but blastocyst rate (53.1 vs. 43.3%) and top blastocyst rate (32.8 vs. 22.5%) is statistically higher in fresh group. Respecting clinical outcome, pregnancy rate (60.9 vs. 58.4%), implantation rate (34.7 vs. 36.1%) clinical pregnancy rate (50.0 vs. 52.7%) and ongoing pregnancy rate (48.6 vs. 51.38%) were similar between group I and II. Although at the beginning it is obvious that vitrification of oocytes has a negative impact in an oocyte donation case, due to lower blastocyst rate, this fact finally does not affect the clinical outcome of the case.
Limitations, reason for caution: The cases in the two examined groups were sibling, originated from the same donors whose oocytes were randomly and equally allocated into these groups and thus, the results of this study can be considered important. It is very important to validate these results in a larger scale.
Wider implications of the findings: The findings of this study are very interesting. Transferring blastocyst derived from vitrified – warmed oocytes instead of embryos from fresh oocytes does not compromise the clinical outcome, although the blastocyst rate is lower. This study validates the clinical efficiency of closed vitrification devices, which are an alternative to vitrify and store oocytes in an aseptic way. Nevertheless, more research is needed in order to improve the embryological parameters of the vitrified oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IAKENTRO Advanced Medical Center.
Trial registration number: The study was approved by the Institutional Review Board (Ref. no. 1/2014, granted 7 January 2014).
Keywords: vitrification, closed system vitrification, human oocytes, oocyte donation

SELECTED ORAL COMMUNICATIONS

SESSION 68: RESEARCH IN CLINICAL ENDOCRINOLOGY

Wednesday 17 June 2015 **14:00–15:15**

O-262 Kisspeptin mediates positive estrogen feedback on gonadotropin secretion in women

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Study question: Does exogenous kisspeptin-10, administered concurrently with appropriately timed estrogen, increase gonadotropin (LH and FSH) secretion, mimicking the physiological mid-cycle surge in women?
Summary answer: In a model of estrogen-induced gonadotropin secretion in women, kisspeptin-10 markedly increased LH secretion, advancing an LH surge and additionally stimulating FSH secretion. This suggests a role for kisspeptin in mediating hypothalamic positive estrogen feedback, and the pre-ovulatory mid-cycle gonadotropin surge triggered by this feedback.
What is known already: The hypothalamic neuropeptide kisspeptin is a key regulator of GnRH and thus gonadotropin secretion. There is evidence from animal-models that kisspeptin neurones mediate sex-steroid feedback signalling. Gonadotropin response to exogenous kisspeptin varies across the menstrual cycle, increasing more than two-fold higher across the follicular phase. We hypothesised that kisspeptin mediates pre-ovulatory positive estrogen feedback in the human and have investigated it by assessing gonadotropin responses to kisspeptin following estrogen priming.
Study design, size, duration: Nine women were treated with transdermal estradiol (200 µg/day) from cycle day 9–11, known to increase LH secretion 48 h later. At 24 h (before the expected LH rise) they were randomised to receive kisspeptin-10 or saline infusion for 7 h: all women received the alternate infusion in a subsequent cycle.
Participants/materials, setting, methods: Women were healthy with regular menstrual cycles. Kisspeptin-10 was infused at 4 µg/kg/h iv. Blood samples were taken before estrogen treatment (time 0), pre and post kisspeptin-10/saline infusion and at 48 and 72 h. Hormone concentrations were compared by ANOVA with Bonferroni multiple comparison post hoc analysis.
Main results and the role of chance: *Model validation*: Estrogen treatment increased LH after 48 h but not 24 h (time 0: 4.4 ± 0.6; 24 h: 3.1 ± 0.3; 48 h: 8.6 ± 1.0 IU/l, $p < 0.0001$). Similarly, FSH secretion increased significantly after 48 h of estrogen treatment (time 0: 2.6 ± 0.3; 24 h: 1.7 ± 0.3; 48 h: 3.3 ± 0.6 IU/l, $p = 0.0017$).

Kisspeptin response: Kisspeptin-10 acutely stimulated LH secretion from 3.8 ± 0.6 to 18.1 ± 4.3 IU/l ($p < 0.0001$) at the end of infusion. LH remained significantly elevated at 48 and 72 h after the start of estrogen treatment (15.3 ± 4.0 and 8.1 ± 1.3 IU/l, both $p < 0.01$ vs. start of infusion). Saline infusion had no effect on LH secretion (3.1 ± 0.3–2.7 ± 0.3 IU/l, ns). Kisspeptin-10 also acutely increased FSH secretion from 1.6 ± 0.1 IU/l pre-infusion to 3.6 ± 0.6 IU/l post-infusion ($p < 0.0001$) while saline had no such effect (1.7 ± 0.3–1.3 ± 0.2 IU/l, ns).

Limitations, reason for caution: This model of positive estrogen feedback may not fully replicate the physiological system. The response to kisspeptin-10 varied between women, suggesting that additional complex signalling pathways might be involved in mediating the mid-cycle surge in gonadotropins.

Wider implications of the findings: These findings add to our understanding of sex steroidal feedback control of hypothalamic function and the mid-cycle gonadotropin surge, key aspects of reproductive biology. Manipulation of this system has potential translational application in regulating GnRH secretion in a wide range of conditions, specifically in promoting or preventing ovulation.

Study funding/competing interest(s): Funding by national/international organization(s) – Wellcome Trust-Scottish Translational Medicine and Therapeutics Initiative.

Trial registration number: NA.

Keywords: kisspeptin, estrogen feedback, gonadotropin, LH

O-263 Whole-exome sequencing identifies mutations in basonuclin 1 as a cause of premature ovarian failure

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Study question: What is the causative gene mutation of the Chinese premature ovarian failure (POF) pedigree?

Summary answer: Whole-Exome Sequencing identified a truncation mutation in basonuclin 1 (BNC1), which was absent in 332 healthy controls, as a cause of POF. The truncated mutant protein exhibited obvious function disorder, and was related to POF in human.

What is known already: POF leads to infertility and premature menopause in women under 40, and increases all-cause mortality. Its known causes are highly heterogeneous. And in most POF cases, the cause remains unknown.

Study design, size, duration: Clinical basic research. A pedigree including seven members with POF, 80 sporadic POF patients, and 332 healthy controls. Five years.

Participants/materials, setting, methods: Our study enrolled a large Chinese pedigree in which seven members were affected by POF in an autosomal dominant manner, 80 sporadic POF patients, and 332 healthy controls. Whole-genome SNP scanning was performed in all the available family 1 members. Whole Exome sequencing was performed in three affected women with POF in family 1, and following Sanger sequencing verification was applied in all family members, sporadic POF patients, and healthy controls.

Main results and the role of chance: We identified a novel 5 bp deletion mutation within the *BNC1* gene completely co-segregated with POF in a Chinese family with POF, and a missense mutation within the *BNC1* gene in three sporadic POF patients. The 5-bp deletion within the *BNC1* gene led to a frameshift in translation and a premature TGA stop codon. The mutated BNC1 protein was truncated, losing its evolutionarily conserved three pairs of zinc fingers. We found BNC1 protein expressed in human oocytes, and *BNC1* knockdown inhibited human oocyte meiosis. The transfection of recombinant plasmids with truncation mutant in HEK293T cells led to obvious nuclear translocation disorder. These results suggest that the *BNC1* mutation resulted in the protein dysfunction, and could cause POF in humans.

Limitations, reason for caution: The size of sporadic POF patients was limited, and need to be expanded in our future research.

Wider implications of the findings: Our study provides the first evidence of a link between BNC1 dysfunction and human POF. Further studies are required to understand the underlying mechanism of ovarian aging.

Study funding/competing interest(s): Funding by national/international organization(s) – The National Basic Research Program of China (No. 2013CB967404, 2012CB944900), the National Natural Science Foundation of China (No. 81270664, 81471421), and the Science Foundation for Distinguished Young Scholars of Zhejiang Province (No.LR14H040001).

Trial registration number: No.

Keywords: POF, gene mutation, whole-exome sequencing, BNC1, nuclear translocation

O-264 Identification of circulating microRNAs in human follicular fluid as biomarkers of ovarian disorders

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Study question: Could circulating microRNAs (miRNAs) in human follicular fluid (FF) be associated with ovarian reserve status and thus, represent new promising biomarkers of female infertility?

Summary answer: Few circulating miRNAs were differentially expressed in FF samples according to women's ovarian reserve status and to controlled ovarian stimulation (COS) protocols. They might constitute potential biomarkers for detecting ovarian disorders in women included in IVF/ICSI program.

What is known already: MiRNAs are small non-coding RNA molecules, involved in the regulation of gene expression. Some miRNAs can be detected in biological fluids, including FF. Circulating miRNAs are already used as diagnostic/prognostic markers of many diseases such as gynecology and pregnancy disorders. Moreover, several circulating miRNAs are regulatory role in ovarian function but their implication in female infertility remains unknown.

Study design, size, duration: This prospective monocentric study included 83 patients (aged 35 ± 4.7 years): 56 women with normal ovarian reserve, 7 with poor ovarian reserve and 20 affected by polycystic ovary syndrome (PCOS). A pool of FF were retrieved for each patient during IVF/ICSI procedure.

Participants/materials, setting, methods: At oocyte retrieval day, all FF were collected and pooled for each patient. MiRNAs were extracted from pooled FF samples and quantified by RT-qPCR, using TaqMan technology. The candidate miRNAs, detected in FF samples were miR 140, miR 230a and miR191. MiRNA levels were compared by using the Mann-Whitney test and Spearman's Rank correlation coefficients were reported.

Main results and the role of chance: Circulating miR-140 in FF was negatively and significantly correlated with serum Anti-Müllerian hormone (AMH) levels and antral follicle count (AFC), evaluated at day 3 of menstrual cycle ($r = -0.3$, $p = 0.02$; $r = -0.41$, $p = 0.001$ respectively). Interestingly, miR-140 was significantly decreased in FF samples from patients with PCOS compared to those with normal ovarian reserve ($p < 0.001$). Likewise, miR-320a were significantly lower in FF samples from women with high basal serum LH levels (>5 IU/l) ($p = 0.007$). Spearman correlation calculation revealed a significant and negative correlation between intra-follicular miR-320a levels and basal LH rates in serum ($r = -0.28$; $p = 0.01$). In contrast, circulating

miR-191 was highly and significantly expressed in FF from patients with high AMH levels (≥5 ng/ml) ($p < 0.001$). Moreover, female infertility or agonist long protocol were significantly related to low miR-140 levels in FF compared to male infertility or antagonist protocol, respectively ($p = 0.02$; $p = 0.02$, respectively). In addition, miR-320a levels in FF were significantly and positively associated with the number of mature oocyte (MII) ($r = 0.25$; $p = 0.046$).

Limitations, reason for caution: The involvement of miR-140, miR-191 and miR-320a in pathogenesis of ovarian disorders and the identification of their potential target genes should be explored by conducting further functional investigations.

Wider implications of the findings: The expression levels of some circulating microRNAs in FF were significantly associated with ovarian reserve status and ovarian response to COS protocols. They provide new useful tools to improve the diagnosis/prognosis of infertile women during IVF/ICSI procedure. The combination of several circulating miRNAs could be more efficient for the diagnosis of PCOS. Moreover, circulating miRNAs might represent promising therapeutic targets in human reproductive system.

Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s). Funding by national/international organization(s). Funding by commercial/corporate company(ies) – This study was partially supported by the University Hospital of Montpellier, INSERM and Ferring Pharmaceuticals. The authors of the study have no competing interests to report.

Trial registration number: NA.

Keywords: circulating microRNA, human follicular fluid, ovarian reserve, ovarian stimulation, polycystic ovary syndrome

O-265 Cardiovascular disease (CVD) in hyperandrogenic postmenopausal women

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Study question: To assess the potential association of postmenopausal androgen levels with the prevalence of CVD (defined as coronary heart disease and/or heart failure and/or stroke) in a population based sample.

Summary answer: Heart failure is more prevalent in postmenopausal women with hyperandrogenism compared to women with normal androgen levels, implicating that women with PCOS might be at greater risk to develop CVD in their postmenopausal years.

What is known already: Hyperandrogenism is one of the three key features of PCOS and has been associated with increased prevalence of the metabolic syndrome and increased cardiovascular risk factors. Hyperandrogenism is probably maintained into the postmenopausal period, which has led to the assumption that women with PCOS are at increased risk for CVD during the postmenopausal years.

Study design, size, duration: A large population-based prospective cohort of 3635 female subjects, aged 55 years or older, with long term follow up. The study was conducted in a defined area in Rotterdam, the Netherlands.

Participants/materials, setting, methods: All patients underwent standardized interviews assessing their general, cardiovascular health followed by a thorough physical examination and asubsequent assessment of steroid levels using LC-MS.

Main results and the role of chance: The median age was 69.6 (range 55.1–105.7). Women with testosterone levels exceeding P_{90} showed an increased prevalence (19/361; 5.3%) of heart failure ($P = 0.047$), compared to women with levels between P_{40} and P_{60} (20/777; 2.6%). However, no significant increase in the prevalence of coronary heart disease (CHD) (17/348; 4.9%, $P = 0.48$), stroke (15/367; 4.1%, $P = 0.87$), or CVD (coronary heart disease, stroke and heart failure combined) (43/348; 12.4%, $P = 0.14$) was found in these women. Surprisingly neither androstenedione nor DHEA levels were correlated with heart failure, CHD or stroke. Hence, CVD was not at all correlated with these hormones. Finally, no correlation between the free androgen index and CVD was found.

Limitations, reason for caution: A potential limitation of the current study is that our cohort is relatively healthy.

Wider implications of the findings: Heart failure is slightly more prevalent in postmenopausal women with hyperandrogenism compared to women with normal androgen levels, indicating that women with PCOS might be at greater risk to develop heart failure in their postmenopausal years. However, no significant increase in the prevalence of coronary heart disease and stroke was found.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Erasmus Medical Centre.

Trial registration number: NA.

Keywords: PCOS, hyperandrogenism, cardiovascular disease, menopause

O-266 Short term estrogen therapy improves endothelial function in menopausal women: a double blinded randomized controlled trial of flow mediated dilation

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Study question: Is Flow Mediated Dilation (FMD) able to detect changes in endothelial function of menopausal women after only 28 days of estrogen therapy?

Summary answer: The use of 0.625 mg of conjugated estrogens for only 28 days is effective in improving vascular Nitric Oxide (NO)-dependent dilation assessed by FMD in menopausal women.

What is known already: Estrogen therapy has been proven efficient in reversing estrogen deprivation features in both laboratory and animal studies but there is still no consensus to which type of hormone therapy, dosage, administration path and time of onset would be optimal for reducing cardiovascular risk and also safe for clinical use.

Study design, size, duration: This was a double-blinded RCT carried out for the past 4 years in Hospital das Clinicas of the Federal University of Minas Gerais, Brazil where participants were selected among over 500 subjects. Sample size calculation determined a number of 30 patients in each group in order to reach a power of 0.80 and an alpha error of 5% in detecting statistically significant difference between the groups.

Participants/materials, setting, methods: 64 healthy menopausal women were selected and randomly assigned into two groups of treatment: 0.625 mg of Conjugated Estrogens were administered in the study group and placebo in the control group. Simple randomization was performed through computer-generated sorting. All participants were blinded to the use of conjugated estrogens or placebo and FMD was assessed by a blinded examiner before and after only 28 days of medication. Mann-Whitney's statistical test was used to compare the results of FMD obtained in both groups. *P-value* lower than 0.05 was considered statistically significant.

Main results and the role of chance: FMD values were statistically different between the groups ($p = 0.025$), where the study group of estrogen therapy showed a FMD value of 0.011 when compared to the placebo group (FMD = –0.082). The two groups were additionally evaluated for homogeneity through the Shapiro-Wilk test in respect to variables that could interfere with endothelial function such as age ($p = 0.729$), Body Mass Index (BMI) ($p = 0.891$), and time since menopause ($p = 0.724$). There was no statistical difference between the groups for any of these variables. Other variables were excluded during selection of the participants such as chronic vascular conditions, smoking, and sedentarism.

Limitations, reason for caution: The main limitation of this study was the small number of patients evaluated, although this number met the sample size requirements previously considered. Another concern that could interfere with the results was the correct use of medication by the patients on a day-to-day basis. Patients were asked specifically whether or not they had taken the capsules as instructed, but there is no objective proof that they did.

Wider implications of the findings: Several studies have been published previously with similar results about estrogenic influence in endothelial function. Studies that will be performed in the future concerning menopause and hormone therapy, in particular RCTs, will benefit from the knowledge that a period

of only 28 days is sufficient to promote endothelial function changes in menopausal women. Such studies will be allowed to be shorter in duration with no loss in the reliability of the outcomes.

Study funding/competing interest(s): Funding by University(ies) – Universidade Federal de Minas Gerais – Hospital das Clínicas.

Trial registration number: This RCT was registered in *Clinicaltrials.gov* under the number NCT01482416.

Keywords: hormone therapy, menopause, flow mediated dilation

SELECTED ORAL COMMUNICATIONS

SESSION 69: SELECTING EMBRYOS BY TIME-LAPSE

Wednesday 17 June 2015 **14:00–15:15**

O-267 The patterns and characteristics of day 3 human embryo fragments generation: a time-lapse study

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Study question: In this study we investigated the generation patterns of cytoplasmic fragmentation in day 3 (D3) human embryos.

Summary answer: D3 embryo fragmentation was generated in cytokinesis of cell cycle. Most of embryos generated fragmentation in a pattern that majority of D3 total fragmentation was generated in the first mitotic division (C1 pattern), especially highly fragmented embryos. Strong unrhythmic cytoplasmic movements occurred before cytokinesis of the first mitotic division were correlated with high total fragmentation degree in D3 embryos.

What is known already: Time-lapse study have suggested that cytoplasmic fragmentation can occur as early as the first mitotic division. Pathogenesis of human embryo fragmentation is still controversial, referring to apoptosis, disordered organization and distribution of cytoskeleton, genetic defects, and so on.

Study design, size, duration: This retrospective and observational study was conducted on 780 bipronuclear (2PN) embryos, obtained from 81 patients undergoing time-lapse system monitoring in our IVF center from May to October 2013. D3 embryo time-lapse parameters were evaluated by a unique operator.

Participants/materials, setting, methods: Embryos (n = 780) were cultured in PRIMO Vision incubators from fertilization to D3. Division behaviors and morphological parameters about generation of cytoplasmic fragmentation were annotated, including the morphologic changes before cytokinesis of the first cleavage, degree of fragmentation generated in each cleavage, and the total degree of D3 embryo fragmentation.

Main results and the role of chance: 536 embryos had different degrees of fragmentation in 780 embryos. According to total fragmentation degree, D3 embryos were divided into four groups: 1–5% (group I), 6–20% (group II), 21–40% (group III), >40% (group IV). Incidence rates of C1 pattern were 57.43% (143/249), 61.68% (132/214), 80.00% (40/50), 100% (23/23), respectively. Significant differences were found in group II, III and IV ($P < 0.05$, respectively). Cytoplasmic movements existed in most embryos during PN period to the first mitotic cytokinesis and subsequent development. 9.36% (73/780) of embryos sufferedp movements before the first cleavage had significantly increased degree of embryo fragmentation in D3, compared with embryos moved lightly and rhythmically, the median and interquartile range were 15% (10%, 27.5%) vs. 5% (0%, 10%) respectively ($p < 0.001$).

Limitations, reason for caution: It was just an observational study, lacking of fundamental research to confirm the relationship between cytoplasmic movements and embryo fragmentation.

Wider implications of the findings: In this study we have analysed the whole generation process of D3 embryo fragmentation. These data show that the first mitotic division is the most crucial period to generation of fragmentation, especially for highly fragmented embryos. Unrhytimic movements occurred before mitotic division, with morphological changing of embryo cytoplasm, probably respresent an erratic cytoskeleton. It may be another evidence of the hypothesis that cytoskeletal disorder is the causation of cytoplasmic fragmentation.

Study funding/competing interest(s): Funding by national/international organization(s) – National Science foundation of China 81222007.

Trial registration number: None.

Keywords: embryo fragmentation, cytoplasmic movements, time-lapse

O-268 The influence of different oocyte insemination techniques (IVF versus ICSI) on early and late morphokinetic parameters: a retrospective study of 449 time-lapse monitored blastocysts

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Study question: How different oocyte insemination techniques (standard IVF versus ICSI) influence early (from fertilization to 9 cells) and late (from compaction to expanded blastocyst) morphokinetic parameters observed during prolonged embryo culture? Is it possible to normalize them so that mixed IVF/ICSI-fertilized embryo cohorts could be analyzed together?

Summary answer: During cleavage stages ICSI-fertilized embryos were developing faster than IVF-fertilized ones whereas at blastocyst stage it was ICSI-fertilized embryos which showed a gradually increasing delay. After normalizing (to the timepoint of PN fading) differences between cleavage-stage parameters disappeared but those observed at the blastocyst-stage increased in favour of IVF-fertilized embryos.

What is known already: Despite the increasing number of published time-lapse monitoring (TLM) studies the influence of different oocyte insemination techniques on morphokinetic parameters has been underinvestigated (Dal Canto 2012, Cruz, 2013). Most TLM studies included ICSI-fertilized embryos exclusively and so far no predictive models have been developed for IVF-fertilized or mixed embryos cohorts.

Study design, size, duration: A 2-year, retrospective study performed between October 2012 and September 2014 in a single private infertility centre. Oocytes stemmed from 186 infertile patients (median age 37.6 ± 4.1 years, range: 28–47) undergoing natural cycle IVF or minimal ovarian stimulation with clomiphene or letrozole coupled with a universal single frozen-thawed blastocyst transfer policy.

Participants/materials, setting, methods: Data from 449 consecutive blastocysts that were monitored in a time-lapse incubator (EmbryoScope, Unisense Fertilitech, Aarhus, Denmark) were analyzed retrospectively. Early (PNf, $t2$–$t9$) and late (start of blastulation and expanded blastocyst) morphokinetic time points were scored according to recently published consensus criteria (Ciray et al., 2014).

Main results and the role of chance: In total 68 and 32% of the whole cohort was fertilized with ICSI and standard IVF, respectively. Standard IVF-fertilized embryos developed significantly slower as reflected by statistically significant delays in early morphokinetic parameters: PNf (-1.68 h), $t2$ (-1.53 h), $t3$ (-1.39 h) and $t4$ (-1.44 h). In contrast at the expanded blastocyst stage IVF-embryos showed faster development (+2.7 to +3.6 h). After normalizing to the timepoint of PNf differences in cleavage-stage parameters disappeared but those at the blastocyst-stage increased even further in favour of IVF-fertilized embryos (morula: +2 h, start of blastulation: +3.4 h, expanded blastocyst stages +4.7 to +5.7 h).

Limitations, reason for caution: Our unselected patient cohort was biased towards advanced-aged, poor-prognosis patients who have undergone mild IVF treatment coupled with single blastocyst transfer which might limit generalizability to other less infertile populations or to centres which use different treatment protocols.

Wider implications of the findings: Differences between cleavage-stage morphokinetic parameters are related to the fact that fertilization occurs with a 1.4–1.7 h delay following conventional IVF (compared to ICSI). However at the blastocyst stage there might be genuine differences between IVF and ICSI-fertilized embryos possibly related to their different intrinsic quality and/or euploidy status. Normalization to a common time point after fertilization might permit the joint analysis of IVF and ICSI-fertilized embryos and increasing the size of studied cohorts.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Kobe Motomachi Yume Clinic.
Trial registration number: NA.
Keywords: morphokinetic parameters, blastocyst culture, intra-cytoplasmic sperm injection, minimal ovarian stimulation, in-vitro fertilization

O-269 Cytoplasmic texture measurements of euploid embryos at a specific stage of development determined by time-lapse culture are indicative of their implantation potential

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Study question: After removing potential confounding effects associated with aneuploidy, are cytoplasmic texture parameters of zygote stage embryos indicative of their implantation potential?
Summary answer: Measures of cytoplasmic texture at the time of 2nd polar body (PB) extrusion are indicative of the implantation potential of euploid blastocyst stage embryos.
What is known already: Although morphokinetic differences between chromosomally normal and abnormal embryos have been identified, many abnormal embryos display characteristics of embryos otherwise capable of initiating full term pregnancies. Similarly, cytoplasmic texture measurements can vary with ploidy status but cannot be used exclusively to differentiate between viable and non-viable embryos. To better understand the relationship between cytoplasm morphology and an embryos implantation potential, it is desirable to restrict investigations to euploid embryos, thus avoiding confounding effects of aneuploidy.
Study design, size, duration: Retrospective study of 66 patients who had embryos screened for aneuploidy, cultured in a time-lapse incubator (Embryoscope), who had single embryo transfers between January 2013 and September 2014.
Participants/materials, setting, methods: Cytoplasmic texture measurements were recorded for 66 embryo images at five developmental stages of the first cell cycle: post injection (Inj), 2nd PB extrusion (PB2), pronuclei appearance (PNa), syngamy (Syn) and just prior to cytokinesis (Cyt). Embryos underwent PGS-CGH on Day 3 and transferred at the blastocyst stage of development.
Main results and the role of chance: Texture measurements were compared for a total of 25 embryos that resulted in the detection of a FH (FH+) with 41 embryos that failed to implant (FH-). No significant differences were observed between FH+ and FH- embryos at the Inj, PNa, Syn or Cyt stages of development. In contrast, 8/11 textural measurements differed ($p = 0.049$ to $p = 0.0003$) at the PB2 stage. ROC curves generated for each textural parameter following logic regression yielded AUC values up to 0.79 with 51/66 (77%) of outcomes correctly predicted.
Limitations, reason for caution: The effects observed are associated with embryos of patients requiring preimplantation genetic screening and may not represent embryos from other patient groups. This study represents a retrospective analysis and further prospective validation of results is necessary.
Wider implications of the findings: By restricting this study to euploid embryos we have removed the potential effects of aneuploidy on morphokinetic timings and textural measurements. As such, we have identified morphological parameters that can be identified using time-lapse culture techniques that are highly predictive of the implantation potential of human embryos. The development of algorithms incorporating textural variables may improve our capacity to select appropriate embryos for transfer or cryopreservation and to assess the impact of various treatment methodologies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No external funding was received for this study. Professor R. J. Hart has received educational sponsorship from MSD, Merck-Serono and Ferring Pharmaceuticals. The other authors have no conflicting interests.
Trial registration number: None.
Keywords: cytoplasm, texture, implantation, embryo, time-lapse

O-270 Time-lapse monitoring of early embryo development may aid in the validation of the effect of sperm DNA fragmentation assay on reproductive outcomes – preliminary findings

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Study question: Can time-lapse study of early embryonic development aid in the validation of the effect of DNA fragmentation assay (DFI – DNA Fragmentation Index) on ART?
Summary answer: Sperm DFI assessed by sperm chromatin structure assay (SCSA) by flow cytometry (FCM) impacts on the embryo-development and reproductive outcome of IVF/ICSI/IMSI in a group of couples with male infertility. Morphokinetics of early embryo development may aid in validation of new cut-off values for DFI.
What is known already: Current literature suggests that, when the sperm DFI value is above 25%, couples with infertility may have greater success with IVF/ICSI/IMSI procedures instead of IUI. However, when sperms with high DFI are involved, there are still uncertainties on the repair capacity of the egg and its further embryo-development.
Study design, size, duration: A retrospective analysis of 23 sperm samples from men undertaking ART in a private IVF center (May–December, 2014) compared the reproductive outcome and morphokinetic parameters (EmbryoViewer-Vitrolife) between patients groups divided by the DFI cut-off value of 25. Mean ± SD and p values with a significance threshold of 0.5 were calculated.
Participants/materials, setting, methods: Semen samples from patients (aged 35.6 ± 4.9 years) were analysed by SCSA (FACSAriaIII-BDBiosciences). Group A had a DFI <25 ($n = 17$) and Group B had DFI ³ 25 ($n = 6$). Twenty six treatment cycles were performed (23% IVF, 50% ICSI, 27% IMSI) and the development of 218 fertilised eggs was monitored by time-lapse imaging (EmbryoScope-Vitrolife).
Main results and the role of chance: All female partners were aged under 36 years and had >4 eggs collected. The following morphokinetic parameters were found to be significantly different in Group A vs. Group B: lower $t3$-tPNF (time to 3 cells minus time to pro-nucleai fading, in hours) (4.73 ± 4.57 versus 9.31 ± 4.57, respectively, $p = 0.0259$, lower $t3$–$t2$ (5.64 ± 7.65, 12.68 ± 9.73, $p = 0.0466$,, lower $t5$–$t3$ (9.23 ± 12.60, 23.46 ± 13.82, $p = 0.0173$, lower $t7$-tPNF (12.66 ± 13.13 vs. 29.31 ± 6.16, $p = 0.0044$, lower $t8$-tPNF (17.08 ± 15.53 vs. 34.51 ± 10.28, $p = 0.0111$) respectively and a reduced value of fragmentation time recording (57.86 ± 7.39 vs. 68.71 ± 6.53, $p = 0.0089$). A significant difference was found between the group of couples with positive (pregnancy rate of 61%) versus negative biochemical pregnancy (DFI of 13.5 ± 5.6 vs. 31.7 ± 23, $p = 0.042$).
Limitations, reason for caution: When the relationship of DFI with the reproductive outcome is assessed, the female bias cannot be completely excluded, although age and ovarian competence have been considered. However, with larger cohorts of patients, a more cautious selection will become possible.
Wider implications of the findings: When strictly controlled female factors and stable incubation conditions are available, the sperm DFI impact on embryo development can be properly assessed and, with larger study cohorts, new cut-off DFI values may be validated and used in conjunction with quantitative morphokinetic parameters for selecting viable embryos.
Study funding/competing interest(s): Funding by national/international organization(s) – National Authority of Scientific Research of Romania (POSCCE Grant 1357 "Infertility a tree pieces puzzle: couple investigation, infertility diagnostic, possible therapy").
Trial registration number: NA.
Keywords: DNA fragmentation index, flow cytometry, embryoscope

O-271 Selection of single blastocyst for transfer using time-lapse monitoring during *in vitro* fertilization in good prognosis patients: a randomized controlled trial

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Study question: In our study we try to answer whether morphological and kinetic information in a combined score, observed via a time-lapse (TL) system improves outcome over standard procedures, when a single blastocyst is selected for transfer (SET). To standardize conditions, groups were cultured in the same incubator.

Summary answer: IVF cycles in which embryos were cultured in TL system and were selected based on combined kinetic and morphological score evaluated on TL images resulted in a 20.5% relative increase in pregnancy over standard morphology-based selection. The ongoing pregnancy rate (PR) was 16% relative higher in the TL group.

What is known already: Most embryology labs select embryos for transfer based on morphology that is evaluated after removing the embryos from the incubator. TL systems provide undisturbed culturing conditions and allow detailed embryo observation. The utility of various kinetic markers to improve outcome has been tested. TL culturing together with embryo selection based on TL parameters was shown to improve blastocyst selection, implantation rate but there is little information on pregnancy/live-birth rates.

Study design, size, duration: Ongoing (since 2013), multicenter randomized controlled trial involving patients <36 years with normal ovarian reserve, undergoing 1st/2nd cycle, who have at least 3 good embryos on day 3 and accept SET. Patients are randomly assigned to PrimoVision monitoring and single blastocyst selection based on TL parameters vs. standard morphology-based blastocyst selection.

Participants/materials, setting, methods: Participants (140 randomized, 20 dropped out) were randomized to TL culture ($N = 59$) and embryo selection based on a composite score made up of kinetic markers and blastocyst morphology vs. standard incubation/evaluation and selection for transfer based on blastocyst morphology ($N = 61$). Patient/cycle parameters and clinical outcome were compared.

Main results and the role of chance: Of the 140 patients 20 dropped out for various reasons. Among those who have completed per protocol patient and cycle parameters are comparable between the groups. The PR is 47.5% in the TL group vs. 37.7% in the control group (20.5% increase, $p = 0.2$). The ongoing PR is 39.5% in the TL and 33.3% in the control group (16% increase, $p = 0.3$).

Embryos in the TL group were selected based on a composite score made up of various early kinetic markers, presence of fragmentation and blastocyst morphology (maximum 17 points). Within the TL group the combined score for embryos resulting in pregnancy is significantly higher when comapared to those not resulting in pregnancy (14.5 vs. 12.3; $p = 0.002$).

Limitations, reason for caution: While we have found a 20% relative increase in PR and a 16% increase in ongoing PR these findings have not reached statistical significance. This is an interim progress report, the study is powered to reach significance with 110 patients per arm.

Wider implications of the findings: IVF is overall a safe treatment but not complication-free. Multiple gestations are undesired following ART. Tools that can aid embryo selection are needed to avoid multiple gestations without compromising PR. The combined scoring of morphology and kinetics in time-lapse systems seems to offer benefits over standard evaluation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Kaali Institute.

Trial registration number: NCT01694641.

Keywords: time-lapse monitoring, embryo selection, single blastocyst transfer

SELECTED ORAL COMMUNICATIONS

SESSION 70: GOOD NEWS IN FEMALE INFERTILITY

Wednesday 17 June 2015 **14:00–15:15**

O-272 The use and success rates of assisted reproductive techniques among female childhood cancer survivors: preliminary results of the DCOG LATER-VEVO study

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Study question: Are there differences in the use and success rates of assisted reproductive techniques (ART) between female childhood cancer survivors (CCSs) and controls?

Summary answer: Preliminary data show that ART techniques are used more often by female CCSs compared to controls with the success rate of ART among CCSs being slightly, but not significantly, lower.

What is known already: Cancer treatment during childhood may impair female fertility. In combination with the general Dutch trend to postpone childbearing to the early thirties, this may put female CCSs at increased risk of requiring ART to achieve a pregnancy. However, information on the number of female CCSs actually using ART as well as on ART success rates is scarce.

Study design, size, duration: The study is part of the DCOG LATER-VEVO study, a nationwide retrospective cohort study on female fertility in Dutch CCSs. The control group consisted of sisters of survivors and females from the general population. Data collection took place between January 2008 and May 2014.

Participants/materials, setting, methods: Data on causes of infertility, the use and success rate of ART (=number of women achieving a pregnancy through ART/number of women who used ART) were assessed by questionnaire. Of the 1,717 survivors and 1,672 controls invited, 1,108 (65%) and 819 (49%), respectively, completed the questionnaire.

Main results and the role of chance: Preliminary data show that, after correction for age at study, CCSs had a higher probability of visiting a gynaecologist due to problems with achieving a pregnancy, compared to controls (OR = 1.7; $p < 0.01$). In addition, CCSs were significantly younger at the time of their first visit ($p < 0.001$). Compared to controls, CCSs more often reported a female factor as the cause of infertility (64.6% CCSs, 34.7% controls; $p < 0.001$). The proportion of CCSs having used any type of ART was 9.2% ($N = 103$) whereas this proportion was 6.1% ($N = 50$) in controls. After correction for age, CCSs had a higher probability of having used any type of ART (OR = 1.8; $p < 0.01$). Success rates of ART were 35% for CCSs and 50% for controls ($p = 0.05$).

Limitations, reason for caution: The study may have been subject to selection bias, since survivors with fertility-related problems might be more likely to participate in the study compared to survivors who do not experience problems in achieving a pregnancy.
Wider implications of the findings: These findings can assist clinicians in counselling childhood cancer survivors about their fertility and the use of ART to achieve a pregnancy. Clinicians should timely refer to reproductive specialists when survivors have a wish to conceive.
Study funding/competing interest(s): Funding by national/international organization(s) – Dutch Cancer Society (grant no. VU 2006-3622) and Foundation Children Cancer Free.
Trial registration number: NTR2922 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC = 2922.
Keywords: ART, IVF, ICSI, childhood cancer survivors

O-273 Therapeutic effect of oil-based-versus water-based contrast for hysterosalpingography (HSG) during the fertility work-up (H2Oil study, NTR3270, a nationwide randomised controlled trial

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Study question: Does tubal patency testing by HSG with an oil-based contrast medium lead to higher ongoing pregnancy rates compared to the use of water-based contrast medium?
Summary answer: Tubal patency testing by HSG with an oil-based contrast medium leads to higher ongoing pregnancy rates during the first 6 months following HSG, compared to the use of water-based contrast medium.
What is known already: Tubal flushing during HSG is suggested to increase pregnancy rates in subfertile women. It is unclear whether the choice of contrast medium used during HSG, water or oil-based, affects this potential therapeutic effect. Prior randomised and non-randomised trials have shown contradicting results.
Study design, size, duration: The H2Oil study is a multicentre randomised controlled trial, which took place in the Netherlands between February 2012 and November 2014. The study was performed in the Dutch consortium for research in women's health, and 27 hospitals participated in this study.
Participants/materials, setting, methods: We studied subfertile women undergoing HSG during their fertility work-up. Participants were randomly allocated to oil-based contrast (intervention group) or water-based contrast (control group). The follow-up period was 6 months and primary endpoint was ongoing pregnancy. Since data on life birth are not all available, this abstract reports on pregnancy rates.
Main results and the role of chance: Between February 2012 and November 2014 we randomised 1119 patients, of whom 557 were allocated to oil-based contrast and 562 to water-based contrast. Mean age was 32 years, mean duration of infertility was 21 months and 68% of the women had primary infertility. Baseline characteristics were comparable between the groups. Hysterosalpingraphy showed bilateral tubal patency in 89% of the women randomised for oil-based contrast versus 90% of the women randomised for water-based contrast. Ongoing pregnancy rates in the first 6 months following HSG were 38,8% in the intervention (oil-based contrast) versus 28,1% the control group (water-based contrast) (RR 1.38, 95% CI 1.14–1.66). There were no adverse events reported.
Limitations, reason for caution: Presented results are preliminary, as data on the primary outcome ongoing pregnancy are still incomplete. Complete follow-up on ongoing pregnancy rates will be available at the meeting.
Wider implications of the findings: HSG with oil-based contrast medium improves pregnancy rates as compared to HSG with water-based contrast medium. These data implicate that flushing of the tubes with oil-based contrast medium should be offered to subfertile women in the fertility work-up.
Study funding/competing interest(s): Funding by hospital/clinic(s) – VU University Medical Center.
Trial registration number: Dutch Trial Register, NTR3270.
Keywords: hysterosalpingography, contrast medium, therapeutic effect, pregnancy rates, RCT

O-274 Chlamydia serology in triaging patients for tubal assessment in female subfertility

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Study question: To assess how implementation of chlamydia antibody testing (CAT) into a clinical setting impacted on triage between tubal patency investigations and expectative management.
Summary answer: In clinical setting chlamydia serology is an appropriate and cost effective means of triage between tubal patency investigations and expectative management.
What is known already: CAT has predictive value in the detection of tubal damage and titres are quantitatively related to the severity of damage. CAT testing adds valuable information to a woman's risk profile based on her medical history. The combination of medical history taking and CAT testing has a better yield for diagnosing tubal disease than either of these alone.
Study design, size, duration: This is a prospective study of a cohort of 543 consecutive patients seen between August 2012 and July 2014.
Participants/materials, setting, methods: CAT testing was offered at initial infertility clinic consultation. Laparoscopy was offered to those testing positive. Those testing negative were offered HyCoSy, if considered for IUI, or proceeded to ovulation induction or expectative management. Theatre and medical records were reviewed for data collection. Statistical analysis was carried out using SPSS.
Main results and the role of chance: Out of 543 patients 110 (22.2%) were positive CAT + ve; 391 (72%) negative CAT - ve and 42 (7.7%) indeterminate CAT-Ind result. 41.8% CAT + ve underwent laparoscopy, out of which 47.8% had positive findings. 72.7% of those who had positive findings at laparoscopy were found to have tubal disease. However, in CAT - ve patients requiring tubal assessment by HyCoSy, 94.6% showed no abnormality. 88% of those testing positive had a past history pelvic infection. When compared to pre-implementation of CATs, there were 35 less laparoscopy done per annum, but extra 10 HyCoSy were performed for the same duration. This equates to about ≤80,000 (≤2,300 per laparoscopy) savings from laparoscopy but additional ≤3,500 (≤350 per HyCoSy) cost for HyCoSy.
Limitations, reason for caution: This study has a small number of participants and a larger cohort study is needed to validate these findings. There is significant number of participants who had the test but were lost to follow up due to spontaneous pregnancy and this may skew the data.

Wider implications of the findings: These findings indicate that implementing CAT into clinical practice can improve triaging of patients to further tubal patency testing and expectative management Therefore this may reduce unnecessary invasive tubal testing by laparoscopy which may translate into extra savings in term of time and money.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – University of Southampton, Complete Fertility Centre.
Trial registration number: Nil.
Keywords: chlamydia serology, tubal patency, laparoscopy, cost effectiveness, HyCoSy

O-275 Live birth in women previously experiencing recurrent implantation failure upon IVF: a retrospective cohort study

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Study question: What is the subsequent live birth rate and time to pregnancy (by conception after IVF/ICSI or by natural conception) in women who experienced recurrent implantation failure (RIF)?
Summary answer: Cumulative live birth rate in women experiencing RIF was 50% (95% CI 44–56%), with a mean time to pregnancy of 64 months.
What is known already: RIF is a distressing situation in which women do not reach a pregnancy within 3 IVF or ICSI treatments after transfer of good quality embryos. Although multiple studies have been performed aimed at identifying the aetiology, prognosis for achieving live birth is not known.
Study design, size, duration: This retrospective cohort study was conducted in two university hospitals in the Netherlands, between January 2008 and December 2012. Two hundred twenty-three women had experienced RIF, defined as no positive pregnancy test after 3 IVF/ICSI treatments including transfer of good quality embryos in women ≤38 years of age, and were included.
Participants/materials, setting, methods: All women diagnosed with RIF were sent a letter inviting their consent for anonymous use of medical file data and a questionnaire enquiring about subsequent treatments, pregnancies and outcome. Data was extracted and analysed to determine the subsequent live birth rate and time to pregnancy during a maximal 5.5 years of follow-up.
Main results and the role of chance: Two hundred twenty-three women were identified to have met the criteria for RIF (7% of treated population), 127 women responded to the invitation letter (57%) and data from 118 women (53%) was available for analysis. During the 5.5 year follow up period the overall cumulative live birth rate was 50% (95% CI: 44–56%). Survival analysis showed a mean time to pregnancy of 64 (95% CI: 55–74) months after diagnosing RIF, with a median of 9 months in those who did achieve a pregnancy during the follow up period. Forty pregnancies (82%) were achieved after further assisted reproductive technique treatments (live birth rate 15%/cycle), five with use of a donor gamete. Nine pregnancies (18%) occurred after natural conception.
Limitations, reason for caution: The retrospective nature of the study and the limited participation rate may have introduced selection bias.
Wider implications of the findings: This study reports favourable pregnancy chances in women experiencing RIF, especially after continuation of IVF/ICSI treatments. These findings can be used when counselling couples after three failed IVF or ICSI treatment cycles despite the transfer of good quality embryos.
Study funding/competing interest(s): Funding by University(ies) – University Medical Centre Utrecht, Utrecht. Academic Medical Centre, Amsterdam.
Trial registration number: NA.
Keywords: implantation failure, IVF, pregnancy

O-276 Indometacin administration can prevent premature follicular repture before timed intra uterine insemination (IUI) and improve the cycle outcome

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Study question: Can administration of indomethacin prevent early follicular rupture before timed intrauterine insemination and does it improves the cycle outcome?
Summary answer: Indomethacin can significantly reduce the incidence of spontaneous follicular rupture before IUI and allow more cycles to have properly timed procedures done.
What is known already: Prostaglandins play an important physiological role in the process of ovulation particularly follicular rupture. It had been used in some trials to prevent spontaneous ovulation in IVF cycles.
Study design, size, duration: A prospective randomized study that was performed on 194 UI cycles for mild male factor and unexplained infertility over an 18 months period.
Participants/materials, setting, methods: Patients were classified into two groups; group A (96 patients) received indomethacin 50 mg every 8 h starting when a leading follicle reached a diameter of 15 mm till the time of human chorionic gonadotropins (hCG) administration and group B (98 patients) a control group where the patients did not receive indomethacin. Patients were followed by vaginal ultrasound daily to check for spontaneous ovulation. hCG was administrated when the dominant follicle reaches a diameter of 18 mm and IUI was performed 36 h later.
Main results and the role of chance: The indomethacin group had a significantly reduced cases of spontaneous follicular rupture till 24 h before schedueld schedueled IUI 4 cases (4.2%) compared to 18 cases (18.4%) in the control group. This was reflected as a non significant increase in the cycle pregnancy rate 15 patients (15.6%) in the indomethacin group compared to 12 patients (12.2%) in the control group.
Limitations, reason for caution: The study needs to expand to a larger number of patients with uniform etiology, type and duration
of infertility.
Wider implications of the findings: Indometacin suppression of follicular rupture can make timing of IUI more convenient for the couple and service provider.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Private infertility center.
Trial registration number: None.
Keywords: IUI, indometacin, timed IUI, follicular rupture

SELECTED ORAL COMMUNICATIONS

SESSION 71: DIAGNOSIS AND SELECTION OF SPERM FOR ART

Wednesday 17 June 2015 **14:00–15:15**

O-277 Processing of semen by density gradient centrifugation selects spermatozoa with longer telomeres for assisted reproductive technology

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Study question: This study was carried out to investigate the role of density gradient centrifugation in the selection of spermatozoa with longer telomeres for use in assisted reproductive technology (ART).
Summary answer: Density gradient centrifugation is a useful technique for the selection of sperm with longer telomeres for ART.
What is known already: The ends of eukaryotic chromosomes contain specialized chromatin structures called telomeres, the length of which plays a key role in early human embryonic development.
Study design, size, duration: Case control study, 105 Semen samples, sperm before and after density gradient centrifugation processing, from July 2014 to November 2014.

Participants/materials, setting, methods: Semen samples were collected from 105 infertile men and then submitted to Sperm Chromatin Dispersion tests (SCD) and quantitative polymerase chain reaction (Q-PCR) to analyze the DNA fragmentation rates and telomere lengths, respectively, in sperm before and after density gradient centrifugation processing.
Main results and the role of chance: After density gradient centrifugation, the average telomere length of the sperm was significantly longer (6.07 ± .22 vs. 5.01 ± 0.94), the average motile sperm rate was significantly higher (77.9 ± 11.8 vs. 44.6 ± 11.2), but average DNA fragmentation rate was significantly lower (11.1 ± 5.9 vs. 25.9 ± 12.9) compared to raw semen. In addition, we found that telomere length was positively correlated with sperm count of the semen ($r_s = 0.58$; $P < 0.01$).
Limitations, reason for caution: Small number of samples and only one reproductive medicine center in our study.
Wider implications of the findings: In this study, we found that density gradient centrifugation could enrich for sperm with high motility and low DNA fragmentation, and for the first time, we found that this commonly used technique for processing semen for ART could effectively recover sperm with longer telomeres.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Science Foundation of China (Grants 31271605 and 31471404) and the National Science Foundation for Young Scientists of China (Grant 31401274)
Trial registration number: No.
Keywords: telomere length, density gradient centrifugation, assisted reproductive technology, sperm, infertility

O-278 Sperm selection using MACS has similar obstetric and perinatal outcomes than standard preparation when ICSI was performed in ovum donation in a prospective and randomized trial

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Study question: Does MACS technology have an effect on children born in ICSI procedures of our ovum program?
Summary answer: Magnetic activated cell sorting does not have any benefit or harmful effect on children born in ICSI cycles of our ovum donation program in unselected male patients.
What is known already: There is still little clinical information available about the wide employment of these selection techniques in *in vitro* fertilization and their impact in final assisted reproduction techniques (ART) outcomes although our findings not show anincrease in live birth deliveries rates when MACS was performed.
Study design, size, duration: Two arms, unicentric, prospective, randomized and triple-blinded trial, a total of 237 infertile couples, October 2010 and January 2013.
Participants/materials, setting, methods: IVI-Valencia. Children born after use of MACS in ICSI (60 deliveries) and control group, only swim up in ICSI (64 deliveries). In 11 and 13 cases from MACS and control group respectively no perinatal and obstetric outcome were obtained. A total of 65 and 66 newborns from MACS and control group respectively were described.
Main results and the role of chance: MACS had no clinically relevant adverse effects on obstetric and perinatal outcomes after adjusting for potential confounders. No differences were found between the both groups in the rate of obstetric problems including premature rupture of membranes 6.1% (CI 95% 0–12.8) vs. 5.9% (CI 95% 0–12.4), 1st trimester bleeding 28.6% (CI 95% 15.9–41.2) vs. 23.5% (CI 95% 11.9–35.1), invasive procedures as amniocentesis 2.0% (CI 95% 0–5.9) vs. 3.9 % (CI 95% 0–9.2), diabetes 14.3% (CI 95% 4.5–24.1) vs. 9.8% (CI 95% 1.6–17.9), anemia 6.1% (IC95% 0–12.8) vs. 5.9% (CI 95% 0–12.4), 2nd and 3rd trimester 10.2% (CI 95% 1.7–18.7) vs. 5.9% (CI 95% 0–12.4), urinary tract infection 8.2% (CI 95% 0.5–15.9) vs. 3.9% (CI 95% 0–9.2), pregnancy-induced hypertension 6.1% (CI 95% 0–12.8) vs. 15.7% (CI 95% 5.7–25.7), birth weight (g) 2684.10 (CI 95% 2499.48–2868.72) vs. 2676.12 (CI 95% 2499.02–2852.21), neonatal height (cm) 48.3 (CI 95% 47.1–49.4) vs. 46.5 (CI 95% 44.6–48.4) gestational cholestasis 0% (CI 95% 0–0) vs. 3.9% (CI 95% 0–9.2) respectively in MACS group compare with control group.
Limitations, reason for caution: We analyzed all the births for which we had notification, and not the whole series of ICSI pregnancies achieved in our Institution during the study period.
Wider implications of the findings: Our data suggest that MACS technology does not increase adverse obstetric and perinatal outcomes in children conceived when this technology was performed, being the largest randomized control trial with live birth reported results with MACS.
Study funding/competing interest(s): None.
Trial registration number: Given that the main intervention was conducted on sperm samples and not human beings, Clinical Trial registration is not needed.
Keywords: MACS technology, children born, obstetric and perinatal outcomes, prospective and randomized trial, ovum donation

O-279 Cytological evaluation of spermatogenesis: a novel and simple diagnostic method to assess spermatogenesis in non-obstructive azoospermia using testicular sperm extraction specimens

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Study question: Can spermatogenesis be evaluated by defining the ratios between Sertoli cells, pachytene spermatocytes and spermatozoa in a cell suspension obtained by testicular sperm extraction (TESE)? Subsequently, are these ratios associated with the outcome of fertility treatment in patients with non-obstructive azoospermia (NOA)?
Summary answer: Pachytene spermatocytes, spermatozoa and Sertoli cells can be easily identified and counted in a cell suspension and their ratios can be successfully used to diagnose the level of spermatogenic impairment. This pilot study indicates that once successful sperm retrieval is achieved, treatment outcome declines when spermatogenesis is impaired in NOA.
What is known already: Most of these NOA-patients have only focal spermatogenesis which results in insufficient numbers of spermatozoa to reach the ejaculate. In approximately 50% of NOA-patients TESE is successful and intracytoplasmic sperm injection (ICSI) is pursued. It is also assumed that in some cases, a genetic cause affects sperm maturation and development. To date, the predictive value of cytological evaluation of spermatogenesis for the TESE-ICSI outcome is unknown.
Study design, size, duration: This retrospective cohort study included 441 consecutive TESE-ICSI cycles in 212 couples, performed between June 2007 and August 2012. For each TESE biopsy the ratios between Sertoli cells, pachytene spermatocytes and spermatozoa were calculated. A control population of 38 vasectomised men was used to define cut-off values for complete spermatogenesis.
Participants/materials, setting, methods: NOA-patients were divided in three subgroups based on the pachytene to sperm ratio (P/Sp) and number of sperm per 100 Sertoli cells (#Sp/100SC). First cycle and cumulative ongoing pregnancy rates were calculated for couples with (partial) maturation arrest and hypospermatogenesis, and compared to rates in NOA couples with complete spermatogenesis.
Main results and the role of chance: The presence of sperm upon cytological evaluation of the TESE specimen is strongly correlated to the observations in the wet prep (Kendall's Tau-b = 0.86). Validation of the cytological diagnoses was performed by comparing the results of cytology to the histological evaluation of spermatogenesis in 40 cases. In 92.5% a perfect match was observed and in the 3 remaining cases cytology corresponded well with the results of the TESE. Couples with complete spermatogenesis have a higher ongoing

pregnancy rate after the first treatment cycle compared to couples with hypospermatogenesis (34 versus 16%; $p = 0.02$) and partial maturation arrest (34 versus 19%; $p = 0.11$).

Limitations, reason for caution: Cause-and-effect cannot be determined in a retrospective study and the predictive value of *cytological* evaluation of spermatogenesis has to be established in a future prospective trial. Due to the embryo transfer strategy in the Netherlands, these results might not apply to patients receiving three or more embryo's per cycle.

Wider implications of the findings: This study shows that the different types of spermatogenic disorder can be easily identified using quantitative *cytological* evaluation of the TESE specimen in the NOA-population. By defining the P/Sp and #Sp/100SC of TESE biopsies, information can be gained regarding the chances of successful TESE-ICSI. This can help couples adjust their expectations and decide about subsequent ICSI cycles or fresh TESE procedures.

Study funding/competing interest(s): Funding by University(ies) – Radboud University Medical Center.

Trial registration number: N/A.

Keywords: infertility, non-obstructive azoospermia, TESE, spermatogenesis, maturation arrest

O-280 Reproduction anomalies in male patients with Bardet Biedl syndrome, an emblematic ciliopathy

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Study question: Bardet-Biedl syndrome (BBS) is a classical ciliopathy with a wide spectrum of clinical features encomprising male microgenitalism. Furthermore, by extrapolation to another ciliopathy, the Kartagener syndrome, BBS patients were supposed to produce immotile sperm, resulting in a severe infertility.

Summary answer: The study focused on the global reproduction function in patients with identified BBS mutations, including genital examination, sperm analysis completed with electronic transmission microscopy, and sexual hormonal status evaluation.

What is known already: BBS is a rare recessive autosomic ciliopathy characterised mainly by many clinical manifestations due to primary cilia dysfunction. There is no publication of BBS fathering. Microgenitalism is one of the usual features. Cryptorchidism is found in more than 90% cases. A frequent low gonadotropic hormone level is mentioned suggesting an impaired hypothalamo-pituitary-gonadal axis. Neither previous sperm description nor motility precision is available. The "ciliopathy" denomination suggests that sperm axoneme of BBS patient is not functional.

Study design, size, duration: The present study is a prospective cohort study provided between 2007 and 2013 and concerns 11 BBS French patients included in PHRC National 2007 – ref HUS No. 4056. BBS mutations were identified in order to determine an eventual link between genotype and phenotype.

Participants/materials, setting, methods: 11 BBS patients beneficed of an andrology examination completed by ultrasonography, sperm analysis and transmission electronic microscopy when the sperm numeration allowed it, and exploration of the hypothalamo-pituitary-gonadal axis including a LH-RH test in addition to the exploration of the gluco-lipidic metabolism and olfactory function.

Main results and the role of chance: BBS patients presented with history of cryptorchidism (5 out of 11), short scrotum (4/8 accepting clinical examination), micropenis (5/8) and, unexpectedly, normal testis size (7/8). Ultrasonography highlighted epididymal cysts or agenesis in cases of extreme hypospermia under 0.5 ml. Kidney cysts or ectasia were observed in these patients but also in BBS patients presenting a normal semen volume. Despite usual severe obesity and impaired olfaction, sexual hormonal levels were normal in all patients except one. Sperm numeration was normal in 8 out of 10 cases of ejaculations. 5–45% of sperm presented a progressive motility. When performed, electronic microscopy did not reveal any homogeneous abnormality.

Limitations, reason for caution: The results should be confirmed in a greater BBS patient cohort, mainly focusing on epididymal cystic abnormalities and on putative sterility of these patients. Functional sperm analysis are expected, especially ART data, despite the difficulty that only a few BBS patients consult for fathering (1 out of 11).

Wider implications of the findings: BBS patients represent a well-defined genetic human model of primary cilium dysfunction with implication in embryology of the male genital tract, especially epididymis, as well as in the androgen fetal production. It results in a disruption of the last androgeno-dependent part of testis descent and a deficit in masculinization of the male genital tubercle, leading to cryptorchidism and short scrotum respectively. Furthermore, male gamete structure seems not impacted by BBS mutations.

Study funding/competing interest(s): Funding by hospital/clinic(s) – This study has been supported by the University Hospital of Strasbourg. No competing interests.

Trial registration number: This study is linked to the National French PHRC 2007 – ref HUS N° 4056 "Physiopathologie ciliaire neurosensorielle et métabolique du syndrome de Bardet-Biedl."

Keywords: Bardet-Biedl syndrome, male genital tract, reproduction, primitive ciliopathy, sperm

O-281 A prognostic model for the outcome of IUI-H in young woman with good ovary response

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Study question: Although a variety of factors have been reported as affecting pregnancy rates (PR) after intrauterine insemination (IUI), there have been conflicting results on prognostic factors. This study aimed to develop a prognostic model for the outcome in patients undergoing their first IUI cycle taking into account male and female infertility factors.

Summary answer: In men with normal sperm samples according WHO 2010, the sperm count, motility and morphology before and after sperm preparation does not influence the IUI outcome. In woman younger than 36 years with good ovarian response, the age and the number of pre-ovulatory follicles recruited are strong predictors of success.

What is known already: Many factors can influence the pregnancy rates after IUI: woman's age, length and type of infertility, sperm quality, number of mature follicles and E2 concentration on the day of hCG. However, results on prognostic factors are still conflicting. Com-pared to IVF-ICSI, IUI is less invasive and associated with lower costs and a low incidence of complications, making it a relatively cost-effective treatment which remains a natural starting point for conveniently selected couples.

Study design, size, duration: Retrospective study of the first cycle of IUI performed in 800 couples between January 2010 and November 2014. Only couples with cervical, anovulation, and unexplained infertility of <4 years length in women younger than 36 years were included. When more than 4 follicles were obtained IUI was converted to an IVF cycle.

Participants/materials, setting, methods: Woman age, body mass index, number of mature follicles and E2 concentration and endometrial thickness were considered as clinical characteristics. Sperm count, motility and morphology (Kruger strict criteria) were asses before and after sperm preparation at the time of IUI. Multivariate logistic regression analysis was performed to evaluated the PR according to clinical and semen characteristics.

Main results and the role of chance: The overall PR was 12%. PR in IVF converted cycles was 42%. No sperm parameters in either the pre or post analysis, nor total motile sperm count or morphology predicted pregnancy. The highest PR (14.7%) was obtained for anovulation and the lowest for unexplained infertility (9.5%). Age was a strong predictor of success ($p = 0.0232$). The number of pre-ovulatory follicles recruited was observed

to be the strongest predictor of IUI success. According to the number of mature follicles the PR was 9.9% for 1, 15.2% for 2 and 20% for 3 follicles ($p = 0.0016$). Acceptable pregnancy rates were obtained when at least 2 mature follicles have developed at the time of insemination. The number of mature follicles and the woman age had the maximum power to predict the PR following IUI.

Limitations, reason for caution: none

Wider implications of the findings: IUI achieves the best results with two or three mature follicles in younger woman. Clinical characteristics of women are unchangeable. However, the number of mature follicles can be increased by increasing the doses of gonadotropins. In order to avoid multiple pregnancy, when more than 4 mature follicles are obtained IUI should be converted to an IVF cycle. Gonadotropin stimulation seems to be an important tool for improving the pregnancy rate in IUI.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital Universitario y Politécnico La Fe, Valencia Spain.

Trial registration number: None.

Keywords: prognostic model, IUI-H outcome, sperm morphology

Posters Presentations

POSTER VIEWING

ANDROLOGY

P-001 Nanotoxicity of mesoporous silica nanoparticles in human sperm following in vitro exposure

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Study question: Given encouraging data in animal models, do nanotoxicity profiles of mesoporous silica nanoparticles (MSNPs) in human sperm permit their use as a versatile multifunctional delivery tool for the *in vitro* transfer of molecular compounds into these specialized cells for investigative and therapeutic purposes.
Summary answer: Short-term *in vitro* exposure of human sperm from fertile donors to MSNPs does not compromise essential sperm function, thereby allowing us to consider this nanomaterial as a potential candidate tool for the internalisation of various molecular constructs into mammalian gametes *in vitro.*
What is known already: Interest is steadily growing in the application of nanoparticle-mediated delivery in reproductive biology, particularly in light of the fact that certain processes involved in gamete development appear to be mediated by natural cell-secreted nanoparticles such as prostatic/epididymal exosomes and granulosa cell microvesicles. MSNPs represent robust and versatile synthetic alternatives to 'natural' nanoparticles that are capable of carrying large amounts of cargo inside animal gametes without acute toxic effects. However, their effect upon human sperm remains untested.
Study design, size, duration: A pilot *in vitro* study, investigating the effects of MSNP exposure upon the motility, viability, and DNA fragmentation status of human sperm, and seeking to evaluate the binding potential of MSNPs with these highly specialised cells.
Participants/materials, setting, methods: Fluorescent MSNPs (~138 nm), synthesised using a surfactant-templated base-catalysed sol-gel reaction, were applied to cryopreserved/thawed sperm from 18 healthy donors in a series of particle/sperm ratios for a maximum of 4 h, followed by computer-assisted sperm analysis (CASA), fluorescence viability staining, sperm chromatin dispersion analysis, and fluorescence microscopy.
Main results and the role of chance: Exposure to MSNPs did not affect the key parameters of sperm motility (average relative% change from controls across all experimental samples after 4 hrs of incubation: +35.8 and +24.0% for total and progressive motility, respectively, $p > 0.05$), the proportion of viable sperm (average relative% change from controls across all experimental samples after 4 hrs of incubation: +21.5%, $p > 0.05$), or DNA fragmentation index (average relative% change from controls across all experimental samples after 4 hrs of incubation: +10.3%, $p > 0.05$). Binding rate between MSNPs and human sperm was dependent upon the time and dose of MSNPs, in contrast to previous findings in animal models, supporting the hypothesis that the effects of MSNPs upon sperm are species-specific.
Limitations, reason for caution: This pilot study focused upon the biocompatibility of MSNPs with human sperm from healthy donors. Therefore, the effects of MSNPs upon infertile/sub-fertile sperm with compromised function, which would represent the main candidate for nanoparticle-mediated supplementation with replacement therapies, remain unknown and should be evaluated in future studies.
Wider implications of the findings: MSNP-mediated delivery represents a promising tool for the transfer of investigative and therapeutic compounds into gametes, which are otherwise remarkably resistant to the uptake of exogenous substances. In applied assisted reproductive technology, biocompatible nanomaterials, including MSNPs, could be used to supplement sub-fertile gametes with fertility-augmenting compounds during *in vitro* culture, such as motility enhancers and/or artificial oocyte activating factors for sperm, or growth factors and other compounds that retain or promote the developmental potential of oocytes and embryos.
Study funding/competing interest(s): Funding by University(ies), Dr Barkalina holds scholarships from Clarendon, Scatcherd European and Cyril & Phillis Long funding schemes. The authors have a patent pending related to the work discussed entitled 'Delivery Method' (PCT/GB13/053394; 20/12/2013).
Trial registration number: NA.
Keywords: nanotechnology, sperm, silica nanoparticles, drug delivery, ART

P-002 Measurement of global methylation and acetylation level before and after swim-up in human ejaculated sperm

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Study question: What changes will take place in global methylation and histone acetylation levels after swim-up preparation of human ejaculated sperm?
Summary answer: The 5-mC levels were significantly lower in the swim-up samples, while no changes were observed in the positivity of six acetylation markers.
What is known already: The swim-up technique could increase the percentage of motile sperms and normal morphology, and DNA fragmentation in sperms was found to be significantly decreased after the swim-up technique. Further, the swim-up technique resulted in a significant decrease in abnormal chromatin structure in sperm.
Study design, size, duration: Prospective experimental study. Normozoospermic semen specimens were obtained from 50 normal healthy volunteers between December 2012 and May 2014.
Participants/materials, setting, methods: The semen samples were processed by the conventional swim-up method. Main outcome measures were sperm motility, strict morphology, DNA fragmentation index (DFI) by TUNEL, abnormal chromatin structure by toluidine blue staining, and immunostaining of 8-hydroxy-2'-deoxyguanosine (8-OHdG), 5-methyl-cytosine (5-mC) and six acetylation markers before and after swim-up.
Main results and the role of chance: Swim-up sperms had significantly elevated motility and normal morphology. DFI and the percentage of abnormal chromatin structure were significantly lower in the swim-up samples; 8-OHdG levels were significantly higher in swim-up samples. The 5-mC levels were significantly lower in the swim-up samples, while no changes were observed in the positivity of six acetylation markers. Elevated normal morphology and decreased DFI and 5-mC levels were observed only in non-smokers; 8-OHdG level was significantly higher in swim-up samples only from smokers. After swim-up, 5-mC levels were positively correlated with DFI ($r = 0.337$, $P < 0.05$).
Limitations, reason for caution: The exact role of 5-mC in human sperms remains unclear. Further large studies are needed.
Wider implications of the findings: This is the first study demonstrating 5-mC reduction and unchanged acetylation patterns in human sperms after swim-up preparation. Our results suggest that measurements of global DNA methylation, DNA fragmentation, and DNA oxidation may be useful in the evaluation of sperm quality.
Study funding/competing interest(s): Funding by national/international organization(s). The Korea Health Care Technology R&D Project, Ministry of Health and Welfare, Korea.
Trial registration number: NA.
Keywords: 5-methyl-cytosine, methylation, acetylation, swim-up, sperm

P-003 Are there differences in semen parameters among ethnic groups and among various professions?

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Study question: Are there differences in semen parameters (volume, count, motility, TMC, WHO and strict morphologies, viscosity, and WBC) among different ethnic groups, and are there any differences when evaluated by profession?
Summary answer: No differences were detected among ethnic groups, or among different professions. However, as a group, men with high BMI had significantly worse parameters compared with men with normal BMI, and there were significant differences in viscosity and volume.
What is known already: Heat affects seminal parameters and there are professions requiring significant time sitting down such as IT and engineering. As far as we know, this is the first study evaluating the effects of various professions, including those that require significant sitting, on semen parameters.
Study design, size, duration: Retrospective study of 901 semen analysis performed between January 2010 and June 2014 at a private suburban Fertility Center.
Participants/materials, setting, methods: All semen analysis (SA) performed at a private suburban Fertility Center by a single technician (GAA). Only the first SA was evaluated. Race and ethnic groups, as well as profession, were collected on all men performing a SA.
Main results and the role of chance: In total, 901 SA including 596 Caucasians (66.1%), 123 South Asians (13.7%), 70 African Americans (AA) (7.8%), 68 East Asians (7.5%), and 44 Hispanics (4.9%) were included. Of these, 276 worked in Business/Finance/Sales (30.6%), 224 worked in Information Technology (24.9%), 77 were engineers (8.5%), 51 worked in government (5.7%), and 37 in Health Care (4.1%). As a group, there was a significant negative effect of BMI on semen parameters ($P < 0.0001$), but there were no differences in age or any semen parameter among different professions.
Limitations, reason for caution: Relatively low numbers of AA, East Asians, and Hispanics. The numbers of minorities are not reflective of the general US distribution.
Wider implications of the findings: As previously reported, higher BMI correlates negatively with semen parameters, however there were no ethnic differences among the main ethnic groups in the US. Different professions are not associated with poorer semen parameters, a reassuring novel finding.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Virginia Center for Reproductive Medicine.
Trial registration number: NA.
Keywords: andrology, ethnic disparities, profession, BMI

P-004 The decline of sperm concentration profile from 1995–2006 in Jos, Nigeria

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Study question: Is there increase in the number of azoospermic patients in Jos, is there increase in the oligoospermic patients What is the drop in sperm concentration over the years in Jos?, Do this sperm concentration abnormalities associated with mining of mineral elements in Jos or infection?
Summary answer: From 1995–2006, there was increase of sperm concentration abnormalities by 41% and decline in mean sperm concentration by 33.14%[21.95 M/ml] and these could be associated with infection, endocrinopathy and environmental factors.
What is known already: Infertility is a global problem to contend with and male factor infertility is on the increase. Male infertility varies from 20 to 50% in various parts of Nigeria. This study is to determine the prevalence of sperm concentration abnormalities and the degree of mean sperm concentration decline in Jos, Nigeria from 1995 to 2006.
Study design, size, duration: Semen samples were collected by masturbation after abstaining for 3–7 days from 2055 male patient of age between 20 and 45 years and who were also attending infertility clinic in the centre for reproductive health research, Jos, Nigeria from 1995 to 2006. 59% represent normal sperm concentration (20 Millions/ml-W.H.O) and 41% represent abnormal sperm concentration.
Participants/materials, setting, methods: Semen samples were collected by masturbation after abstaining for 3–7 days from 2055 male patient of age between 20 and 45 years and who were also attending infertility clinic in the centre for reproductive health research, Jos, Nigeria. The semen samples were analysed for sperm concentration, using Neubauer counting chamber in line with world health Organization procedures (1999).
Main results and the role of chance: Result We had 13% azoospermia,28% oligoospermia,59% normal sperm concentration, mean sperm concentration in 1996 and 2006 are,66.23 M/ml,44.28 M/ml (p-values of <0.00) The mean sperm concentration decline by 33.14% [21.95 M/ml]
Limitations, reason for caution: We do not have actual number of children by secondary infertile couples.
Wider implications of the findings: The drop in sperm concentration could be as result of high level of mining of minerals and a lot of people are exposed to this process in Jos, Nigeria. This mining process has disrupts the process of spermatogeneis in human over a period of time. Thus reduction of sperm concentration in male patients, in Jos, Nigeria between 1995 to 2006
Study funding/competing interest(s): Funding by hospital/clinic(s) – Centre for Reproductive Health Research.
Trial registration number: NA.
Keywords: male infertility, mean sperm concentration

P-005 Dietary habits and reproductive health – results of a sociological case-control study

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Study question: Male infertility is a multifactorial disease with a number of potential contributing causes. How nutrition could have a beneficial impact on sperm count, motility, and ultimately, fertility?
Summary answer: Infertile Caucasian-European individuals have worse recreational and nutritional habits compared to fertile individuals. Fertile individuals reported more regular consumption of vegetables, legumes, fruits and eggs.
What is known already: Many nutritional therapies have been shown to improve sperm counts and sperm motility including carnitine, selenium, zinc and vitamin B12. Arginine – essential for the synthesis of putrescine, spermidine and spermine – gives a significant improvement in sperm count and motility after taking 4 g/day for three months**.** Numerous antioxidants are used in treating male infertility such as vitamin E, glutathione, coenzyme Q10 and vitamin C whose deficit correlates with damage to the sperm DNA.
Study design, size, duration: A cohort of 1134 consecutive individuals [n = 324 (28.6%) infertile; n = 810 (71.4%) fertile] anonymously completed a questionnaire related to nutritional and recreational habits, for above one year. Infertility was defined according to WHO definition.
Participants/materials, setting, methods: Descriptive statistics was applied to describe the overall cohort. Complete data collection was available for 585 (51.6%) women [mean (SD) age: 34.27 (6.44) years; range: 20–66)] and 549 (48.4%) men [mean (SD) age: 37.21 (9.97) years; range: 18–73)].
Main results and the role of chance: Both male [33.4 vs. 38.3; p = 0.001] and female [33.7 vs 34.7; p = 0.04] fertile patients were younger than infertile individuals. Infertile individuals were more frequently smokers (p = 0.02); a higher rate of infertile individuals were current alcohol drinkers ($p < 0.001$), and consumed more alcohol/week than fertile drinkers (p = 0.04). Fewer fertile people were coffee drinkers (p = 0.003). A higher proportion of infertile individuals reported a past use of illicit drugs (p = 0.02). Fertile individuals more frequently reported regular consumption of vegetables (p = 0.04), legumes (p = 0.001), fruits ($p < 0.001$), and eggs (p = 0.02), but less frequently consumed sweets (p = 0.04). No significant dietary differences were observed between fertile and infertile individuals in terms of cereals, red meat, poultry, and fish. A greater rate of fertile individuals thought that diet can significantly impact fertility (p = 0.02).

Limitations, reason for caution: We could substitute the clinical questionnaire with a diet diary in order to allow patients to be more accurate. In addiction some biological markers both in females and males could be used to objectify the improvement of fertility due to the diet. Also the time of follow-up might be increased in order to perceive how nutrition correlates to the number of pregnancies.
Wider implications of the findings: Literature has proposed as one of the possible etiologies of idiopathic male infertility the disruption of the delicate balance between oxidant and antioxidant elements. Probably food rich in antioxidants such as vegetables and fruit could protect polyunsaturated fatty acids and phospholipids from oxidative damage.

In addiction several studies have found that oligoelements such as zinc, available in legumes and cereals, and selenium, contained in eggs, correlate with proper testosterone levels and with stability of mid-piece.
Study funding/competing interest(s): Ospedale San Raffaele.
Trial registration number: NA.
Keywords: nutrition, infertility, diet, oligoelements, antioxidants

P-006 Who is the most suitable candidate for clomiphene treatment? Findings of an observational survey in a cohort of primary infertile men

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Study question: We assessed seminal outcomes after clomiphene treatment; and, the most suitable candidate for clomiphen in a cohort of white-European men presenting for primary couple's infertility.
Summary answer: Clomiphene treatment was associated with an improvement of sperm concentration in a cohort of white-European men presenting for primary couples infertility. Patients who most benefited from this therapy are young, with a normal BMI and a short period of infertility.
What is known already: Clomiphene citrate is a well-tolerated anti-estrogen thought to increase sperm parameters in males attempting to conceive. Clomiphene has been widely used in idiopathic oligoasthenoteratozoospermia without proven evidence for its benefit. Further research is needed to clarify when clomiphene citrate is indicated in the treatment of male infertility.
Study design, size, duration: Observational study. Complete data from 307 consecutive men treated with clomiphene were analyzed.
Participants/materials, setting, methods: Comorbidities were scored with the Charlson Comorbidity Index (CCI). Semen analysis values were assessed based on the 2010 WHO reference criteria. Descriptive statistics tested the association between seminal outcome after therapy and clinical characteristics; logistic regression models tested the association between clinical parameters and clomiphene-related seminal improvement.
Main results and the role of chance: 194 (63.2%), 190 (61.9%) and 146 (47.6%) men presented with oligospermia, astenozoospermia and teratozoospermia. After 3 months of clomiphene, sperm concentration ($p < 0.001$) and total sperm progressive motility ($p < 0.001$) improved. Improvement of sperm concentration was more frequently reported in younger individuals ($p < 0.001$), with a lower BMI score ($p = 0.002$), and with a higher left testicular volume ($p = 0.03$). Similar findings were also found for sperm motility (all $p < 0.05$). Young patients ($p < 0.001$), with normal BMI ($p = 0.02$), and with a greater left testicular volume ($p = 0.003$) were more likely to fall within the normal range of sperm concentration and motility (all $p < 0.05$) after treatment. At MVA, age (OR 0.86; $p = 0.005$), BMI (OR 0.8; $p = 0.023$) and length of infertility (OR 0.95; $p = 0.46$) achieved independent predictor status for concentration improvement after treatment.
Limitations, reason for caution: The sample size is too small to draw general conclusions. We did not evaluate hormonal changes after clomiphene treatment.
Wider implications of the findings: This study confirmed a statistically significant increase in sperm concentrations after clomiphene and pointed out the importance of patient age and duration of infertility as predictors of seminal parameters improvement after treatment.
Study funding/competing interest(s): None.
Trial registration number: NA.
Keywords: male infertility, clomid, treatment

P-007 Testis development in the absence of SRY: chromosomal rearrangements at SOX9 and SOX3

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Study question: Is testis development possible in the absence of SRY.
Summary answer: SOX3 duplications may substitute SRY in some XX subjects.
What is known already: 46,XX disorders are congenital conditions in which, in the presence of a female karyotype, the development of gonadal and anatomical sex is atypical, ranging from various degrees of ambiguous genitalia to phenotypic males with azoospermia. These conditions are poorly characterized, at least in subjects whose DNA does not contain SRY, the gene triggering testis differentiation in mammals.
Study design, size, duration: Case report.
Participants/materials, setting, methods: We analyzed by conventional and molecular cytogenetics an SRY-negative unrelated 46,XX subject.
Main results and the role of chance: The patient, a 30 years old normal adult male, was investigated because of infertility. Physical examination showed normal male secondary sexual characteristics and bilateral gynecomastia. Laboratory investigations showed azoospermia, low serum testosterone and increased FSH and LH. The subject carried partially overlapping 17q24.3 duplications about 500 kb upstream of SOX9, inherited from a normal father. Breakpoints cloning showed that duplications were in tandem whereas the 17q in the reciprocal translocation was broken at about 800 kb upstream of SOX9.
Limitations, reason for caution: exceptional case, additional evidence are necessary.
Wider implications of the findings: in the absence of SRY, altered expression of genes crucial to gonadal development, such as SOX9 and SOX3, may invert the expected embryonic plan.
Study funding/competing interest(s): Military Hospital of Tunis.
Trial registration number: 122/2014 46, XX.
Keywords: disorders of sex development, infertility, non-coding 38 regulatory elements

P-008 Low birth weight is associated with a higher rate of health-significant comorbidities and worse seminal parameters – results of a cross-sectional study in primary infertile patients

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Study question: We assessed prevalence of, and clinical and seminal impact of different categories of weight at birth in a cohort of white-European men presenting for primary couple's infertility.
Summary answer: Low birth weight (LBW) was associated with a significant higher rate of adult diseases. Clinical, endocrine and semen parameters were significantly worse in the LBW group.
What is known already: Several theories pointed out the association between LBW and the subsequent risk of developing cardio vascular diseases, high blood pressure and type II diabetes mellitus in adulthood. The raise of the developmental origins of adult disease has positioned low birth weight as a significant health issue.
Study design, size, duration: Cross sectional study. Complete data from 757 consecutive infertile men were analysed.
Participants/materials, setting, methods: Birth weight ≤ 2500, 2500–4200, and ≥ 4200 g were considered as LBW, normal (NBW) and high (HBW). Comorbidities were scored with the Charlson Comorbidity Index (CCI). Semen analyses were assessed based on 2010 WHO criteria. Descriptive statistics detailed the association between semen parameters and clinical characteristics and the birth weight categories.
Main results and the role of chance: LBW, NBW and HBW were found in 52 (6.9%), 605 (79.9%) and 100 (13.2%) men, respectively. LBW reported a higher prevalence of comorbidities ($p < 0.001$). Likewise, hypercholesterolemia

($p = 0.04$) and hypertriglyceridemia ($p = 0.01$) were more frequently reported in LBW. LBW had a lower mean testicular volume ($p = 0.02$). LBW men showed a higher rate of both asthenozoospermia ($p = 0.02$) and teratozoospermia ($p = 0.02$). Overall, ejaculated volume ($p = 0.006$), sperm motility ($p = 0.02$) and normal morphology ($p = 0.04$) were significantly reduced in the LBW group. Likewise, LBW patients presented higher FSH levels ($p = 0.04$) but lower circulating testosterone levels ($p = 0.03$) as compared with the other groups. At MVA, LBW achieved independent predictor status for a higher CCI value (OR 3.7; $p < 0.001$), lower sperm motility (OR 2.7; $p < 0.04$), and lower normal sperm morphology (OR 2.3; $p < 0.04$).
Limitations, reason for caution: The sample size is too small to draw general conclusions. We did not evaluate the impact of birth weight on seminal parameters in non-infertile men.
Wider implications of the findings: This is the first study focused on the impact of birth weight on clinical and seminal parameters in a cohort of infertile men. This study is in line with previous data reporting that small for gestational age subjects have pituitary-gonadal axis function that tends toward hypogonadism.
Study funding/competing interest(s): Ospedale San Raffaele.
Trial registration number: NA.
Keywords: male infertility, birth weight

P-009 Sperm retrieval at the time of tumor resection in azoospermic men with congenital adrenal hyperplasia and bilateral testicular adrenal rest tumors: a case series

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Study question: Is it feasible to offer sperm retrieval at the same surgical setting as tumor resection in azoospermic men with congenital adrenal hyperplasia (CAH) and bilateral testicular adrenal rest tumors (TARTs)?
Summary answer: Yes. After appropriate hormonal treatment, sperm retrieval should be performed concomitantly with tumor resections.
What is known already: CAH men have impaired cortisol and aldosterone production resulting in increased adrenocorticotropic hormone (ACTH) production leading to adrenal hyperplasia and adrenal androgen overproduction. Some will develop benign TARTs, typically bilateral and originating in the rete testis. TARTs commonly result in obstructive azoospermia and destroy normal testicular tissue. There are reports of testicular aspiration for use with in-vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) with the tumors in situ and reports of men remaining azoospermic after tumor resections.
Study design, size, duration: Case series, between October 2014 and December 2014, of two men with CAH, bilateral TARTs, and azoospermia.
Participants/materials, setting, methods: Bilateral TART resections and sperm retrievals were performed concomitantly in both men.
Main results and the role of chance: Both men had successful sperm retrievals, which were cryopreserved for future use with IVF/ICSI, at the same settings as bilateral TART resections. This eliminates the need for multiple procedures for these patients. The role of chance is possible, as this is a small sample size in this relatively uncommon scenario; however, successful sperm retrievals in these two patients is encouraging.
Limitations, reason for caution: This is a small sample size with this relatively uncommon scenario and needs to be validated with higher powered studies.
Wider implications of the findings: To our knowledge, this is the first report of men with CAH and bilateral TARTs undergoing successful sperm retrievals concomitantly with bilateral TART resections. As it has previously been shown that men remain azoospermic following TART resections, sperm retrieval and tumor resection in one surgical setting would seem to be the optimal approach rather than subjecting such patients to two separate procedures. Wider implications are that men in this scenario should undergo these procedures concomitantly.
Study funding/competing interest(s): No funding was required.
Trial registration number: NA.
Keywords: congenital adrenal hyperplasia, sperm retrieval, TESE, testicular sperm extraction, testicular adrenal rest tumor

P-010 Diabetes mellitus, spermatogenesis progression and sperm function: does a spoonful of sugar compromise male fertility?

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Study question: Is hyperglycemia, the major effector of the diabetes mellitus (DM) pathology, capable of promoting male reproductive dysfunction alone?
Summary answer: While high glucose levels impair some aspects of spermatogenesis, possibly through an effect on Sertoli cell functionality, mature sperm function does not seem to be affected at least *in vitro*. Nevertheless, the lack of available glucose in sperm vicinity severely suppresses their function, potentially jeopardizing fertilization.
What is known already: DM is associated with the emergence of several clinical complications, including reproductive dysfunction (RD). Alterations in testicular histology and decreased standard sperm parameters have been described in diabetic men. Given the multifactorial nature of DM-induced physiological changes, it remains unclear which are the mechanisms accountable for the reported RD.
Study design, size, duration: Normozoospermic sperm samples (minimum $n = 4$) were exposed to different D-glucose concentrations for 48 h (37°C, 5%CO_2) to mimic physiological and hyperglycemic conditions. Additionally, spermatogenesis progression ($n = 3$) was assessed using testis fragments of C57BL/6 mice pups following 3 weeks in culture under D-glucose concentrations (35°C, 5%CO_2). Osmotic controls were performed with L-glucose.
Participants/materials, setting, methods: Healthy individuals were recruited at the Human Reproduction Service from University Hospitals of Coimbra and animals were obtained from CNC animal facility. Motility, viability (eosin Y), mitochondrial membrane potential (MMP; JC-1), superoxide production (mitoSOX), capacitation status (detection of phosphotyrosines) and acrosome reaction (AR; PSA-FITC) were assessed daily. Organ culture was performed according to the interphase gas-liquid methodology. Histomorphometric analysis was determined using Image J software.
Main results and the role of chance: After evaluation of several sperm parameters, results highlight the absence of direct effects promoted by 25 mM and 50 mM D-glucose when compared to control (5 mM D-glucose; $p > 0.05$) following 24 and 48 h of treatment. Interestingly, data obtained with the non-metabolizable L-glucose showed a severe impairment of sperm function when compared to respective D-glucose concentrations. Indeed, an increase in reactive oxygen species production ($p < 0.05$) paralleled by a suppression in sperm motility ($p < 0.05$) and capacitation ($p < 0.05$) were already detected after 24 h of treatment while decreased MMP ($p < 0.05$), acrosomal integrity ($p < 0.01$) and viability ($p < 0.05$) were found later on. Organ culture experiments, showed that high glucose levels increase Sertoli cell number ($p < 0.05$) while decreasing tubular luminal area ($p < 0.01$), therefore suggesting an impairment of this somatic cell vital for spermatogenic control.
Limitations, reason for caution: This is an *in vitro* study, and caution must be taken when extrapolating to an *in vivo* situation.
Wider implications of the findings: This study indicates that high glucose levels *per se* influence male reproductive function but only at the spermatogenesis level, stressing the importance of other factors involved in the DM. Furthermore, it highlights the importance of available glucose for spermatozoa functionality, in accordance with previous reports. Additionally, our results lead us to suggest that the adverse *in vivo* effects of the disease at the sperm level might be explained by a cellular hypometabolism in diabetic conditions.
Study funding/competing interest(s): This study was supported by the Portuguese National Science Foundation (FCT) through the project EXPL/BEX-BCM/0224/2012 and CNC funding (FCT, PEst-C/SAU/LA0001/2013-2014). SA is also a recipient of a FCT fellowship (SFRH/BPD/63190/2009).
Trial registration number: NA.
Keywords: diabetes mellitus, hyperglycemia, male reproductive function, human sperm, spermatogenesis progression

P-011 Evaluation of the effect of cadmium and lead concentration in seminal plasma on sperm DNA fragmentation

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Study question: The aim of this research is to specify the influence of lead and cadmium on the semen DNA fragmentation.
Summary answer: It has been shown that the fragmentation of sperm DNA intensifies with the increasing concentration of Lead and Cadmium in seminal plasma.
What is known already: Lead (Pb) and cadmium (Cd) are two of the well-known reproductive toxicants which humans are exposed to occupationally and environmentally, and can lead to negative effects on the sperm chromatin DNA integrity. 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: In order to determine the percentage of fragmented DNAin sperm, the SCD test was used (sperm chromatin dispersion), according to the instructions provided by the producers [Dyn-Halosperm® kit, Halotech DNA SL, Madrid, Spain]. The Pb and Cd measurements were performed by the electrothermal-atomic absorption spectrometry method.
Participants/materials, setting, methods: The presented study concerned 125-infertile men aged 25–35, and was conducted in 2013 and 2014 in the 'Ovum-Reproduction and Andrology' Non-Public Health Care Unit in Lublin, (Poland).The study has been approved by the ethics committee of the Institute of Rural Health in Lublin.
Main results and the role of chance: We have established that a statistically significant positive correlation occurs between the intensification of sperm DNA fragmentation and the concentration of Pb (r = 0,187, p = 0,032). Also, a positive correlation has been observed between damaged chromatin and the concentration of Cd (r = 0,185, p = 0,033).
Limitations, reason for caution: None.
Wider implications of the findings: The pollution of the environment with heavy metals, like Pb and Cd, may lead to decreased fertility of men by damaging the integrity of sperm chromatin. This phenomenon may affect the epigenetic changes of future generations.
Study funding/competing interest(s): International Scientific Association for the Support and Development of Medical Technologies.
Trial registration number: Local institutional registration.
Keywords: lead, cadmium, sperm DNA fragmentation

P-012 Effect of seminal transforming growth factorß1 (TGFß1) and glutathione on apoptosis in spermatozoa from Tunisian infertile men

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Study question: Human seminal plasma is a natural reservoir of biochemical constituents that cause apoptosis of sperm. The presence of TGFß1 and glutathione in the seminal plasma plays an important role in the death of the sperm by apoptosis. Our present study is the first approach carried out in Tunisia.
Summary answer: glutathione and seminal TGFß1 are important risk factors that can lead to a succession of morphological and functional alterations in sperm cells resulting in the necrozoospermia. Their rise in seminal plasma can lead to poor results of IVF.
What is known already: TGFb1 induces the production of free radicals or reactive oxygen species (ROS) which is derived from non-functional spermatozoa and leukocytes. The TGFß1 causes an increase rate of the oxidized glutathione (GSSG) with respect to the reduced intracellular glutathione (GSHr). Thus an increase in the production of ROS and a decrease of reduced glutathione indicates that the rate of TGFß1induit oxidative stress in the walls sperm. These ROS are largely due to DNA fragmentation.
Study design, size, duration: The study involved 120 men aged 28–56 years and characterized by idiopathic infertility. The men were divided into three groups: normozoospermics that were considered as controls (n = 40), asthénotératozoospermics (OAT; n = 45) and necrozoospermics (Necro; n = 35).
Participants/materials, setting, methods: For each patient, we evaluated sperm parameters according to WHO standards, TGFß1 rate by ELISA and the oxidized and reduced glutathione levels in seminal plasma method. The percentage of sperm DNA fragmentation was performed by the TUNEL technique.
Main results and the role of chance: With the significant increase in TGFß1 and DFI rates in groups and asthenoteratozoospermics necrozoospermics, there were positive correlations between these substances and sperm quality [TGFß1 / count; r = +0.42, $p < 0.05$), TGFß1 / Mobility; r = 0.494, $p < 0.001$) and TGFb1 / Morphology; r = -0.38, $p < 0.05$). In addition, high levels of reduced glutathione (GHSR) and oxidized (GSSG) seminal were observed in the control group, but there were conflicting associations that reflect the effects of antioxidants on sperm parameters. However, we found a significant increase in DFI in groups with abnormal semen analysis in particular necrozoospermic group. We showed negative associations between DNA fragmentation and the seminal parameters.
Limitations, reason for caution: These results suggest that glutathione and seminal TGFß1 are important risk factors that can lead to a succession of morphological and functional alterations in sperm cells resulting in the nécrozoospermie. Their rise in seminal plasma can lead to poor results of IVF.
Wider implications of the findings: the originality of this study lies in the fact that the TGFb1 and the seminal glutathione significantly increase the Necrospermia way through the voice of ROS. These ROS promote DNA fragmentation and triggeted the process of apoptosis.
Study funding/competing interest(s): Laboratory cytogenetic, molecular genetics and biology of human reproduction; University Hospital Farhat Hached Sousse Tunisia.
Trial registration number: Glutathione and seminal TGFß1 are important risk factors that can lead to a succession of morphological and functional alterations in sperm cells resulting in the necrozoospermia. Their rise in seminal plasma can lead to poor results of IVF.
Keywords: sperm, DNA, apoptosis, TGFb1, necrospermia

P-013 Sperm DNA fragmentation index and female age are two critical parameters in predicting IUI outcome

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Study question: To demonstrate whether DNA fragmentation index (DFI) as well as the other parameters including age can be used as main predictors of intrauterine insemination (IUI) outcomes.
Summary answer: IUI prediction will be possible through measuring sperm DNA damage and female age. We offered three equations with high validity to predict IUI outcomes.
What is known already: There is a need for new markers as well as sperm parameters that might predict pregnancy outcome and the risk of adverse reproductive events. Markers of sperm DNA integrity may help differentiate fertile from infertile men, but the clinical value of sperm DNA integrity testing remains to be defined.
Study design, size, duration: A prospective and cohort study was conducted at Royan Institute from May 2010 to April 2012. 576 Couples were diagnosed with idiopathic infertility referred for IUI.
Participants/materials, setting, methods: The choice of IUI method was based upon infertility diagnosis. Couples were diagnosed with idiopathic infertility by specialist in our center. For each couple, only one treatment cycle during the study period was included in the analysis. In order to obtain sufficient number of spermatozoa for sperm chromatin structure assay (SCSA) analysis, only those men having sperm concentration of at least 1×10^6 sperm/ml in neat semen were included. Moreover, men with ≤ 50 years old; women with ≤ 40 year of age, body mass index (BMI) < 30 kg/m^2 and female baseline FSH <12 IU/l were considered eligible for the study. Semen samples were collected at the day of IUI.
Main results and the role of chance: We compared mean of quantitative variables between pregnancy outcomes, assessed bivariate relationship between

continuous variables. Cut-off point for DFI was determined and ROC curve was used to measuring accuracy of predictive models of pregnancy outcomes. There were statistically significant differences regarding DFI in groups with and without biochemical pregnancy (21.7 ± 0.56 versus 34.86 ± 0.42, mean ± SE, $P < 0.001$). Odds ratio for DFI (95% confidence intervals) were 71 (66–76), 69 (64–74) and 56 (49–65) for biochemical and clinical pregnancy and delivery, respectively. Measured cut-off point for DFI was equal to 24.5%. In order to predicting IUI outcomes, it was created three equations on the basis of DFI and female age.

Limitations, reason for caution: There were not any limitations.

Wider implications of the findings: There are two merits in present study as compared with similar studies. First thing is that our study is the largest research about predictive value of DFI in IUI outcomes which has been ever done. Secondly, for the first time, we offered three formulas to predict IUI outcomes so that with being available the amounts of DFI and female age are resulted in partly accurate prediction. Besides, we have studied effects of sperm processing on our results that will be submitted later.

Study funding/competing interest(s): Funding by hospital/clinic(s) –Royan Institute.

Trial registration number: This study was not RCT.

Keywords: IUI, female age, pregnancy outcomes, sperm DNA damage

P-014 Predictive value of serum inhibin-B levels as an indicator of the presence of testicular spermatozoa in non-obstructive azoospermia

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Study question: What is the predictive value of serum Inhibin-B levels as an indicator of the presence of testicular spermatozoa in non- obstructive azoospermia (NOA), compared with other markers as AMH, FSH and testicular volume.

Summary answer: Inhibin-B as a potential marker of persistent spermatogenesis seems to reach a satisfactory clinical utility, and in association with other parameters such as AMH, FSH and testicular volume could allow to improve predictive value of male assessment in NOA.

What is known already: Inhibin-B is glycoproteins of the transforming growth factor-β superfamily, is produced almost exclusively by the Sertoli cells and has been proposed as direct markers of their function and indirect markers of spermatogenesis.

Study design, size, duration: Study design: prospective clinical study.
Size: 228 patients with idiopathic NOA.
Duration: from October 2009 to October 2013.

Participants/materials, setting, methods: Participants: 228 Middle Eastern males with idiopathic NOA.
Setting: Faculty of Medicine, Damascus University and Orient Hospital.
Materials and methods: Inhibin-B in Serum evaluated before sperm retrieval, in addition to AMH, FSH and testosterone. Testicular volume measured by sonography.
Interventions: Fine Needle Aspiration (FNA) and Testicular Sperm Extraction (TESE).

Main results and the role of chance: Spermatozoa were retrieved in 87 patients (38.16%), which classified as group I and were absent in 141 patients (61.84%) in group II. Mean serum Inhibin-B ± SD was significantly higher in group I than group II, (71.77) versus (27.49) respectively ($P < 0.001$), The odds ratio of Inhibin-B was 1.0409 (95% CI: 1.0286 to 1.0533). Serum Inhibin-B demonstrated that the best inhibin B value threshold for discriminating between successful and failed sperm retrieval was 35 pg/ml, with a sensitivity of 75.86 (95% Confidence interval [CI] : 65.5–84.4) and specificity of 80.85 (CI: 73.4–87.0) for the prediction of the presence of testicular spermatozoa as determined by the receiver operating characteristic (ROC) curve analysis and area under curve (AUC).

Limitations, reason for caution: The diagnosis of idiopathic non-obstructive azoospermia was based on clinical finding and several parameters as FSH, testicular volume and histo-pathology of a previous testicular biopsy (if available) although no standard method was used. And our study might reflect selection bias due to our referral pattern.

Wider implications of the findings: In the literature, attempts have been made to compare serum FSH and Inhibin-B levels as predictors of the recovery of testicular spermatozoa: Inhibin-B proved to be superior (Liu YC et al., 2006; Nagata Y et al., 2005) or equal (Tunc L et al., 2006; Halder A et al., 2005) to FSH, while in a other studies (Tsujimura A et al., 2004; Bohring C et al., 2002) combination proved to be better than any hormone alone.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Study Funding: from the scientific research budget of Faculty of Medicine and Orient Hospital.

Trial registration number: NA.

Keywords: inhibin B, sperm retrieval, spermatogenesis, azoospermia

P-015 Interferometric phase microscopy for label-free morphological evaluation of sperm cells

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Study question: Is interferometric phase microscopy (IPM) superior to bright field microscopy (BFM) in evaluating sperm cell morphology for ICSI?

Summary answer: IPM is superior to label free BFM and equivalent to label based BFM in identification of sperm cell morphological abnormalities according to the WHO criteria.

What is known already: Approximately 70 million people a year worldwide need assisted reproductive techniques to bare children. To improve fertilization rates, the most suitable sperm cell has to be chosen. Sperm morphology, evaluated qualitatively by label free bright field microscopy, is a good predictor for fertilization success. Interferometric phase microscopy is able to quantitatively image a sample without staining and with great accuracy and In recent years, several initial researches have demonstrated utilization of IPM for sperm morphological characterization.

Study design, size, duration: We evaluated 350 sperm cells by IPM and label free BFM according to the WHO criteria. Furthermore, presence and relative size of head vacuoles was also compared. Label based BFM was considered the most accurate test for sperm cell abnormalities and IPM and label free BFM were compared to it.

Participants/materials, setting, methods: Continuous variables were compared by the student's *t*-test and Wilcoxon sign-rank test. Categorical variables were compared with χ^2-test of independence and Mcnemar test. Sensitivity and specificity of IPM and label-free BFM were calculated compared to label-based BFM.

Main results and the role of chance: No statistically significant difference was identified in the continuous variables evaluated with IPM and label-based BFM. In contrast, head width, acrosome area and midpiece width were statistically different between IPM and label-free BFM.

Categorical variables evaluated with IPM were not found to be independent of the variables evaluated by label-based BFM. In contrast, the variables evaluated with label-free BFM were statistically independent of those found by IPM.

All the vacuoles that were not identified with IPM were single and smaller than 5% of the sperm cell heads. However, 50% of the vacuoles that were missed by label-free BFM were identified by IPM.

Sensitivity and specificity of IPM was higher than those of label free BFM for all WHO criteria.

Limitations, reason for caution: The number of sperm cells needed to be evaluated was chosen to identify a 10% difference in any of the continuous variables at $p < 0.05$ and power of 80%. our main limitation is that we examined fixed cells and not live motile cells.

Wider implications of the findings: Portable IPM is a novel and affordable method for observing and analyzing sperm cell characteristics. This method is found equivalent to label-based BFM and superior to the commonly used label-free BFM in assessing static parameters of sperm samples and thus may be incorporated in the ICSI procedure.

Study funding/competing interest(s): Funding by University(ies) – Department of Biomedical Engineering, Tel Aviv University.

Trial registration number: NA.

Keywords: interferometry, ICSI, sperm, interferometric phase microscopy

P-016 An "in vitro" prepubertal porcine mini-testis: a new model to study the effects of gonadotropins on Sertoli and Leydig cells interactions

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Study question: At the present there is no reliable 'in vitro' model to achieve better understanding about gonadotropins effects on Sertoli and Leydig cells, to be possibly used for treatment of male infertility.
Summary answer: The aim of our study was to:
- set-up an 'in vitro' pre-pubertal porcine mini-testis as a useful tool.
- study the effect of gonadotropins (FSH and LH) on the mini-testis model.
What is known already: Gonadotropins (FSH, LH), whose secretion is induced by the hypothalamic hormone GnRH, are the principal regulators of the testis functions. Pre-pubertal age is characterized by physiologic hypogonadotropic hypogonadism state with the exception of AMH that is upregulated by FSH and down-regulated by androgen AMH was then proposed for potential marker of Sertoli cells function in the pre-pubertal age. Moreover, in this age range, most of damage, subsequently leading to male infertility will start.
Study design, size, duration: Set-up of an *'in vitro'* mini testis; evaluation of gonadotropins effects on Sertoli and Leydig cells by: (a) ELISA assay for AMH, inhibin B and testosterone secretion in the medium; (b) Real Time PCR analysis of FSH-r, inhibin B, AMH and MAPkinases (Erk1/2, AKT).
Participants/materials, setting, methods: Sertoli and Leydig cells, obtained by 15–20 days old neonatal pigs, were isolated and evaluated in terms of purity by AMH (unique pre-pubertal SCs marker), INSL3 (Leydig cells marker), ASMI (peritubular cells marker) and PGP9.5 (gonocytes and spermatogonial cells marker) immunocytochemistry. Finally, purified Sertoli and Leydig cells were co-cultured to obtain the 'mini-testis'.
Main results and the role of chance: We have obtained highly purified pre-pubertal Sertoli and Leydig cells culture.

In addition, our 'in vitro' mini-testis, demonstrated to be a functional model for studying the effects of gonadotropins on the cells.

In fact, AMH secretion was down-regulated in our *'in vitro'* mini testis by FSH treatment, while inhibin B increased after FSH and FSH/LH stimulation. Testosterone production was increased only by LH treatment.

Preliminarily, we have shown that ERK1/ERK2 expression was upregulated by FSH and FSH/LH stimulation while FSHr expression was down-regulated by FSH and increased by FSH/LH treatment; AKT was upregulated in all conditions.
Limitations, reason for caution: No.
Wider implications of the findings: These preliminary data could help to better understand the interactions between gonadotropins, Sertoli and Leydig cells possibly to be possibly used for treatment of male infertility.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – this work was supported by a grant from Mr. Gary Harlem (Altucell Inc. 3 Astor Court, Dix Hills, New York, NY). Gonadotropins were supplied by Merck-Serono (London, UK).
Trial registration number: NA.
Keywords: gonadotropins, Sertoli cells

P-017 An investigation of seminal plasma concentration of CRISP-1 as a marker for determining the type of azoospermia using a novel ELISA

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Study question: Evaluation of the ELISA for the quantitative detection of human Cysteine-Rich Secretory protein 1 (CRISP-1) in seminal plasma as a non-invasive method for the distinction between obstructive and nonobstructive azoospermia.
Summary answer: The quantitative measurement of seminal CRISP-1 using BioVendor ELISA could potentially differentiate the cause of azoospermia.
What is known already: Cysteine-Rich Secretory Protein 1 is a member of the cysteine-rich secretory proteins (CRISPs) family. CRISPs show a strong expression bias to the mammalian male reproductive tract and have been implicated in many aspects of male germ cell biology spanning haploid germ cell development, epididymal maturation, capacitation, motility and the actual processes of fertilization. Human CRISP-1 is produced throughout the epididymis and plays important role in sperm-egg fusion process and inhibition of sperm capacitation.
Study design, size, duration: We developed a novel enzyme immunoassay for human CRISP-1 and measured CRISP-1 in seminal plasma samples from 24 normospermic donors, 19 asthenospermic donors, 23 oligospermic donors, 13 azoospermic donors and 2 donors after vasectomy.
Participants/materials, setting, methods: The BioVendor Human Cysteine-Rich Secretory Protein 1 ELISA was used for the quantitative measurement of CRISP-1 in seminal plasma. This sandwich ELISA is based on two specific polyclonal anti-human CRISP-1 antibodies. The standard used in this kit is the recombinant human CRISP-1 protein produced in *E. coli*.
Main results and the role of chance: Significant differences in mean concentration of CRISP-1 in seminal plasma samples of men with different diagnosis were observed. CRISP-1 was present at very low level in samples from donors after vasectomy. All seminal plasma samples from normospermic donors were CRISP-1 positive, whereas CRISP-1 was absent in two samples of azoospermic patients indicating the obstructive form of azoospermia.
Limitations, reason for caution: Only limited number of seminal plasma samples was used to produce these results. Therefore, a larger number of azoospermic semen samples is needed to make these results more reliable.
Wider implications of the findings: Previous findings indicate vasectomy has an effect on CRISP-1 expression. Moreover, the results have shown that the seminal plasma samples from normospermic and nonobstructive azoospermic donors are CRISP-1 positive, whereas CRISP-1 is absent or present at low levels in samples from patients with obstructive azoospermia. Therefore, the BioVendor Human CRISP-1 ELISA for the quantitative measurement of human CRISP-1 in seminal plasma could be used as a promising non-invasive method for the distinction between obstructive and nonobstructive azoospermia.
Study funding/competing interest(s): Funding by hospital/clinic(s) – funding by national/international organization(s) – Ministry of Health, Czech Republic – DRO (FNBr).
Trial registration number: FNBr, 65269705.
Keywords: seminal plasma, azoospermia, ELISA, CRISP-1

P-018 The relationship between body mass index and scrotal temperature

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Study question: How closely does body mass index (BMI) correlate with scrotal temperature among male partners of subfertile couples?
Summary answer: BMI has a moderate and positive association with scrotal temperature.
What is known already: Scrotal temperature plays a role in spermatogenesis. Obesity may alter sperm parameters via testicular heat resulting from increased scrotal adiposity.
Study design, size, duration: A correlation study was performed using data collected from male partners of subfertile couples who had visited an infertility center between March 2013 and November 2014. Participants were classified into three groups according to their BMI (< 25.0, 25.0–29.9, and > 30 kg/m^2).
Participants/materials, setting, methods: Data of 471 (aged 25–56) subjects were included. Scrotal temperature index (STI) was mean left and right skin temperature difference (ΔT) between the thigh and testicle. Pearson's correlation coefficient between BMI and STI was calculated, and mean STI was compared using analysis of variance (ANOVA).
Main results and the role of chance: Among 471 men, 280 men being underweight and normal weight, 175 overweight and 16 obese. Pearson's correlation coefficient was 0.346, which indicated a moderate positive correlation between BMI and STI ($p < 0.001$). We then performed subgroup analysis using BMI. Mean STI and standard deviation (mean ± SD) of the underweight and normal, overweight, and obese groups were -1.50 ± 0.95, -1.08 ± 0.83, and -0.57 ± 0.93, respectively. ANOVA indicated significant differences of STI; the obese and overweight groups had higher STI than the underweight and normal groups ($p < 0.001$).

Limitations, reason for caution: This was an exploratory study, and results should be interpreted cautiously because of other possible confounding factors (i.e., sedentary lifestyle or daily activities) that affect scrotal temperature. Only 16 men were obese (3.4%), too few to draw general conclusions.
Wider implications of the findings: Increased BMI is associated with higher scrotal temperature. High scrotal temperatures could negatively affect the sperm parameters of obese patients. To discuss this causal relationship, further assessment of the role of weight loss or gain in scrotal temperature changes is warranted.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: body mass index, scrotal temperature, thermography

P-019 Human papilloma virus and sperm DNA fragmentation

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Study question: The aim of the present study was to analyse the effects of HPV infection on semen parameters, focusing our attention to DNA fragmentation.
Summary answer: Our results suggest that HPV infection is not responsible of an a impairment of sperm quality and of an a higher DNA fragmented index (DFI).
What is known already: Human papillomavirus (HPV) infection is a common sexually transmitted disease. It is related to anogenital warts and neoplasia. In males, HPV DNA has been found in external genitalia, in the urethra, in the ductus deferens, epididymis, testis and in the seminal fluids. Literature reports that the presence of HPV in a semen sample could modify spermatic parameters as volume, viscosity, pH, count, motility, and viability, but very few works links HPV infection to DNA fragmentation.
Study design, size, duration: From January to December 2014, at the Unit of Human Reproduction of the Hospital S. Luca, Vallo della Lucania (SA), 250 infertile couple were treated. 118 medical records were retrospectively analysed. Statistical analysis was performed using T Student test to compare sperm parameters between men affected and not with HPV infection.
Participants/materials, setting, methods: The patients underwent a conventional semen analysis.

Using the SCD test (Sperm Chromatin Dispersion test) we valuated the amount of DNA fragmented sperm expressed in DFI (DNA fragmented index).

To test the presence of HPV we used a highly sensitive nested polymerase chain reaction assay followed by HPV genotyping.
Main results and the role of chance: 21 (16, 15%) of the total semen samples were HPV-positive. Median value of sperm concentration was 42,19x106/ml and 46,75x106/ml in HPV-negative and HPV-positive groups, respectively (P = 0,62). HPV-non infected and HPV-infected semen samples had similar rapid progressive motility (medians 7,0% vs 7,1%; P = 0,88) and similar morphology (median 7,8% vs 6,3%; P = 0,23).

The amount of DNA fragmented sperm was similar into two groups (29,90 vs 30,6%; P = 0,69).
Limitations, reason for caution: None.
Wider implications of the findings: Seminal HPV infection was frequently associated with a lower quality, although other studies did not find any effect on sperm parameters. No studies focused attention on the relationship between HPV infection and DNA sperm fragmentation. Our results suggest that HPV infection is not responsible of an a impairment of sperm quality and an a higher DFI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – San Luca Hospital Vallo della Lucania (SA).
Trial registration number: NA.
Keywords: sperm fragmentation, human papilloma virus, seminal parameters

P-020 The latest decade sperm quality evaluated and education in Taipei citizen

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Study question: Since 2003, Department of Health, Taipei city administration has offered citizen married, prior to get pregnant, to receive sperm quality examination. Through further analysis and detailed data, we realize more health situation of our male citizens upon suitable ages for getting married in recent 10 years.
Summary answer: The more high-educated persons show higher-abnormal proportion.
What is known already: It's helpful to find problems with non-eugenics and infertility as soon as possible if men can take the sperm quality evaluated prior to get pregnant.
Study design, size, duration: From 2005/01 to 2014/12, there're more than 12,500 persons recruited for our study.
Participants/materials, setting, methods: The men have to abide sexual abstinence for 3–5 days, and then their semen were collected into the sterile specimen containers by masturbation. All semen samples were allowed to liquefy about 15–30 minutes at room temperature. After liquefaction, semen-sample analyses were displayed by the computer aided sperm analysis detector to evaluate sperm concentration and motility. Sperm morphology was identified by manual examination.
Main results and the role of chance: In our study, the average ejaculate quantity of is 4cc; the sperm number of per milliliter of ejaculate is 131 (10^6/ml); the sperm morphology is 50% and the sperm motility is 53%. The percentage of deficient sperm quality was increased in latest decade. The major abnormal state is low motility. The sperm motility was going down with each passing year from 2005.
Limitations, reason for caution: nil.
Wider implications of the findings: There're various factors to affect the sperm quality. It can be age, working under high temperature operating environment, toxic substances, environmental chemicals, and medication. We found the most abnormal conditions are low motility and less sperm concentration. The more high-educated persons show higher-abnormal proportion. Perhaps many high-educated persons are elder, long office hours, received more environmental chemical and medication, meanwhile lack of exercise in Taipei.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Taipei City Hospital.
Trial registration number: 103XDAA00095.
Keywords: sperm quality, education

P-021 Role of FSH level, testicular volume and histology in choosing the appropriate surgical technique of sperm retrieval for patients with non-obstructive azoospermia

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Study question: Are there clinical characteristics that may support the urologist in choosing the appropriate surgical technique for individual patients with non-obstructive azoospermia?
Summary answer: According to multivariate analysis, patients with FSH level higher than 12 mIU/ml, testicular volume lower than 7.1 ml and histology different from hypospermatogenesis were more likely to obtain a successful sperm retrieval (SSR) when microdissection testicular sperm extraction (microTESE) was chosen instead of the standard testicular sperm extraction (TESE) technique.
What is known already: FSH and inhibin B level combined with testicular volume seem predictive of SRR in patients undergoing TESE, but no clinical characteristic can predict the outcome of microTESE.
Study design, size, duration: Retrospective cohort study on 558 normoandrogenic infertile patients with non-obstructive azoospermia (NOA), evaluated from July 2002 through July 2009.
Participants/materials, setting, methods: 558 patients with NOA referred to a university hospital underwent TESE (n = 356) or microTESE (n = 202). Serum FSH level and testicular volume, assessed by ultrasound, were obtained from all patients. Testicular histology was performed on a fragment of subcapsular parenchyma by examining at least 100 different sections of tubuli seminiferi.

Main results and the role of chance: Patients in microTESE group had significantly higher FSH serum level compared to that of TESE group ($p < 0.01$), significantly higher incidence of histology of Sertoli cell-only syndrome (SCO) ($p < 0.0001$) and lower incidence of histology of hypospermatogenesis (HYPO) ($p < 0.05$), while testicular volume was comparable among groups. Sperm retrieval rate was also comparable among groups (44.4% in TESE group and 39.6% in microTESE group, $p = 0.27$). Neither FSH nor histology alone resulted predictive of SSR in either TESE or microTESE group. However, multivariate analysis showed that microTESE provided significantly higher sperm retrieval rate compared to TESE (35.6 vs. 22.3%, $p < 0.05$) in a subgroup of 162 patients with FSH level > 12 mIU/ml, testicular volume <7.1 ml and histology different from HYPO.

Limitations, reason for caution: The success of microTESE in terms of sperm retrieval was shown to increase with the number of the procedures performed, therefore it probably increased from the beginning to the end of the study period. Histology can be obtained only during surgery, therefore it may be useful only in patients with a previous testicular biopsy.

Wider implications of the findings: The pre-surgical combined evaluation of patients FSH serum level, testicular volume and histology could help in categorizing NOA patients spermatogenic pattern. According to our results, microTESE is indicated in those patients with a more severe impairment of spermatogenesis, as predicted by higher FSH level, lower testicular volume and histology suggestive for SCO or maturation arrest, while patients with less severe spermatogenesis impairment could be assigned to TESE, an easier and less invasive surgical procedure.

Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: non-obstructive azoospermia, TESE, microTESE, FSH

P-022 Endometrial stromal cell-secreted factors improve sperm motility

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Study question: The aim of the study was to examine the influence of conditioned medium from *in vitro* cultured endometrial stromal cells (EnSCs) on sperm movement characteristics in cases with normozoospermia.

Summary answer: The incubation of spermatozoa from normozoospermic semen samples with EnSC conditioned medium significantly enhances the analyzed parameters: progressive motility; VCL (curvilinear velocity); VSL (straight line velocity); VAP (average path velocity) and hyperactivation. These results suggest that EnSC could facilitate the movement of sperm through the uterus by secreting motility-stimulating factors.

What is known already: Highly motile state of spermatozoa for extended durations is essential for their fertilizing capacity. Moreover, hyperactivation is necessary for the penetration through the cumulus oophorus and zona pellucida. Previous studies have reported an improvement in sperm motility parameters following co-culture with different cell types including Vero cells, skin fibroblasts, oviductal and endometrial cells. However, it remains unclear whether the beneficial effect is associated with cell-to-cell contacts or is due to the secretion of various factors.

Study design, size, duration: This 6-month prospective study includes semen samples from 10 normozoospermic donors (WHO, 2010). Semen samples were incubated with EnSC conditioned medium (1:1 mixture) for 4 h (37°C/5% CO_2). Control aliquots were incubated with DMEM/10% FCS. Sperm motility parameters (progressive motility; VCL; VSL; VAP; hyperactivation) were assessed using a CASA system.

Participants/materials, setting, methods: EnSCs were isolated from healthy donors and cultivated in DMEM/10% FCS. Conditioned medium was collected from a monolayer of EnSCs cultured for 48 h.

All sperm samples were prepared by DGC and divided into two aliquots. The first served as a control and the second was incubated with EnSC conditioned medium.

Main results and the role of chance: There was a significant improvement in the mean values of all motility parameters tested in the semen samples, treated with EnSC conditioned medium, compared to the controls (incubated with DMEM/10% FCS):

- progressive motility – 52,3 versus 41,5% (control), $p < 0.05$
- VCL – 62,3 μm/s versus 50,4 μm/s (control), $p < 0.05$
- VSL – 27,8 μm/s versus 22,9 μm/s (control), $p < 0.05$
- VAP – 34,5 μm/s versus 28,7 μm/s (control), $p < 0.05$
- hyperactivation – 22 versus 15,4% (control), $p < 0.05$

Limitations, reason for caution: These results should be verified on a larger number of samples (the study is in progress). Semen samples with pathological spermiogram parameters have to be included.

Wider implications of the findings: These findings may help to better understand the intimate mechanisms by which endometrium and its cellular components actively support the sperm during their passage to the site of fertilization.

In ART, the *in vitro* treatment with EnSC secreted motility-stimulating factors could have beneficial effects on sperm movement characteristics, especially in cases with pathological spermiogram parameters and, thus, could improve the fertilizing capacity of the semen.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Ob/Gyn Hospital "Dr. Shterev", Sofia, Bulgaria.
Trial registration number: NA.
Keywords: endometrial stromal cells, cell-secreted factors, sperm motility

P-023 Human sperm lipid fingerprinting by mass spectrometry – a pilot study

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Study question: Is the lipid profile of human sperm a prognostic tool of sperm quality?

Summary answer: Specific lipids that are differentially represented depending on the male age, sperm DNA fragmentation index and incidence of large nuclear vacuoles (LNV) sperm were detected. The MS fingerprinting may be a valuable, non-invasive tool for the prediction of semen sample quality.

What is known already: Semen analysis is the best tool available to assess male fertility; nonetheless, many infertility cases remain undiagnosed. Lipids are basic semen components that are involved in energy metabolism and in the events that lead to fertilization. In general, sperm fatty acids are suggested as markers of fertility disorders in men. Lipidomics is an emerging field of biomedical research. Not much is known about the association between sperm lipid profile and other assays for sperm quality.

Study design, size, duration: This prospective study enrolled 27 male patients seeking for reproductive treatment. Semen samples were analysed by motile sperm organelle morphology examination (MSOME), DNA fragmentation test and lipid profile. Samples were divided in quartiles according to patient's age, DNA fragmentation index and incidence of sperm with LNV.

Participants/materials, setting, methods: Mass spectra fingerprinting were acquired using a 7.2T LTQ FT Ultra-MS. Data were analysed using the Partial Least Square Discrimination Analysis (PLS-DA) combined with variable influence on projection (VIP) scores. The lipid profiles were compared between the different groups of male age, MSOME and sperm DNA fragmentation results.

Main results and the role of chance: Regarding male age, the model detected an increase of 4 lipids in the 1st quartile group (range: 28–32 years), 6 lipids in the 2nd quartile group (range: 33–36 years), 4 lipids in the 3rd quartile group (range: 37–42 years) and 1 lipid in the 4th quartile group (range: 43–54 years), with an accuracy of 60.0%. Regarding DNA fragmentation index, the model detected an increase of 5 lipids in the 1st quartile group (range: 7.7–12.5%) and 10 lipids in the 4th quartile group (range: 18.5–31.5%), with an accuracy of 84.6%. Regarding the incidence of LNV sperm, the model detected a significant increase of 10 lipids in the 1st quartile group (range: 2–6%) and 5 in the 4th quartile group (range: 22–42%), with an accuracy of 85.0%.

Limitations, reason for caution: The main limitation of this study is the low number of patients included.

Wider implications of the findings: The understandings that are provided by lipidomics studies of disrupted systems are expanding at a rapid rate. Several lipidomics publications in diverse fields of medicine are demonstrating the ability of these platforms to identify clinical biomarkers. While in its infancy, lipidome of human sperm may provide detailed insights into how lipid composition and metabolism affects sperm physiology and fertilization, and expand the scope of potential male infertility markers.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fertility – Centro de Fertilização Assistida.
Trial registration number: NA.
Keywords: lipid, mass spectrometry, MSOME, OMICS, sperm

P-024 Comparison of pregnancy rates with intrauterine insemination with different abnormal semen parameters and all combinations of those parameters by the 2010 World Health Organization guidelines

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Study question: To evaluate the effect on pregnancy rates in relation to one or more abnormal semen analysis parameters based on the 2010 World Health Organization (WHO) semen analysis guidelines.
Summary answer: Only low total sperm was a significant predictor for pregnancy when not controlling for other effects ($p = 0.04$). When controlling for confounding effects, only low total sperm count, low motility and low volume predicted pregnancy ($p = 0.049$). The parameters composing total motile sperm count failed to predict pregnancy ($p = 0.33$).
What is known already: Semen analyses consist of parameters including: volume, sperm concentration, progressive motility, and morphology. In prior studies total motile sperm count has consistently been shown to be a predictor of pregnancy. However, a pubmed search failed to detect any studies which classified semen analysis at the time of insemination in the presence of normal or abnormal parameters by the 2010 WHO semen analysis guidelines, and a combination of those parameters while investigating effects on pregnancy.
Study design, size, duration: A retrospective analysis was performed on 2.5 years of data collected at Stanford University. A total of 981 couples underwent 2231 IUI cycles. Couples had at least one year of either primary or secondary infertility with their current partner. Patients were treated with clomiphene citrate, letrozole or gonadotropin IUI.
Participants/materials, setting, methods: Data was classified as normal or abnormal by the 2010 parameters. Parameters analyzed included volume (1.5 ml), concentration (15mil/ml), minimum total count (39mil), forward motility (32%). Subjects with morphology <4% were treated with ICSI and were unavailable for analysis. All statistical analyses were performed using chi squared analysis. Data is presented as means ± SD.
Main results and the role of chance: Pregnancy rates were 13–25%. When comparing either normal or abnormal volume (3.2 ± 1.5 ml vs. 0.91 ± 0.25 ml, $p = 0.28$), concentration (59 ± 42 vs. 9.8 ± 3.5 mil/ml, $p = 0.11$), forward motility (58 ± 15 vs. 19 ± 8%, $p = 0.11$) or total sperm count (177 ± 142 vs. 24 ± 10mil, $p = 0.04$), only forward motility had a different likelihood of pregnancy. Mean parameters differed in each of the above groups ($p < 0.0001$, in all cases). To control for effects of the other parameters groups were subdivided in to all possible combinations for normal of abnormal volume, concentration, total sperm count, and forward motility. Statistically similar pregnancy rates were found in all cases except, abnormal total count (3.0 ± 2.3mil), volume (0.8 ± 0.3 ml) and motility (17 ± 8%), ($p = 0.049$). The combination of parameters making up total motile sperm count (volume 0.8 ± 0.2 ml, concentration 8.6 ± 3.2mil, motility 17.8 ± 8.7%, $p = 0.33$) failed to predict pregnancy.
Limitations, reason for caution: This is a retrospective study. Since Kruger-Tyberg strict morphology was unavailable, its role in pregnancy rates cannot be determined.
Wider implications of the findings: A reorganization of the semen analysis report should be done emphasizing total amount of sperm present and de-emphasizing concentration of sperm, possibly even removing it from reported results. However, even with abnormal semen analysis results likelihood of pregnancy remained excellent, at least 13% per cycle, in this study.
Study funding/competing interest(s): Funding by University(ies) – funding by national/international organization(s) – NIH grant 5K12HD01249 and the Stanford University Medical Scholars Program.
Trial registration number: NA.
Keywords: semen analysis, pregnancy rates, intrauterine insemination

P-025 Establishing a threshold level for sperm DNA quality in the semen of donors versus patients using the sperm chromatin dispersion test

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Study question: In an attempt to establish a threshold level of sperm DNA fragmentation (SDF), baseline levels of SDF and sperm DNA longevity (SDL) were compared between patients undergoing their first seminogram at an IVF clinic and a control donor population.
Summary answer: Control donor sperm exhibited baseline SDF values 2.2x lower and SDL levels 2.8x higher than that observed in patients undergoing their first seminogram.
What is known already: SDF is a controversial parameter gaining acceptance as a potential predictor of fertility. While SDF values of 30 to 40% are typically considered as being predictive of poor fertility, some patients with high levels of SDF are still capable of obtaining a successful pregnancy, particularly when ICSI is used. The level of SDF in sperm donors is generally considered to be lower than in patients undergoing their first seminogram, but this assumption has not been analyzed in detail.
Study design, size, duration: Multicentre, independent, blind study that used two main criteria for cohort configuration: (1) First seminogram of patients attending a clinic for assisted reproduction and (2) Individuals selected as semen donors. Cryopreserved sperm samples were used to assess DNA damage in both cases.
Participants/materials, setting, methods: Multicentre study of 5 independent IVF centers and two Universities. Materials: 110 sperm donors and 875 patients. SDF was evaluated at T0 (baseline) and following incubation for 24 h at 37ºC, with sub-sampling performed after 2, 6 and 24 h incubation. SDF was assessed using the sperm chromatin dispersion test (SCDt).
Main results and the role of chance: Donor sperm exhibited baseline SDF values 2.2 times lower than patients undergoing their first seminogram; DNA stability of the donor sperm was 2.8 times higher than that observed in patients when the sample is tested after 2 h of sperm incubation at 37ºC. The results of this investigation revealed that a baseline SDF value of 8% would be regarded as a threshold level to produce discrimination between both groups with a sensitivity of 65% and specificity of 82%. Similarly, a DNA quality loss of approximately 1.9% per h, would be regarded as a threshold level to produce discrimination between both groups with sensitivity of a 77% and a specificity of 65%. Baseline SDF and SDL levels behaved as independent variables.
Limitations, reason for caution: With respect to the proposed threshold baseline SDF level of 8 and 1.9% per h of DNA quality loss documented in the present study, 20% of patient population would be regarded as possessing donor quality sperm.
Wider implications of the findings: Proposed threshold values for SDF and SDL can be used for the identification of high quality sperm donors.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – University Autónoma of Madrid, 20849 Madrid, Spain. University of Queensland, Australia. CEFIVA, clinic. CREA, clinic, Centro Gutenberg, clinic. GINEMED, clinic. TAMBRE, clinic.
Trial registration number: This investigation is a part of the experimental protocols included in the research project BFU-2013-44290-R, as approved by the University in accordance with the participating IVF clinics.
Keywords: male factor, semen donor, sperm DNA damage

P-026 Was there a decline in sperm quality in the last 20 years in Portugal? A retrospective study

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Study question: The aim of this study was to evaluate if, similarly to the observed in many European countries, there was a decline in sperm quality in Portugal since the last 20 years (1994–2013).
Summary answer: In fact, in the last 20 years, there was a decrease in sperm quality, namely volume of the ejaculate, sperm concentration and quantity, and sperm morphology. On the opposite, sperm motility seems to have increased.
What is known already: In the last decades, a decrease in fertility along with a decrease in birth rate have been observed in the Portuguese population. However, it is not totally known which factors are responsible for that decreases, once social and economic factors only explain part of it. In several European countries, a decrease in sperm quality has been observed, where environmental pollutants seems to be the cause. In Portugal, the hypothetic change in sperm quality was never monitored.
Study design, size, duration: Cross sectional. 3146 sperm quality analysis from five time points of the last 20 years: (1st time point: years 1994/1995/1996, $n = 698$; 2nd time point: years 1999/2000, $n = 484$; 3rd time point: year 2005, $n = 698$; 4th time point: year 2009, $n = 640$; 5th time point: year 2013, $n = 626$) were retrospectively analysed and compared.
Participants/materials, setting, methods: Sperm quality analyses in five time points (years 1994/1995/1996; 1999/2000; 2005; 2009; 2013) were retrospectively analysed. Parameters like patient age; color, aspect, pH and volume of the ejaculate; presence of leukocytes and sperm agglutinates; sperm concentration and quantity, motility and morphology were recorded. Time point groups were compared.
Main results and the role of chance: When time point groups were compared, a decrease in volume was observed. In 1994/1995/1996 there was a higher volume (3.58 ml) than in the others: 1999/2000 (3.3 ml, $p = 0.056$), 2005 (3.29 ml, $p = 0.006$), 2009 (3.27 ml, $p = 0.004$), 2013 (3.3 ml, $p = 0.01$). Similarly, a decrease in sperm concentration/quantity was observed: 1994/1995/1996 presents higher concentration/quantity (81.97×10^6/ml and 279.35×10^6, respectively) than the others: 1999/2000 (54.5×10^6/ml and 175.03×10^6, respectively, $p < 0.001$), 2005 (43.7×10^6/ml and 147.45×10^6, respectively, $p < 0.001$), 2009 (51.55×10^6/ml and 168.79×10^6, respectively, $p < 0.001$), 2013 (68.68×10^6/ml and 194.77×10^6, respectively, $p < 0.001$). The percentage of patients presenting normal sperm morphology values also decreased ($p < 0.001$). Contrarily, sperm motility seems to have increased: 1994/95/96 (48.13%), 1999/2000 (52.01%, $p = 0.069$), 2005 (58.78%, $p < 0.001$), 2009 (56.85%, $p < 0.001$), 2013 (64.18%, $p < 0.001$). No differences were observed for the other parameters analysed.
Limitations, reason for caution: Criteria for some sperm parameters have changed in the last 20 years, especially sperm morphology. Taking this limitation into account, sperm morphology values were considered as normal or abnormal regarding to the reference value used in each time point.
Wider implications of the findings: As in other European countries, the sperm quality of the Portuguese population seems to have decreased in the last 20 years. For the volume of the ejaculate and the sperm morphology, these decreases occurred after the year 2000, however for sperm concentration/quantity this was already visible after 1996. Surprisingly, sperm motility seems to have increased. Larger studies should be conducted in order to validate these findings and identify the causes for these changes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Coimbra hospital and university center. Nothing to declare in terms of competing interest.
Trial registration number: NA.
Keywords: fertility decline, sperm quality, sperm concentration/quantity, sperm morphology, sperm motility

P-027 The impact of sperm nuclear chromatin condensation and ploidy status on the outcome of assisted reproduction techniques

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Study question: Do sperm nuclear chromatin condensation and ploidy status affect *in vitro* fertilization (IVF) / intracytoplasmic sperm injection (ICSI) outcome?
Summary answer: Sperm nuclear chromatin condensation and ploidy status were significantly associated with the fertilization rate, the embryo quality and the pregnancy rate of IVF/ICSI cycles. Consequently, sperm chromatin condensation and ploidy are critical parameters for the evaluation of semen samples before IVF or ICSI application.
What is known already: WHO parameters such as sperm concentration, motility and morphology are mainly taken into account in assisted reproduction. However, high percentages of IVF/ICSI cycles remain unsuccessful, suggesting the need of additional parameters, such as sperm nuclear chromatin condensation and ploidy, to estimate sperm contribution in assisted reproduction technique failures.
Study design, size, duration: Two hundred couples undergoing IVF cycles as well as 30 couples with male factor infertility and low sperm flow cytometry (SFC) parameters and 60 couples with male factor infertility and relatively high SFC parameters undergoing repetitive ICSI cycles in a period of four years constituted the study population.
Participants/materials, setting, methods: Conventional semen analysis and SFC analysis after Acridine Orange and Propidium Iodide staining, for the respective evaluation of sperm nuclear chromatin condensation and ploidy, were performed in the 290 men. The fertilization rate, the embryo quality and the pregnancy rate per cycle were the IVF/ICSI outcome measures.
Main results and the role of chance: In the IVF group, couples with lower euploidy rates and lower percentages of spermatozoa with fully condensed chromatin, presented with decreased fertilization rates ($p < 0.006$), diminished grade A embryo rates ($p < 0.004$) and decreased pregnancy rates ($p = 0.007$), compared to couples with higher SFC parameters. As concerns the ICSI group, the 65 ICSI cycles of the 30 couples with low SFC parameters were characterized by lower fertilization rates ($p < 0.004$), decreased grade A embryo rates ($p < 0.003$) and increased grade C embryo rates ($p < 0.01$), compared to the 86 cycles of the 60 couples with relatively high SFC parameters. Significantly elevated arrested embryos rates ($p < 0.001$) and decreased clinical pregnancy rates ($p = 0.019$) were observed in couples with severe SFC parameters undergoing ICSI. The above associations were independent from the conventional semen parameters.
Limitations, reason for caution: Larger population and multicenter studies are needed to verify our preliminary results.
Wider implications of the findings: SFC analysis may help in the discrimination of semen samples with elevated pregnancy potential post-IVF from those in need of ICSI application as well as in the identification of semen samples with reduced pregnancy likelihood post-ICSI, in which the use of testicular spermatozoa is potentially preferable. The combination of SFC with sperm selection methods could help in the fractionation and isolation of spermatozoa with normal genetic constitution and complete nuclear chromatin condensation from defective semen samples.
Study funding/competing interest(s): Funding by national/international organization(s). This study has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).
Trial registration number: NA.
Keywords: assisted reproduction, chromatin condensation, pregnancy rate, sperm aneuploidy, sperm flow cytometry

P-028 Seminal transforming growth factor ß1, insulin-like growth factor I and epidermal growth factor status in male idiopathic infertility

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Study question: This study aims' at investigating the relationship between seminal plasma growths factors (TGF β1, IGF I and EGF) and semen parameters, sperm DNA fragmentation, and ART outcome among idiopathic infertile men and normal men.
Summary answer: The increase of Seminal TGFβ1 levels was significantly associated with low semen characteristics such as total sperm count, progressive motility and normal morphology, and with high rate of sperm DNA fragmentation in idiopathic infertile men. Nevertheless, we didn't found any correlation between growth factors and IVF or ICSI outcomes.
What is known already: It is becoming apparent that seminal growth factors have a definite role in fertility and their modulation can cause infertility in both men and women. These factors have also been shown to have both positive and negative effects on the function of spermatozoa in vitro. Therefore, their exploration may provide useful information in cases of male idiopathic infertility.
Study design, size, duration: A prospective study conducted during one year that included 104 couples undergoing ART cycles (IVF or ICSI). Two groups were identified according to semen analysis and infertility related factor. A first group ($n = 67$) with only male infertility factor (idiopathic OAT) and a control group ($n = 37$) with normal semen parameters.
Participants/materials, setting, methods: A part of each sample was prepared using a discontinuous sperm gradient after parameters analyze, for oocyte insemination or injection and the remaining pellet was fixed for subsequent measure of DNA fragmentation index (TUNEL assay).Subsequently, seminal plasma growth factors concentrations were measured by ELISA using commercially available kits.
Main results and the role of chance: The mean concentration of seminal plasma T**GF** β1 was significantly higher in OAT group 234.68 ± 41.32 vs. 97.25 ± 36.85 ng/ml in controls ($p = 0.001$). T**GF** β1 levels were correlated negatively with sperm total count, motility ($r = -0.437$, $p = 0.000$; $r = -0.556$, $p = 0.000$) but positively with teratozoospermia ($r = 0.705$; $p = 0.000$). No evident correlation was found between these growth factors and ART outcomes such as fertilization, cleavage, top embryos and pregnancy rates. Idiopathic infertile men showed a high degree of DNA damage20.21% as compared to controls 12.96% ($p = 0.02$). A strong significant negative correlation was found between DNA fragmentation and all semen parameters ($p < 0.01$). Moreover, a significant positive correlation was noted between seminal TGF β1 and sperm DNA fragmentation levels ($r = 0.350$, $p = 0.01$) but not with IVF or ICSI outcomes.
Limitations, reason for caution: EGF tented to be higher in OAT group but the difference was not significant. EGF levels were lower among patients with clinical pregnancy compared to those with ART failure but IGFI levels were higher in controls and in cases of pregnancy. This may due to our small sample size.
Wider implications of the findings: Seminal TGFβ1 may have a direct or indirect role in spermatogenesis-steroidogenesis and its derangement may be involved in male idiopathic infertility especially when mediated through low semen characteristics and high DFI which can be explained by reactive oxygen species (ROS) generated with excessive seminal TGFβ1 that leads to DNA disintegration and therefore to increase sperm DNA damage. However, the exact molecular mechanism of seminal TGF β1on spermatic cells inducing ROS production requires further investigation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University Farhat Hachad Hospital-unit of reproductive medicine.
Trial registration number: NA.
Keywords: growth factors, semen parameters, DNA fragmentation, ART outcome

P-029 Efficacy of follicle-stimulating hormone treatment in male idiopathic infertility: a meta-analysis

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Study question: To comprehensively evaluate whether follicle stimulating hormone (FSH) administration to the male partner of idiopathic infertile couples improves pregnancy rate, both spontaneous and after assisted reproductive technique (ART), using a meta-analytic approach and including controlled clinical trial.
Summary answer: The administration of FSH to infertile men is reported in the literature since 1991, improving fertilisation and pregnancy rate. A significant increase in pregnancy rate after ART and male treatment with FSH was already shown in several studies, since FSH improves the sperm quality. FSH treatment could improve sperm quality and pregnancy rate in idiopathic infertile men.
What is known already: The Cochrane Collaboration recently estimated the overall effect of FSH treatment of the man in couples attending ART, enrolled in randomised, controlled, clinical-trials. That meta-analysis demonstrated that FSH treatment significantly improves spontaneous pregnancy rate, whereas no improvement of pregnancy rate was observed after ART, using fixed and strict inclusion criteria. They excluded all trials in which the randomization was not provided leading to potential loss of useful information that could help clinicians in their routinely practice.
Study design, size, duration: We conducted a comprehensive literature search for controlled clinical trials in which FSH was administered for male idiopathic infertility, compared with placebo or no treatment. The randomization was not considered as inclusion criterion. We considered studies in which men with idiopathic infertility or subfertility were enrolled, chronicallty treated with any type of FSH, compared with placebo or no treatment.
Participants/materials, setting, methods: We found 15 controlled clinical studies. Concerning the type of FSH, eight studies included in the meta-analysis used recombinant FSH, whereas seven studies used purified FSH. Pregnancy rate, when evaluated, was considered spontaneous or after ART. Selected trials gave details about 1275 infertile-men, 614 treated with FSH and 661 not-treated.
Main results and the role of chance: Among the 15 studies included, nine studies evaluated the spontaneous pregnancy rate, resulting in an overall improvement of about 4.5 (CI 2.17–9.33 and $I^2 = 0\%$) ($p < 0.001$). Eight studies evaluated pregnancy rate after ART, showing a significant improvement of about 1.60 (CI 1.08–2.37 and $I^2 = 43\%$) ($p = 0.002$). Sub-dividing studies according to the FSH preparations (purified or recombinant), the pregnancy rate improvement remained significant ($p = 0.007$ and $p = 0.002$, respectively). Eleven studies considered sperm quality after FSH treatment, finding a significant improvement of sperm concentration (mean improvement of $2.66x10^6$ millions/mL, with CI 0.47–4.84, $p = 0.02$), but not of sperm motility (mean improvement of $1.22x10^6$ millions/mL, with CI -0.07–2.52, $p = 0.06$). Finally, three trials evaluated testicular volume, showing a non-significant increase in men treated (mean increase of 1.35 mL, with CI -0.44–3.14, $p = 0,14$).
Limitations, reason for caution: The heterogeneity of studies, together with the high risk of biases in this field of research could limit the strength of these results.
Wider implications of the findings: The results of controlled clinical trials available in literature indicate an improvement of pregnancy rate after FSH administration to the male partner of infertile couples, both spontaneous and after ART.
Study funding/competing interest(s): Funding by University(ies) – University of Modena and Reggio Emilia.
Trial registration number: NA.
Keywords: FSH, idiopathic male infertility, reproduction

P-030 Are obese men subfertile?

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Study question: Does obesity affects male fertility potentials?
Summary answer: BMI was proved, in our study, to have a significant effect on sperm concentration.
What is known already: Overweight and obesity can affect female fertility, in men the negative effects on reproductive system attributed to obesity are less evident and have been less often studied.
Study design, size, duration: Prospective, 439 male partners of couples presenting for evaluation of infertility, in the period from January 2013 to November 2014.
Participants/materials, setting, methods: Semen samples from 439 male partners of couples presenting for evaluation of their infertility, were collected. Men were divided into three BMI groups; normal, overweight and obese. Samples has been analyzed based on the WHO 5th edition guidelines. Meticulous scrotal examination and scrotal ultrasound were performed. Hormonal profile, including testosterone, FSH, LH and prolactin was done to exclude any possible causative hormonal factor.
Main results and the role of chance: Mean BMI was 29.67 ± 5.89. ANOVA testing revealed no significant differences in semen parameters between the 3 different BMI groups. Also, pair-wise multiple comparisons were found to be

non-significant ($P > 0.05$). The distribution of patients with normal sperm concentration per BMI group was as follows: normal weight, 46/75 (61.33%); overweight, 114/179 (63.69%); and obese, 116/185 (62.70%). BMI had a negative correlation with semen volume, sperm concentration, sperm motility and sperm morphology. However, this correlation reached a significant level only between BMI and sperm concentration ($r = 0.101$; $p = 0.035$).
Limitations, reason for caution: One semen analysis was performed in some cases.
Wider implications of the findings: Weight reduction may help to improve the semen quality.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Salman bin Abdulaziz University Hospital.
Trial registration number: IRB: SAU-2014-U-8-30PI.
Keywords: sperm concentration, body mass index (BMI), obesity

P-031 The effects of indirect cigarette smoke inhalation and the antioxidant effects of curcumin on the morphology of spermatozoa and Leydig cells of the adolescent rats

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Study question: What are the combined morphological and functional effects of; indirect cigarette smoke inhalation that causes oxidative stress and curcumin on spermatozoa recovered from epididymis and the Leydig cells of the adolescent rats?
Summary answer: Cigarette smoke has adverse effects on spermatogenesis, and Leydig cell's morphology by provoking cellular stress. Curcumin plays an important role in suppressing the negative effects of cigarette smoke by inhibiting the cellular oxidative stress.
What is known already: Scientific research points out that toxic substances in cigarette smoke damage DNA of spermatozoa, Leydig cells and Sertoli cells. This affects the male fertility negatively. Curcumin has a wide range of pharmacological and biological activities. Numerous studies proved its antioxidant action and significant protective effect on the testes tissue.
Study design, size, duration: Two months old 32 male Wistar albino rats weighing 150–200 g divided equally into four groups of; control, cigarette smoked (CS), curcumin and CS + curcumin. Rats exposed to CS for 2 h per day for 28 days. DMSO and curcumin dissolved in DMSO; administrated to control and experiment groups respectively by gavage.
Participants/materials, setting, methods: Fluid samples obtained from tail of epididymis evaluated in Makler chamber to count spermatozoa and smears prepared to examine the morphology of spermatozoa. Testis specimens prepared with appropriate methods for light and electron microscopy. Statistic results of the cell counts, sperm morphology, and body weights analyzed by one-way ANOVA.
Main results and the role of chance: Light and electron microscopical examinations showed that cigarette smoke (CS) have adverse effects on spermatogenesis. The spermatozoa count reduced associated with increased number of abnormally formed spermatozoa. Invaginations and enfoldings of nuclear envelope, dilated SER, mitochondrial injury with reduced amount of crista, diminished lipid droplets, groups of SER, lipid droplets and mitochondria located in the periphery of cell, and multiple pinocytotic vesicles were the ultrastructural changes of Leydig cells. However, the antioxidant effects of curcumin; increased the number of spermatozoa and reduced the amount of abnormal ones. Ultrastructural morphology of the Leydig cells was similar in CS and CS + curcumin groups. Thus, curcumin plays an important role in decreasing the negative effects of cigarette smoke on sperm counts and abnormalities.
Limitations, reason for caution: In previous studies, the negative effects of oxidative stress on Leydig cells meliorated by antioxidant properties of curcumin, but in our study only sperm count and morphology improved while Leydig cell morphology showed similarities with the ones in cigarette smoke group.
Wider implications of the findings: Smoking causes most prominent social and health problems, specifically in young population of all countries. Evaluation of the cigarette smoke on the urogenital system is considerably important. Curcumin acts as an effective compound playing a significant role in suppressing negative effects of cigarette smoke on testis tissue due to its anti-oxidative property.
Study funding/competing interest(s): Funding by University(ies) – Research Fund of Istanbul University.
Trial registration number: Project No. 22934.
Keywords: curcumin, testes, cigarette, Leydig, microscopy

P-032 The superiority of swim down technique compared to density gradient technique in preparing semen samples before intracytoplasmic sperm injection (ICSI): a prospective randomized clinical trial

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Study question: Does swim down sperm preparation technique increase the blastulation rate and the ongoing pregnancy rate compared to density gradient sperm preparation technique in ICSI cycles?
Summary answer: There was a statistical significant increase in the blastulation rate (71%) ($P < 0.0001$) and the ongoing pregnancy rate (64%) ($P < 0.0001$) when using swim down sperm preparation technique before ICSI.
What is known already: Density gradient centrifugation while considered as a conventional technique for ICSI sperm preparation, recommended by the WHO laboratory manual 2010; it is not efficient enough to produce sperm populations free of DNA damage, because the technique are not physiological and not modeled on the sperm selection processes occurring at the female genital tract (Henkel 2012). Migration based protocols including the swim down, based upon the migration of the motile sperms down the discontinuous gradient.
Study design, size, duration: A prospective randomized multi-centric clinical trial of 890 couples enrolled into the study after singing the informed consent and after approved from ethical committees of the participating centers at the period from February 2013 to December 2014.
Participants/materials, setting, methods: Semen samples of 890 couples in two ICSI centers (Al Baraka Fertility Hospital, Manama Bahrain) and (Ibn Sina IVF Center- Ibn Sina Hospital, Sohag Egypt) were allocated randomly into two groups; group I prepared using direct swim down over a 100% silica containing media (ALL Grad® 100', Life Global®) and group II prepared using the conventional density gradient technique.
Main results and the role of chance: There were no statistical significant differences between both studied groups as regard mean age, body mass index, dose of FSH/HMG used, number of neither oocytes collected nor number of stimulation days. The blastulation rate in-group I show statistically significant increase (71%) compared to (53%) in-group II ($P < 0.0001$). We could also document a statistically significant difference increase in the pregnancy rate in-group I (64%) compared to (54%) in-group II ($P < 0.0001$).
Limitations, reason for caution: We excluded moderate to severe oligozoospermia cases, since the yielded sperm count is lesser in swim-down compared to the density-gradient technique. The inappropriate use of swim down-100% technique with non-progressive motility sperm due to very poor outcome. We did not study the effect of sperm exposure to Silica solution 100% solution for lengthy periods.
Wider implications of the findings: Utilizing sperm natural motility for sperm preparation can be applied at any ICSI units. Avoiding multiple centrifugation steps using swim down-100% considering the sperm natural powers for separation can reflect a semi natural sperm preparation for ICSI. Application for virally infected semen can also be evaluated.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Al Baraka fertility Hospital – Ibn Sina IVF Center- Ibn Sina Hospital.
Trial registration number: NCT02328534.
Keywords: Swim down semen preparation density gradient, swim-down, semen preparation, density gradient, silica solution

P-033 Is there any role of medical treatment or varicocele repair in infertile men who failed initial testicular sperm extraction?

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Study question: Is there any role of medical treatment or varicocele repair in infertile men who failed initial testicular sperm extraction (TESE)?
Summary answer: The aim of this study was to investigate whether there is any role of medical treatment or varicocele repair in azoospermic men who had previous negative TESE procedure.
What is known already: Empirical medical therapy and varicocele repair have been controversial in infertile men with non-obstructive azoospermia. In addition, no evidence based medical treatment has been reported to induce spermatogenesis in men who failed initial TESE procedure.
Study design, size, duration: During a 4 years period, the study included 181 azoospermic men, who failed TESE procedure, who then underwent repeat micro-TESE procedure. All patients were evaluated and treated by a single clinician.
Participants/materials, setting, methods: The patients were divided into 6 groups according to treatment type. Of the patients, 76 had clinical palpable varicocele on either one or both sides, and 105 had no varicocele. Of the 76 patients with varicocele, 58 had microsurgical subinguinal varicocele repair (Vx): 42 had additional medical treatment (recombinant HCG, anastrozole, or clomiphene citrate), and 16 were followed without additional medical therapy. Eight patients received medical therapy with no Vx treatment, and 10 were only followed with no medical and Vx treatment. Of the 105 men with no clinical varicocele, 90 received medical therapy, and 15 were followed with no treatment. Sperm retrieval rates on micro-TESE were compared according to the treatment groups.
Main results and the role of chance: The mean age was 33.44 ± 6.82 years (21–51), and mean treatment duration prior to re-do micro-TESE was 10.4 ± 3.42 months (6–24). Testicular sperm retrieval rates were 30.9% (13/42) in the men with varicocele who had Vx repair + medical treatment, 12.5% (1/8) in the men with varicocele who had no Vx treatment, but medical treatment, 18.7% (3/16) in the men with varicocele who had only Vx treatment with no medical treatment, 0% (0/10) in the men with varicocele who were followed with no Vx treatment or medical treatment, 18.8% (17/90) in the men with no varicocele who had only medical treatment, and 6.7% (1/15) in the men with no varicocele who were followed only with no medical treatment.
Limitations, reason for caution: None.
Wider implications of the findings: Benefit of pre-medical treatment prior to micro-TESE is limited in selected cases. Men with varicocele who failed initial TESE had higher sperm retrieval rate with varicocele repair than men having no varicocele repair. The highest sperm retrieval rate was achieved with varicocele repair plus medical treatment with recombinant HCG.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Private IVF center.
Trial registration number: NA.
Keywords: male infertility, medical treatment, micro TESE, varicocele, sperm

P-034 The cytotoxic effects of membrane lipid peroxidation products on human spermatozoa

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Study question: The cytotoxicity of malondialdehyde (MDA) was compared to that of two other common products of lipid peroxidation, 4-hydroxynonenal (4-HNE) and acrolein (ACR), in human spermatozoa.
Summary answer: While treatment of spermatozoa with 4-HNE and ACR consistently elicited cytotoxic effects in a dose dependent manner, congruent treatment with MDA failed to produce any effect on cell viability or oxidative status.
What is known already: Spermatozoa are particularly susceptible to oxidative stress largely due to high membrane content of polyunsaturated fatty acids (PUFAs). Reactive oxygen species (ROS) preferentially attack PUFAs and form a variety of highly reactive lipid peroxy radicals and aldehydes, such as MDA, 4-HNE, and ACR. Though MDA is used as a marker of lipid peroxidation, little is known about its cytotoxic effects as compared to other common lipid aldehydes in spermatozoa.
Study design, size, duration: Semen samples of healthy human volunteers were collected through the University of Newcastle's donor program. Pooled samples were treated with increasing doses of MDA, HNE, or ACR for 2 and 24 h, with an untreated replicate serving as a control. Treatments were conducted on at least three separate samples.
Participants/materials, setting, methods: Cytotoxicities at six concentrations (0, 12.5, 25, 50, 100, 200 μM) were assessed *in vitro* under physiological conditions using fluorocytometric assays for mitochondrial and cytoplasmic ROS production, caspase activation, lipid peroxidation and DNA damage in the form of 8-hydroxydeoxyguanosine (8-OHdG) formation. Sample motility and vitality were also assessed at each concentration.
Main results and the role of chance: After 2 h of incubation, 4-HNE and ACR induced a dose dependent reduction in motility ($p < 0.01$) and vitality ($p < 0.001$), while increasing mitochondrial ROS production ($p < 0.01$) in a dose dependent manner. Treatment with 200 μM 4-HNE alone increased lipid peroxidation and caspase activation after 2 h. After 24 h, both 4-HNE and ACR induced significant, dose-dependent increases in cytoplasmic superoxide formation ($p < 0.001$), mitochondrial ROS formation ($p < 0.001$), caspase activation ($p < 0.001$), 8-OHdG formation ($p < 0.001$), and reduction in motility and vitality ($p < 0.001$). MDA produced no observable cytotoxic effect compared to untreated controls in any of the aforementioned assays. Compared to controls, motility and vitality were unaffected by incubation with MDA after 24 h. MDA, at concentrations used, had little cytotoxicity in spermatozoa as compared to 4-HNE and ACR.
Limitations, reason for caution: MDA is a tautomer and its reactivity is pH dependent, increasing in acidic environments. However, sperm are exposed to near-neutral pH during spermatogenesis and storage. Since all experiments were conducted in a neutral environment, the observed cytotoxicities of MDA, 4-HNE, and ACR probably reflect those experienced by spermatozoa *in vivo*.
Wider implications of the findings: The observed cytotoxicities of 4-HNE and ACR are consistent with previous findings in spermatozoa, as are the similar patterns observed between the two molecules. However, cytotoxicity of MDA on spermatozoa had not previously been reported. Though aldehyde byproducts of lipid peroxidation are generally accepted to adversely impact cellular health, these data suggest considerable cytotoxic differences between species. As aldehydes are used as markers of lipid peroxidation, differing reactivity may influence biomarker preference in future experiments.
Study funding/competing interest(s): Funding by University(ies). The study was funded by the University of Newcastle. P. G. is the Managing Director of, CellOxess LLC, which has a commercial interest in the detection and resolution of oxidative stress. A.P. and R.M. are employees of CellOxess LLC, which has a commercial interest in the detection and resolution of oxidative stress.
Trial registration number: NA.
Keywords: 4-hydroxynonenal, malondialdehyde, lipid peroxidation, oxidative stress, spermatozoa

P-035 Validation of the American Society for Reproductive Medicine guidelines/recommendations in Caucasian-European men presenting for couple's infertility

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Study question: To retrospectively validate the American Society for Reproductive Medicine (ASRM) guidelines/recommendations concerning the endocrine evaluation in Caucasian-European men presenting for couple's infertility.
Summary answer: Current findings showed that ASRM guidelines/recommendations for male infertility work-up may not be suitable for Caucasian-European men. One out of four hypogonadal men may miss out on a proper endocrine assessment when adhering to the ASRM guidelines/recommendations prompting the EAU guidelines suggestion to have tT assessed in every infertile patients.
What is known already: The assessment of the hormonal milieu is not of unequivocal importance in the male infertility workup. We aimed to retrospectively validate the American Society for Reproductive Medicine (ASRM) guidelines/recommendations concerning the endocrine evaluation in Caucasian-European men presenting for couple's infertility.
Study design, size, duration: Complete socio-demographic, clinical and hormonal data from 1056 consecutive infertile men were analysed. Cross-sectional study. Semen analysis values were assessed based on the 2010 WHO reference criteria.

Participants/materials, setting, methods: Hypogonadism was defined as total testosterone <3 ng/ml (Endocrine Society classification criteria). ASRM indications (sperm concentration <10 million/mL; impaired sexual function; other findings suggesting a specific endocrinopathy) were used to predict hypogonadism in our cohort and compared with a novel nomogram including patient age, BMI, and left testis volume predicting hypogonadism.
Main results and the role of chance: Biochemical hypogonadism was diagnosed in 156 (14.8%) men. Overall, 669 (63.4%) patients would have necessitated tT assessment according to ASRM criteria; of these, only 119 (17.8%) were actually hypogonadal according to the Endocrine Society classification criteria. Conversely, 37 (23.7%) out of 156 patients with biochemical hypogonadism would have been overlooked. The overall predictive accuracy, sensibility, and specificity of the ASRM guidelines were 58%, 76%, and 39%, respectively. Our logistic regression-based nomogram, was not quite reliable enough to predict hypogonadism, despite demonstrating a significantly higher predictive accuracy (68%, $p < 0.001$) than ASRM guidelines.
Limitations, reason for caution: The cross-sectional nature of the study.
Wider implications of the findings: These results provide evidence that ASRM guidelines may not be suitable in a Caucasian-European setting.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: ASRM, hypogonadism

P-036 Evaluation of varicocele patients using the testicular real-time elastography

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Study question: Are there any correlations between the testicular real-time elastography and varicocele, varicocele grades, hormones and semen parameters?
Summary answer: There were significant correlations between the testicular real-time elastography and varicocele, varicocele grades, and some semen parameters.
What is known already: To our knowledge this is the first study correlates real-time elastography with varicocele, varcocele grades, hormones and semen parameters.
Study design, size, duration: Prospective study, involved 50 patients with left varicocele and 20 controls,
Participants/materials, setting, methods: 70 testes (50 left varicoceles and 20 controls) were evaluated using real-time elastography (Hitachi HI VISION Avius® system) to identify testicular volume, strain ratio and tissue elasticity score. Serum FSH, LH, free testosterone, total testosterone, prolactin and estradiol were assessed. Semen analysis was checked using computer-assisted sperm analysis.
Main results and the role of chance: Main testicular volume was 16.17 ± 4.86 ml for the varicocele patients vs 24.46 ± 6.34 ml for the controls ($p < 0.001$). The main strain ratio was 0.40% ± 0.06% for the varicocele patients vs 0.33% ± 0.03% for the controls ($p < 0.001$). The elasticity score in the varicocele patients was significantly higher than the controls ($p < 0.001$). There was a positive correlation between the grade of varicocele and both elasticity score ($r = 0.884$, $p < 0.0001$) and strain ratio ($r = 0.992$, $p < 0.001$). There were also negative correlations between normal sperm forms and both strain ratio ($r = -0.398$, $p = 0.004$) and elasticity score ($r = -0.394$, $p = 0.005$). No more correlations were detected between elastography parameters and hormones or other sperm parameters.
Limitations, reason for caution: none.
Wider implications of the findings: new study.
Study funding/competing interest(s): Funding by University(ies) – Alexandria University.
Trial registration number: NA.
Keywords: varicocele, real-time elastography, testes, strain ratio, elasticity score

P-037 MIcro dissection TESE, a series of more than 300 men with non obstructive azoospermia from a developing country : obstacles encountered, success achieved and the path forward

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Study question: How to implement micro dissection testicular sperm extraction (TESE) for men with non obstructive azoospermia (NOA) in a developing country, the expected obstacles in implementation and the overall success?
Summary answer: It is feasible to implement the technique in developing country like India. The difficulties encountered included the cost of setting up the technique, the training of the surgeon and convincing the other reproductive medicines specialists and general gynaecologists to refer the indicated men for the procedure.
What is known already: Microdissection TESE is a well known modality of surgical sperm retrieval in men with NOA preferred over conventional TESE and testicular sperm aspiration (TESA).It has been practiced since 1990s and has gained momentum in last few years. Though practice of this procedure started mainly in developed countries, the developing nations are slowly, but surely catching up.
Study design, size, duration: We performed a retrospective analysis of 300 men with NOA who have undergone micro dissection TESE from 2012–2014 at Craft hospital. The sperm retrieval rate and Intracytoplasmic sperm injection (ICSI) outcome were analysed. We also analysed the difficulties encountered in the implementation of the technique and steps taken to overcome them.
Participants/materials, setting, methods: 300 men with NOA underwent microdissection TESE for the first time in Craft hospital and research center. The procedure was done a day prior to the oocyte pick up. Initially, the larger testis was first and proceeded to the other side if no sperms were retrieved on the first side.
Main results and the role of chance: We were able to implement the procedure with considerable ease. The main hinderance was the cost of the procedure to the couple, which was dealt with dedicate counselling. The sperms were retrieved in 151/300 (50.33%) of men with non obstructive azoospermia undergoing microdissection TESE. The highest recovery rate was seen in men with testicular histopathology of hypospermatogenesis followed by maturation arrest and sertoli cell syndrome. ICSI was done for all but two couples due to extremely poor sperm morphology. For the 149 couples who underwent ICSI, fertilization rate of 75.1% was achieved. The clinical pregnancy rate and live birth rate were 29 and 26% respectively. There was one case of post operative testicular hematoma. There were no major complications in any other case.
Limitations, reason for caution: Since this was a case series, there is no control group to which the findings can be compared to.
Wider implications of the findings: Microdissection TESE as an sperm acquisition method for NOA can be successfully implemented in developing countries in units with dedicated andrology unit. It is associated with more than 50% sperm retrieval rate with low complication rate.

The hinderances are minor ones including cost, training, learning curve and dissemination of the necessary information to general gynaecologists.
Study funding/competing interest(s): Funding by hospital/clinic(s) – craft hospital.
Trial registration number: NA.
Keywords: non obstructive azoospermia, micro TESE

P-038 Is a second sperm analysis necessary in every infertile man?

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Study question: To develop 3 nomograms capable of predicting findings of a second pathological semen analysis.
Summary answer: Current findings showed that half of infertile men could ideally be spared from a second semen analysis in order to confirm pathological sperm parameters. This could speed up the diagnostic work-up, without a consistent risk of overtreatment.
What is known already: Current guidelines suggest andrological investigation in infertile men after at least two pathological semen analyses, with a potential considerable delay in couples who need infertility work-up.
Study design, size, duration: Complete data from 647 consecutive infertile men were analysed. Cross sectional study.

Participants/materials, setting, methods: Testicular volume was assessed with a Prader orchidometer. Two consecutive semen analyses carried out 3 months apart were requested for each patient (2010 WHO criteria). Three different nomograms (A, B, C) were developed and internally validated in order to predict pathological concentration (pC), total progressive motility (PT), and morphology (M) at the second semen analysis following a previous pathological one.
Main results and the role of chance: A) Left testicular volume, baseline concentration and number of baseline pathological sperm parameters (all $p \leq 0.03$) were independent predictors of oligospermia at second semen analysis. Nomogram predictive accuracy (PA): 88%. **B)** Baseline concentration and PT (all $p \leq 0.03$) were independent predictors of asthenozoospermia at second semen analysis. Nomogram PA:74%. **C)** Baseline normal M, baseline PT, number of baseline pathological semen parameters, and left testicular volume were independent predictors of teratozoospermia at second semen analysis found at MVA. Nomogram PA: 73%. Using a nomogram-derived probability of oligospermia at second sperm analysis > 70%, would spare 58% of repeating a second sperm analysis to confirm oligospermia. Similarly a 65% cut-off for asthenospermia and a 70% cut-off for normal M, would spare 39.1% and 32.8% men respectively from repeating sperm analysis.
Limitations, reason for caution: The cross-sectional nature of the study.
Wider implications of the findings: These results could speed up the diagnostic work-up, without a consistent risk of overtreatment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: sperm analysis, workup

P-039 How to predict success in ART: the impact of sperm cell origin on intracytoplasmic sperm injection (ICSI) outcomes of ejaculated versus testicular spermatozoa

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Study question: Is there a relationship of sperm origin, testicular [in testicular sperm aspiration (TESA)] and ejaculate with ART outcomes in couples treated with ICSI?
Summary answer: It's possible to achieve similar pregnancy and spontaneous abortion rates via ICSI with fresh non-ejaculated spermatozoa, although the fertilization, cleavage and sometimes the implantation rates, are significantly lower than those of counterparts using freshly ejaculated spermatozoa from men with different sperm characteristics.
What is known already: The literature evidence is quite limited, and conflicting reports exist regarding whether the ART outcome is affected by sperm origin; studies have reported either a decrease or no difference in pregnancy outcomes with ICSI in cases of non-obstructive azoospermia (NOA) and obstructive azoospermia (OA), respectively, and the results are similar to those reported with ICSI using ejaculated sperm.
Study design, size, duration: Retrospective cohort study at private IVF centre from 2002 to 2014: the results of 2818 fresh ICSI cycles using TESA and ejaculated sperm were compared. Main outcomes measures were fertilization, cleavage, implantation, pregnancy and spontaneous abortion rates. A complete workup was conducted to exclude female factor influence.
Participants/materials, setting, methods: A total of 426 TESA-ICSI cycles using fresh testicular aspirated sperm from men with different origin of azoospermia (obstructive and non-obstructive) and temporary ejaculation failure (TEF) and 2392 ICSI cycles using fresh ejaculated sperm from men with different sperm characteristics (normozoospermia, oligoasthenoteratozoospermia and cryptozoospermia) were included in the analysis.
Main results and the role of chance: Mean female age, number of inseminated oocytes and transferred embryos were similar among the groups. Overall, in ICSI-TESA cycles, fertilization, cleavage and implantation rates were significantly lower than in ICSI-ejaculated cycles (62.0 vs 72.6%, $p < 0.0001$; 94.0 vs 97.1%, $p < 0.0001$; 12.4 vs 16.9%, $p = 0.001$, respectively), but the differences tend to reduce with the lowering quality of sperm in ICSI-ejaculated subgroups (from normozoospermia to cryptozoospermia). On the contrary, the pregnancy and spontaneous abortion rates were similar among the overall groups (23.4 vs 28.7%, $p = 0.058$; 12.9 vs 16.1%, $p = 0.522$, respectively). Moreover, among ICSI-TESA cycles, NOA obtained significantly lower fertilization, implantation and pregnancy rates than OA (59.9 vs 64.7%, $p < 0.05$; 9.6 vs 17.0%, $p < 0.05$; 18.8 vs 34.0%, $p < 0.05$, respectively), while TEF obtained the lowest laboratory and clinical outcomes.
Limitations, reason for caution: It is a retrospective cohort study with different sample size in the groups. In addition, the study design with respect to statistical power, cases number recruitment and lack of data on ectopic and multiple pregnancy rates could be improved on.
Wider implications of the findings: Although pregnancy and spontaneous abortion rates in TESA-ICSI cycles are clinically satisfactory, they are lower than in ICSI-ejaculated cycles. This difference might be related to the severity of sperm defects, to major implications during spermatogenesis and to the 'incomplete' maturation process of testicular spermatozoa. Therefore, future investigation focusing on the functional and genetic integrity of ejaculated spermatozoa versus those retrieved from testicle would help identify the most competent gamete source for ART.
Study funding/competing interest(s): Funding by hospital/clinic(s) – no competing interests declared.
Trial registration number: NA.
Keywords: intracytoplasmic sperm injection (ICSI) outcomes, ejaculated sperm, testicular sperm, obstructive/non-obstructive azoospermia, temporary ejaculation failure

P-040 Treatment with zinc, D-aspartate and coenzyme Q10 protects bull sperm against exogenous oxidative damage

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Study question: Do antioxidants Zinc, Coenzyme Q10 (CoQ10) and the micronutrient D-aspartate (D-Asp), present in dietary supplement Genadis, (Merck Serono), protect bull spermatozoa against exogenous oxidative damage?
Summary answer: Results demonstrated that in vitro treatment of bull spermatozoa with the xanthine-xanthine oxidase system (X-XO) determines a rapid and time-dependent decrease of sperm motility and kinetics, and a concomitant increase of sperm DNA fragmentation that are prevented by incubation with Zinc, D-Asp and CoQ10.
What is known already: Sperm oxidative stress in vivo and in vitro is thought to impair semen quality, fertilization and embryo developmental competence. We recently demonstrated that Zinc, D-Asp and CoQ10 have protective effects on human and bull sperm motility and DNA fragmentation in vitro in the absence of exogenous oxidative stress. Moreover, treated bull spermatozoa promoted the development of 8-cell embryos and good quality blastocysts endowed with a lower percentage of DNA fragmented blastomeres.
Study design, size, duration: The study was performed on 10 frozen/thawed bull semen samples. Each experiment included three groups: control, treated with X-XO alone or in combination with Zinc, D-Asp and CoQ10. Sperm kinetics was evaluated by computer assisted semen analysis (CASA) and DNA fragmentation measured by the TUNEL assay in all samples.
Participants/materials, setting, methods: Frozen/thawed bull spermatozoa were incubated for 2 h in culture medium (control), supplemented with 0.5 mM xanthine-0.05 U/ml xanthine oxidase alone or in combination with 10 μg/mL Zinc chloride, 500 μg/mL D-Asp, and 40 μg/mL CoQ10. Samples were analyzed by CASA at 0,30,60 and 120 min. DNA fragmentation was measured after 2 h of treatment.
Main results and the role of chance: Time 0 total (74 ± 9.6%), and progressive (72 ± 10.9%) motility and kinetics (VCL 122.8 ± 11.8, VSL 81.8 ± 9.49, VAP 96.7 ± 9.6 μm/sec) significantly decreased after 2 h of incubation with X-XO (control 2 h vs treated: motility 59 ± 15.2 vs 32 ± 12.1, progressive motility 52 ± 13.9 vs 23 ± 10.9, $P < 0.01$). Interestingly, treatment with X-XO and Zinc, D-Asp and CoQ10 prevented the drop of motility (2 h, total 60 ± 12%, progressive 47 ± 13.9%) and the increase of sperm DNA fragmentation (control time 0, 2,2; control 2 h, 5,2; X-XO 2 h, 8,4; X-XO plus Zinc, D-Asp and CoQ10, 4,5; $P < 0,01$).
Limitations, reason for caution: The study was carried out in vitro using frozen/thawed bull spermatozoa. Data could not reflect what occurs in vivo and/or in the human species.

Wider implications of the findings: Data demonstrated that antioxidants protect bull spermatozoa from exogenous oxidative stress in vitro. The supplementation of sperm culture media with Zinc, D-Asp and CoQ10 could protect spermatozoa from oxidative stress during in vitro handling. The use of an animal model will allow to understand the role played by sperm exogenous oxidative damage and its prevention by Zinc, D-Asp and CoQ10 on embryo developmental competence.
Study funding/competing interest(s): Funding by University(ies) – funding by commercial/corporate company(ies) – Merck Serono.
Trial registration number: NA.
Keywords: oxidative stress, spermatozoa, motility, DNA fragmentation, antioxidants

P-041 The quest for infertility genes: identification of two new genes involved in azoospermic and oligozoospermic patients by whole exome sequencing in Turkish consanguineous families

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Study question: Our currently restricted understanding of basic mechanisms driving human gametogenesis severely limits the improvement of proper clinical care of infertile patients. The goal of our team is to identify genes through whole exome sequencing that, when mutated, cause a male infertility phenotype that adversely affects gametes production.
Summary answer: Using exome sequencing, we identified mutations in two genes; a no-stop mutation in an X-linked gene, which causes the production of an extended protein by turning the stop codon into amino acid coding codon, and a stop-gained mutation in an autosomal gene leading to the production of a truncated protein.
What is known already: The World Health Organization estimates that one in four couples in developing countries is confronted with infertility. Despite 37 years of assisted reproductive activities, a significant number of cases remain idiopathic. Considering the high predicted numbers of genes involved in male gametogenesis, it is likely that most 'idiopathic' forms have genetic origin. At present, only a dozen of genes has been identified as being responsible for an infertility phenotype in men when mutated.
Study design, size, duration: This study has been performed at IGBMC, Strasbourg, France in collaboration with Bahceci Health Group, Istanbul, Turkey. Two Turkish consanguineous families having at least two well documented male infertility cases as well as two or more unaffected brothers, 86 Turkish men with spermatogenic failure and 107 fertile controls were included.
Participants/materials, setting, methods: Saliva samples were used as DNA source. A whole exome sequencing of two affected patients per family was performed by IGBMC microarray and sequencing platform. Suspected mutations were confirmed by Sanger sequencing first on family members, then on fertile controls and affected men with the same phenotype.
Main results and the role of chance: In family one, all affected males present a no-stop mutation which causes loss of the normal translational termination, leading to a 23 amino-acid extension of an X-linked gene. In family two, all affected males present, on the contrary, a stop-gained mutation on an autosomal gene, resulting in early translational arrest in the first 1/3 of the protein. Dramatic testicular size reduction was observed especially in affected males of the second family as well as in the corresponding KO mouse line. No mutation was found either in 107 healthy Turkish fertile controls or in 86 azoospermic and oligozoospermic Turkish infertile men. Additional tests were performed to define the effect of the mutations on the expression, localization and partners of the corresponding protein.
Limitations, reason for caution: Our study contains only a limited number of Turkish patients. Mutation screening should be continued on larger groups of infertile patients, including men of other ethnicities. Also for obvious ethical reasons, no in-vivo works were possible and only in-vitro experiments were done.
Wider implications of the findings: Understanding the genetic basis of male infertility has large implications not only for understanding the cause of infertility but also for determining the prognosis and management of such couples, and to provide maximally adapted therapeutics. It will then be possible to develop a diagnostic test and propose a molecular diagnosis to patients showing similar phenotype. It also opens the possibility to carry on more basic research on human spermatogenesis.
Study funding/competing interest(s): Funding by national/international organization(s) – Agence nationale de la recherché (ANR) and l'Agence de BioMédecine.
Trial registration number: NA.
Keywords: male infertility, whole exome sequencing, stop codon mutations, azoospermia, oligozoospermia

P-042 Making the embryologist's life easier during intracytoplasmatic sperm injection (ICSI): use of dimethylxanthine theophylline (SpermMobil®) for testicular sperm samples

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Study question: Is it possible to facilitate the embryologist's work during ICSI when dealing with testicular biopsy samples (TESE) in the absence of motile sperm? Would the use of dimethylxanthine theophylline compromise fertilization, implantation and pregnancy rates, or affect perinatal outcomes?
Summary answer: The use of a commercially available compound improved fertilization but not implantation and pregnancy rates among the groups. Perinatal outcomes (gestational age at birth, GA, weight and height) was also similar among the groups with the live birth of 21 children so far.
What is known already: Derivatives of theophylline increase intracellularly the levels of cyclic AMP, thus stimulating sperm motility and reducing the time required to find viable motile sperm. There has been only a limited number of studies showing the effects of SpermMobil® on pregnancy rates and only one live birth report after the use of this compound.
Study design, size, duration: ICSI cycles using TESE from January 2012 to October 2014 were analyzed retrospectively. 57 cycles did not require the use of SpermMobil® (group A), whereas 42 TESE (group B) presented no motility and were treated with SpermMobil® before ICSI. Two patients were excluded due to lack of motile sperm.
Participants/materials, setting, methods: TESE were treated mechanically and enzymatically, cryo-preserved, and thawed for therapy. Female partners underwent controlled ovarian stimulation and harvested oocytes were injected with motile sperm only. Embryos were cultured for up to 5 days. Statistical analysis were performed by Fischer's exact test, t-test, or Mann Whitney test.
Main results and the role of chance: Fertilization rates were higher in group 2 (65.22 versus 53.11%, $p = 0.0431$), showing a beneficial effect of SpermMobil®. There were no statistically significant differences among the groups regarding pregnancy (34.78 and 44.11%) and implantation (21.91 and 27.11%) rates among groups A and B, respectively, although group B tended to have higher rates. In group A, there were two biochemical pregnancies and two aborts, whereas in group B two aborts and 4 still ongoing pregnancies. GA,

weight and height at birth also did not differ among the groups (A, *n* = 12 newborns; B, *n* = 9 newborns). The embryologist´s work was also facilitated, as the time required to obtain enough motile sperm decreased.
Limitations, reason for caution: This is a retrospective analysis of ICSI outcomes using testicular motile spermatozoa only. As the benefits of this compound have been already reported, we opted not to make a truly negative group (no spermatozoa motility, no SpermMobil® treatment) not to jeopardize patient's treatment outcome.
Wider implications of the findings: This is the first report of multiple healthy births after the use of this chemical compound. The use of such a compound may facilitate not only the embryologist's work, by reducing the time needed to recover enough motile sperm, but also optimize patient's treatment outcomes by increasing the number of fertilized oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kinderwunschzentrum Ulm.
Trial registration number: NA.
Keywords: TESE, ICSI, theophylline, perinatal outcome

P-043 Antioxidant activity of CAPE (Caffeic acid phenethyl ester) in vitro can protect human sperm deoxyribonucleic acid from oxidative damage

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Study question: Oxidative stress is an important factor which influences fertility potential of spermatozoa by lipid peroxidation and formation of stable peroxidation products like malondialdehyde (MDA) in seminal plasma which may result in sperm dysfunction. Can CAPE Supplementation In Vitro Protect Human Sperm Deoxyribonucleic Acid From Oxidative Damage?
Summary answer: The results of this study emphasize the susceptibility of human spermatozoa to oxidative injury in vitro that preincubation of spermatozoa with the antioxidant CAPE offers protection against oxidative deoxyribonucleic acid (DNA) damage in vitro.
What is known already: Zini et al. have found that preincubation of spermatozoa with lycopene (5 µmol/L) effectively protected spermatozoa from DNA damage caused by subsequent incubation with H2O2.
Study design, size, duration: Prospective study. 30 control (fertile male donors), 30 treatment group (Oligoasthenoteratozoospermia male donors). Semen samples were obtained and allowed to liquefy at room temperature. After centrifugation and washing protocol spermatozoa were incubated in single step medium with 10, 50 and 100 µmol/L CAPE for 2 h at 36.0 °C.
Participants/materials, setting, methods: After incubation period, MDA levels of seminal plasma and spermatozoa were measured. Spermatozoa with fragmented DNA, as detected by Anilin Blue Assay with light microscopy, and morphology (spermatozoa structure) were analyzed by transmission microscopy.
Main results and the role of chance: Significant increase in percent DNA fragmentation index before the centrifugation (0.57 ± 0.15 respectively). Incubation of samples with 100 µmol/L CAPE resulted in a significantly lower percent DNA fragmentation index than samples incubated without CAPE (0.42 ± 0.12 respectively). ($P < 0.001$). Values are calculated, results are given as mean ± SD.
Limitations, reason for caution: Subject number has been limited with 30 participants. A limitation to our study is the relatively small sample size.
Wider implications of the findings: The results of this study emphasize the susceptibility of human spermatozoa to oxidative injury in vitro. The data suggest that preincubation of spermatozoa with the antioxidant CAPE offers protection against oxidative DNA damage in vitro. These data also highlight the differential effects of CAPE on sperm DNA integrity.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Zeynep Kamil Gynecology and Maternity Training and Research Hospital, IVF-ET Unit, Istanbul, Turkey.
Trial registration number: Basic science.
Keywords: sperm DNA damage, antioxidant, reactive oxygen species, CAPE

P-044 Evaluating the use of ubiquitin, a marker of defective sperm, as a potential infertility marker and selection tool within human sperm

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Study question: Can we confirm a relationship between the presence and levels of ubiquitin (Ubiq) in human sperm samples (SS) and their ability to succeed and result in a clinical pregnancy after an ICSI treatment with own and donated oocytes?
Summary answer: Percentage of stained cells as well as Ubiq protein quantity in sperm from infertile males achieving or not pregnancy by means of ICSI are similar, irrespective of the oocyte source, after controlling for clinical and individual confounders, leading to conclude that Ubiq involvement in sperm function seems limited hitherto.
What is known already: Ubiq is a highly conserved protein of 76 aminoacids which function is to tag proteins that should be degraded by the proteasome inside the cell. However, some groups proposed the idea of sperm surface ubiquitination occurring along the epididymis. They suggested that defective spermatozoa are tagged and therefore eliminated along the epididymis, while some of them could escape from this system, and consequently, ubiquitinated spermatozoa could be found within the ejaculate and fail fertilization.
Study design, size, duration: Nested cases and controls study analyzing spermatozoa from 21 ejaculates that achieved clinical pregnancy (*P*+) and spermatozoa from 18 SS that did not achieve it (P-), after undergoing ICSI treatments with own or donated oocytes prospectively collected, where Ubiq was measured by means of flow cytometry.
Participants/materials, setting, methods: From SS prepared for ICSI treatments, aliquots were fixed and stored for ulterior analysis once the pregnancy results were known. SS were incubated with Anti-Ubiq-Ab followed by secondary FITC-conjugated-Ab. Ubiq positively stained cells and mean fluorescence intensity was quantified and groups compared using T-test and logistic regression controlling confounding parameters.
Main results and the role of chance: Mean volume, concentration, motility and total motile count were 2.3 ml CI 95%(2.0–2.6), 56.1 mill/ml CI 95% (41.9–70.4); A + B 45.3% CI 95% (40.1–50.5), 59.9 mill CI 95% (43.6–76.2), respectively. In prepared sperm, 12.0mill/ml CI 95% (9.8–14.1), A + B 96.7% CI 95% (95.5–97.9) and 4.3 mill CI 95% (3.2–5.4). A mean number of 1.8 CI 95% (1.7–1.9) embryos were transferred, in 25 donation and 14 own oocytes'cycles. Women's mean age was 38.4y CI 95%(37.0–39.7). Ubq + ve cells were 60.3% CI 95% (47.5–73.2) in P+, and 56.2% CI 95% (41.7–70.6) in P-, while mean staining intensity was 186.14 CI 95% (74.2–298.1) in P+ vs. 211.1(133.2–288.9) in P-. The odds ratio (OR) of P+/P- depending on the Ubq% of stained cells was 1.01CI 95% (0.98–1.03), B = -0.05, while staining intensity OR = 1.00 CI 95% (1.00–1.00), B < 0.001. Once adjusted for potential clinical confounders (day of embryo transfer, own or donated oocytes, age, female etiology, sperm features, embryos transferred, etc…), Adj (OR) Ubiq% of stained cells was 1.01CI95%(0.98–1.02), B = 0.05, while staining intensity Adj (OR) = 1.00 CI 95% (1.00–1.00), B < 0.001.
Limitations, reason for caution: Other sperm factors not included within this analysis could be biasing the studied relationships, given that sperm function has been demonstrated to be multiparametric. Further confirmation of our results is needed from other studies, confirming or not if our results are extrapolable to other subpopulations or different assisted reproduction protocols.
Wider implications of the findings: Ubiquitin is a sperm membrane protein, specific of malfunctioning sperm, that may be potentially used as an infertility marker, thus suitable for the development of a sperm selection tool based on the use magnetic activated cell sorting (MACS) technology to eliminate Ubq+ve cells and improve ART results. Nevertheless, there is a previous need to confirm the link between the protein in sperm and clinical results with ICSI, and this has not been accomplished yet.

Study funding/competing interest(s): Funding by national/international organization(s) – Department of Industry, Innovation, Trade and Tourism and FEDER funding (EU)(IG-2011/0000681 and IG-2012/0000497).
Trial registration number: NA.
Keywords: ubiquitin, spermatozoa, infertility marker, male fertility, flow cytometry

P-045 Virtual slide is a relevant tool for training when assessing human sperm morphology

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Study question: Semen smears are traditionally used for training and QC in sperm morphology. However, it does not allow the evaluation of exactly the same cells between operators. Virtual slide is based on high-definition on-screen morphology assessment on numbered spermatozoa, allowing training, e-learning and QC, with comparison with a correction grid.
Summary answer: Yes, virtual slide is an efficient tool for training and QC in sperm morphology assessment, allowing operators to work on identified spermatozoa and compare themselves with correction grid.
What is known already: Sperm analysis suffers from intra and inter-operator variability, highlighting the need for training and QC in andrology laboratories. External quality control programs should concern sperm concentration, motility and morphology evaluation. Whereas sperm concentration and motility can be correctly assessed for QC with fixed-sperm suspension and movies respectively, morphology assessment is generally based on semen smears, which raises the issue of individual sperm classification and availability of corrected answers for trainers.
Study design, size, duration: Between 2006 and 2013, 5 e-learning case studies including sperm morphology assessment via virtual slide were proposed to the 470 Spermionet e-learning facility members.
Participants/materials, setting, methods: Participants had to classify 100 spermatozoa on a virtual slide available online obtained after high-definition scanning of a Shorr-stained smear. Each spermatozoa was numbered and participants had to classify them according to David classification. Individual sperm classification based on consensual evaluation by a panel of experts was presented afterwards.
Main results and the role of chance: There was an acceptable agreement between participants and consensual values provided by the steering committee of Spermionet. More than 50% of the participants had morphology results within the acceptability range according to Ricos tables.
Limitations, reason for caution: E-learning cases based on sperm morphology assessment performed up to now have not included WHO classification. In addition to e-learning facility for training, external QC programes for sperm morphology will also be based on virtual slide starting from 2016.
Wider implications of the findings: Virtual slide provides an opportunity to improve and standardize training and external QC for sperm morphology. It could easily be used for skill follow-up within lab's staff. Virtual slide could eventually also be used for training and QC in sperm vitality assessment.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Biologie Prospective.
Trial registration number: NA.
Keywords: semen analysis, andrology, quality control

P-046 Is there a future for human round spermatid injection (ROSI)?

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Study question: Can an improved human round spermatid injection into the oocyte (ROSI) be considered a worthy clinical treatment despite the fact that conventional ROSI has been recognized as being ineffective for non-obstructive azoospermia due to its extreme low success rates?
Summary answer: In this study ROSI fully proved to be effective clinically when there is proper identification of round spermatid and oocyte activation.
What is known already: To date, the application of ROSI in clinical IVF has had disappointing results due to difficulties in accurate identification of round spermatids among other round spermatogenic cells and insufficient oocyte activation.

Patients who may be candidates for ROSI should receive careful and thorough pretreatment counseling to ensure they are clearly informed of the limitations and potential risks of the procedure.
Study design, size, duration: A total of 44 non-obstructive azoospermic men whose first MD-TESE conducted by andrologists showed no testicular spermatozoa or late staged spermatids but had round spermatids found at our hospital and received ROSI from September 2011 to October 2014. Study approved by the Japanese Ministry of Health, Labour and Welfare.
Participants/materials, setting, methods: Round spermatids, cytologically selected after thawing, were injected into ooplasm which was activated 10 minutes before the injection by electrical stimulation with an alternating current pulse of 2V/cm for 8s + direct current pulse of a single 1.2 kV/cm for 99 µs.
Main results and the role of chance: The percentage of occurrence of 1PN, 2PN and 3PN after electrical stimulation were 80% (8/10), 20% (2/10) and 0 respectively. Fertilization and rate to develop over 4 cell stage were 59.0% (315/534), 56.7% (303/534) respectively. The pregnancy rate per transferred cycle, miscarriage rate and birth rate in fresh embryo transfer cycles and freezing-thawed transfer cycles were [16.3%(16/98), 68.8% (11/16), 5.1% (5/98)], [36.8% (7/19), 42.9% (3/7), 21.1% (4/19)] respectively.

No abnormal karyotype and genomic imprinting abnormalities (IGF2, H19, SNRPN) were identified in any of the newborn babies. All of 4 female and 7 male babies are healthy and no serious physical or cognitive disorders have been reported so far.
Limitations, reason for caution: The limitation of this clinical study concerns the small size of the study group, patients who had received MD-TESE by other andrologists and sperm could not be found but had round spermatids found at the second MD-TESE performed at our hospital.
Wider implications of the findings: Application of ROSI in clinical human IVF is still considered experimental. However, this study presents evidence that ROSI has a high potential to help many non-obstructive azoospermic men whose most advanced spermatogenic cells are round spermatids.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Saint Mother Obstetrics and Gynecology Clinic and Institute for ART.
Trial registration number: UMIN Clinical Trials Registry UMIN000006117.
Keywords: round spermatid injection, oocyte activation, non-obstructive azoospermia, MD-TESE

P-047 Molecular atlas of different stages of human spermatogenesis: Sertoli cells, spermatogonia and sperm

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Study question: The aim of this study is to establish the molecular atlas of human spermatogenesis. We started to optimize the methods for isolation of cell populations that are involved in spermatogenesis (especially Sertoli cells, spermatotogonia and sperm) and performed transcriptomic and immunocytochemistry analyses to better understand the human spermatogenesis.
Summary answer: Our results showed that it is possible to establish the gene expression profiles of selected cell populations of spermatogenesis with microarrays validated by Fluidigm real-time PCR. Detailed differential and functional bioinformatics analysis was followed by mapping and varifying the cellular localization of candidate genes by in situ hybridization and immunohistochemistry.
What is known already: Because of several technical limitations no detailed knowledge on the molecular profile of different stages of human spermatogenesis and the cell types involved is available at present. This would be an

important prerequisite to further develop strategies for diagnosis and treatment of male infertility by assisted reproduction, to better understand the manifestation of testicular cancer and to develop better opportunities for individualized diagnosis and therapy of cancer in the future.
Study design, size, duration: In this study, five groups of human spermatogenic cells were analyzed using transcriptomics and immunocytochemistry:

1.) laminin and alkaline phosphatase (AP)-enriched spermatogonia.
2.) lectin-enriched Sertoli cells (patients with obstructive azoospermia, with sperm),
3.) lectin-enriched Sertoli cells (patients with non-obstructive azoospermia, no sperm).
4.) testicular sperm (azoospermia),
5.) ejaculated sperm (normospermia).

Participants/materials, setting, methods: Each group of human spermatogenic cells was analyzed for its gene expression profile in three biological replicates using microarrays. The most interesting genes were further validated by qPCR and statistically evaluated using Principal Component Analysis (PCA), Student *T*-test, and one-way ANOVA to elucidate differences between groups.
Main results and the role of chance: Specificity of laminin/alkaline phosphatase-enrichment for germ cell lineage and lectin-enrichment of Sertoli cells were confirmed by UTF1/VASA and VIM/SOX9 expression, as revealed by immunohistochemistry. Principal component analysis (PCA) of the microarray data revealed that all cell populations clearly separated from each other. Expressions of specific genes related to Sertoli cells, sperm and spermatogonia in selected populations of cells confirmed the 'character' of selected cells. Bioinformatics elucidated the functional differences between the various groups of cells. According to F-test analysis, the most interesting genes were confirmed by Fluidigm real-time PCR. The validation experiments were also performed on various sections of testicular samples using automation of non-radioactive in situ hybridization in combination with cell type-specific immunohistochemical markers and some new interesting cell type-specific gene expression patterns were identified.
Limitations, reason for caution: The testicular biopsies used for this study were from patients with obstructive and non-obstructive pathologies. It was possible to select the enriched populations of cells that may still contain some other types of cells.
Wider implications of the findings: Our results provide a new set of genes that could serve as important biomarkers to better understand the human spermatogenesis in the future, especially in association with male age, testicular cancer (seminoma) and fertility/infertility to explore it in a more detail. Moreover, the selected populations of spermatogenic cells could serve as a model to study the effects of xenoestrogens and their potential involvement in infertility and manifestation of testicular cancer.
Study funding/competing interest(s): Funding by national/international organization(s). This study was funded by the German Federal Ministry of Education and Research (BMBF; bilateral collaborative research project) and the Slovenian Research Agency (ARRS).
Trial registration number: The study was performed after approvals of the Ethical Committee of University of Heidelberg and Slovenian National Medical Ethics Committee.
Keywords: human spermatogenesis, human sertoli cells

P-048 Prevalence of human papillomavirus (HPV) sperm infection and fertility outcome in 200 couples candidate to assisted reproduction techniques

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Study question: The objectives of this study were to evaluate the prevalence of human papillomavirus (HPV) semen infection in males of infertile couples and to compare the reproductive outcome in infected and non infected patients.
Summary answer: Our results demonstrated a very high prevalence of HPV sperm infection in infertile patients. Positive HPV patients had no spontaneous pregnancy and a lower cumulative pregnancy rate both by intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI).
What is known already: Several studies showed the bond of HPV to sperm and/or exfoliated cells and its association with altered sperm parameters. Moreover, some authors demonstrated that infected sperm are able to transmit viral genes to oocyte and that HPV may cause pregnancy loss by DNA fragmentation and apoptosis of embryonic cells. Finally, a recent study performed in couples undergoing ICSI, showed a significant increase of pregnancy loss when HPV DNA was present at sperm level.
Study design, size, duration: Cross-sectional clinical study. Between 2012 and 2014, 182 consecutive infertile patients of couples candidate to assisted reproduction were evaluated at our unit. We included subjects with altered sperm parameters, at least 2 years of unprotected sexual intercourse without conception, and normal female partners with negative PAP test.
Participants/materials, setting, methods: All patients were evaluated for sperm parameters according to world health organization guidelines 2010 and HPV detection on semen was made by fluorescence in situ hybridization (FISH). Patients were followed from the time of diagnosis, through the ART period, to date.
Main results and the role of chance: Among patients, 38 (20.8%) had HPV semen infection (group A) and 144 had not (Group B). Infected males showed the virus bound to sperm or to exfoliated cells or to both cells in 52.6, 26.3 and 21.1% respectively. During diagnosis period, 12 spontaneous pregnancies were recorded only in Group B. IUI was performed in 16 and 49 couples, ICSI in 22 and 83 couples from groups A and B respectively and cumulative pregnancy rates were 13.1 and 38.9% ($p < 0.01$). We recorded 5 pregnancies in group A and 56 in group B and the follow up showed respectively: 1 and 16 healthy born, 2 and 38 ongoing pregnancies, 2 and 2 miscarriages. Interestingly, abortions of group A regarded only patients with infection on sperm.
Limitations, reason for caution: The exact mechanism by which the presence of HPV in sperm is able to reduce pregnancy rate remains unknown and it is worthy of further investigation. Moreover, a larger group of infertile patients with HPV semen infection should be considered to drive final conclusions.
Wider implications of the findings: The high prevalence of HPV sperm infection observed in infertile patients suggests a role for this infection in male infertility. We had no ongoing pregnancies in patients with HPV infection at sperm level. Results from this study rise concern about the possible role of HPV on natural and assisted reproduction outcome. Screening for HPV should be considered in the workup of infertile patients and before ART.
Study funding/competing interest(s): Funding by University(ies) – University of Padova.
Trial registration number: Protocol Number 2331P.
Keywords: male infertility, HPV sperm infection, HPV and fertilization, reproductive outcome, assisted reproductive technology

P-049 The influence of croton caudatus Geiseler (Tukul Takal) on spermatogenic activity in the sexual dysfunction-induced rats

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Study question: The study was designed to investigate the effect of *Croton caudatus* Geiseler supplementation on the spermatogenic activity and their histological parameters in the sexual dysfunction-induced rats.
Summary answer: The findings demonstrated that the supplementation of *C. caudatus* roots extract improves the sperm count and quality in Bisphenol A-induced rat model. The histological parameters also revealed that the extract improves the spermatogenesis activity within the seminiferous tubules and does not exhibit toxicity effect to liver and kidney.
What is known already: Sexual dysfunctions cover a wide variety of problems that may be due to low sperm count, poor sperm quality, or both. Advances in phytomedicine have allowed for more efficient and cost-effective treatment of infertility. However, to date, least information is available on the anti-infertility

properties of *C. caudatus* Geiseler. Furthermore, no scientific data about the mechanisms of reversible infertility of this herb have been reported.
Study design, size, duration: Thirty male Wistar rats (9 weeks old) were randomly divided into five groups, namely; the negative control (distilled water); the positive control (200 mg/kg BPA), and the treatment groups which received 16, 32, and 64 mg/kg doses of *C. caudatus* root extracts + 200 mg/kg BPA, orally for twenty one days.
Participants/materials, setting, methods: After twenty one days of treatment, blood were collected and proceeded for liver and renal function test, as well as testosterone level. Sperm parameters were examined using Hamilton Thorne Sperm Analyzer and testis were harvested for histological assessments.
Main results and the role of chance: Overall, results showed *C. caudatus* at 64 mg/kg significantly ($p < 0.001$) increased the total sperm count and progressive cells motility; as well as decreased abnormal sperm morphology. The testosterone hormones were slightly decreased but within normal range. The histological results revealed that normal morphology of germ cells organized in concentric layers of seminiferous tubules. Abundant and compact spermatogenic cells were found on the lumen of seminiferous tubules which indicate the influence of spermatogenesis process that facilitated by this herb.
Limitations, reason for caution: Whilst these animal experiments suggest that this herb improved the spermatogenesis activity, sperm count and motility; as well as reduced sperm abnormalities, future clinical trials should be done in human for scientifically proven.
Wider implications of the findings: Data from this study may serve as an important indicator for anti-infertility agent from this herb. Practising natural treatment for infertility then be carried out for the sterile males, thereby creating a more cost-effective option for infertility treatment. Therefore, combination of sperm parameter, immunological aspects, DNA integrity and understanding of the mechanisms as how this herb influences the sperm activity would give a better insight regarding anti-infertility potential of this herb.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). This research was supported by the Research Acculturation Grant Scheme (RAGS) No: 600-RMI/RAGS 5/3(45/2014), Ministry of Education, Malaysia to Razif Dasiman, Fatimah Sham, Fatin Nadzirah Zakaria and Nina Keterina Hashim. The authors declare no competing interests.
Trial registration number: NA.
Keywords: croton caudatus Geiseler, spermatogenic activity, sexual dysfunction, bisphenol A, anti-infertility

P-050 The effect of microsurgery inguinal varicocelectomy by secondary infertile male patients with clinical varicocele

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Study question: Is Micro Surgery Inguinal Varicocelectomy effective in secondary infertile male patients?
Summary answer: Microsurgery inguinal varicocelectomy is an effective therapy method by secondary infertile patients because of it's improving effect on semen parameters which results in rising of pregnancy rates.
What is known already: Varicocele is the most common correctable causes of male infertility.
Study design, size, duration: During the last four years, sixty primary infertile and thirty secondary infertile patients whom were referred to andrology clinic due to infertility and undergone Microsurgery Inguinal Varicocelectomy operation because of varicocele presence, were retrospectively investigated.
Participants/materials, setting, methods: Patients were assessed according to age, duration of the infertility history, grade of varicocele, pre and postoperative semen parameters and pregnancy rates.
Main results and the role of chance: It was detected that sperm motility and number of total motile sperms with primary infertile group and number of spermia, motile spermia and sperm motility by secondary infertile group have statistically significantly increased after surgical procedure ($p < 0,05$). Pregnancy rate by secondary infertile group was observed as higher as the rate in the primary infertile group which was not statistically significant.
Limitations, reason for caution: The number of participants, retrospective study.
Wider implications of the findings: Pregnancy rate by secondary infertile group was observed as higher as the rate in the primary infertile group which was not statistically significant.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Firat University Hospital.
Trial registration number: NA.
Keywords: varicocele, secondary infertility, microsurgery inguinal varicocelectomy.

P-051 Pharmacological stimulation of sperm motility in microdissection testicular sperm extraction (micro-TESE) using theophylline in patients with non-obstructive azoospermia (NOA)

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Study question: To evaluate whether theophylline improves sperm motility and treatment outcomes in micro-TESE in patients with NOA.
Summary answer: Theophylline allowed the selection of live sperm from immotile testicular sperm for intracytoplasmic sperm injection (ICSI), significantly improving fertilization and blastocyst formation rates in patients with NOA.
What is known already: Micro-TESE was shown to be the most effective method of sperm retrieval in patients with NOA. However, few spermatozoa are present in these patients, and, as a rule, those are immotile. Theophylline has been known to be reliable in stimulating testicular spermatozoa. Micro-TESE and theophylline stimulation of sperm motility may significantly increase success rates in patients with NOA.
Study design, size, duration: The study included 43 patients with NOA. Spermatozoa retrieved from all 43 patients by micro-TESE were immotile. Theophylline activation was performed in 24 cases, and then motile sperm was used for ICSI (treatment group). In 19 cases there was no theophylline activation (control group).
Participants/materials, setting, methods: Sperm motility was stimulated by GM501 SPERMMOBIL (Gynemed), according to the manufacturer's protocol. Fertilization, blastocyst formation, and clinical pregnancy (% per ET, % per micro-TESE) rates were compared in the two groups.
Main results and the role of chance: Following activation, 13.44 ± 7.26% of spermatozoa became motile. The fertilization rate (63.41% [104/164] vs. 29.87% [46/154], $p = 0.001$) and blastocyst formation rate (64.42% [67/104] vs. 43.47% [20/46], $p = 0.03$) were significantly higher in the treatment than in the control group. Clinical pregnancy rates per ET (28.57% [6/21] vs. 10.52% [2/19], $p = 0.27$) and per micro-TESE (25% [6/24] vs. 10.52% [2/19], $p = 0.28$) were higher in the treatment than in control group, but the difference was not statistically significant.
Limitations, reason for caution: Micro-TESE and oocyte retrieval were performed simultaneously, using fresh spermatozoa for ICSI. This study is limited by its statistical power in pregnancy rates which is not very high due to the sample size (43 patients).
Wider implications of the findings: Pharmacological stimulation of sperm motility in micro-TESE, using theophylline in patients with NOA, significantly improved fertilization and blastocyst formation rates, as well as improving pregnancy rates comparable to those achieved with spermatozoa from semen.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for Reproductive Medicine MAMA, Moscow.
Trial registration number: NA.
Keywords: micro-TESE, pharmacological stimulation, theophylline, ICSI, NOA

P-052 Age independent of body mass index negatively affects semen quality

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Study question: To find effects of age and body mass index on semen quality (volume, concentration, total number, motility, rapid movement, progressive movement, normal morphology).
Summary answer: Controlling for body mass index, age is negatively associated with semen volume, total number, motility, rapid movement, and progressive movement. Controlling for age, body mass index is associated with none of semen parameters.
What is known already: Age alone has been known to negatively affect semen quality while body mass index alone does not significantly affect semen quality. However, it is unclear whether age would have differing effects on semen quality at various levels of body mass index.
Study design, size, duration: We investigated the effects of age and body mass index on semen quality via a retrospective cohort study in male patients attending a fertility clinic at King Chulalongkorn Memorial Hospital. One thousand three hundred and seventy-seven patients were included in the study.
Participants/materials, setting, methods: We used multivariate regression models to test for effects of age and body mass index concurrently on each of sperm analysis parameters (volume, concentration, total number, motility, rapid movement, progressive movement, normal morphology). Statistical significance was determined at $p < 0.05$.
Main results and the role of chance: Our participants' mean age was 36.8 years (SD = 6.4). Mean body mass index was 24.9 (SD = 3.6). Controlled for body mass index, age was significantly, negatively associated with semen volume (coefficient = -0.15; $p < 0.001$), total number (coefficient = -0.07; $p = 0.018$), motility (coefficient = -0.16; $p < 0.001$), rapid movement (coefficient = -0.19; $p < 0.001$) and progressive movement (coefficient = -0.19; $p < 0.001$) but not with concentration and normal morphology. Controlled for age, body mass index did not associated with semen volume, concentration, total number, motility, rapid movement, progressive movement and normal morphology.
Limitations, reason for caution: Our study participants were from one fertility clinic. Future studies to include more diverse study population and with fertility outcomes in addition to semen quality can be helpful to validate our findings.
Wider implications of the findings: There was limited evidence on effects of age and body mass index on semen quality in Asian population. This study shows that age but not body mass index is associated with semen quality. With advantages of multivariate regression models, this study showed and confirmed that, independent of body mass index, age is negatively associated with semen quality. This information can be helpful in patient education and counselling.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society (Bangkok, Thailand)
Trial registration number: NA.
Keywords: age, body mass index, semen quality

P-053 Effect of leukocytospermia and *in vitro* zinc supplementation on DNA integrity and seminal oxidative stress

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Study question: To investigate whether leucocytes and *in vitro* zinc supplementation could interfere with sperm DNA integrity and seminal oxidative stress.
Summary answer: Even a low amount of leukocytes may lead to sperm quality impairment whereas zinc may enhance sperm quality.
What is known already: Oxidative stress is caused by the unbalance between amounts of reactive oxygen species and seminal antioxidant scavenging systems. It seems to be one of the main causes leading to male infertility.
Study design, size, duration: A prospective study performed on 94 semen samples.
Participants/materials, setting, methods: The nitroblue tetrazolium test was carried out for superoxide anion estimation, the TUNEL assay for DNA fragmentation and the acridine orange assay for DNA denaturation. Total antioxidant status and enzymatic antioxidants: superoxide dismutase and glutathione peroxidase were measured using an assay kit. Lipid peroxidation was evaluated by Malondialdehyde production.
Main results and the role of chance: The level of leucocytes was positively correlated with superoxide anion generation ($r = 0.46$; $p < 0.001$) and DNA denaturation ($r = 0.605$; $p = 0.013$). Samples containing leucocytes had a significantly higher DNA fragmentation index compared to control samples. Our results showed that zinc concentration of 6 µmol/L significantly reduced DNA fragmentation ($p = 0.014$) and seminal antioxidant status ($p < 0.001$). Both 2 and 6 µmol/L zinc concentration increased superoxide dismutase level and decreased superoxide anion production ($p < 0.001$). The impact of zinc on both glutathione peroxidase level and lipid peroxidation amount was not significant.
Limitations, reason for caution: DNA fragmentation assay and superoxide anion generation were investigated for each of the 98 samples. While oxidative stress parameters were assayed for only 35. DNA denaturation was performed for 24 samples.
Wider implications of the findings: The findings on the deletirious effect of leukocytospermia are contoversial. we demonstrated that even a few amount of leukocytes leads to sperm quality impairment. So in case of leukocytospermia it could be beneficial to associate zinc to antibiotics. Zinc could also improve assisted reproductive techniques outcomes when added to embryo culture media.
Study funding/competing interest(s): Funding by University(ies) – Laboratory of Histology Embryology and Cytogenetic (UR 12 ES 10), Faculty of Medicine, Street Avicenna, Monastir 5019, University of Monastir, Tunisia.
Trial registration number: NA.
Keywords: leukocytospermia, zinc, male infertility, DNA integrity, sperm

P-054 Seminal fluid biomarkers and IUI clinical outcome

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Study question: We questioned whether assessment of fructose level in the seminal plasma of subfertile men can serve as a biomarker coadjutant to standard semen analysis. In addition, we questioned whether fructose and total antioxidant capacity (TAC) can be used to gain information on spermatogenic competence and to predict pregnancy outcome.
Summary answer: Our analysis detected an inverse correlation between fructose level and male age unconnected to sperm parameters. Oligo-/oligoathenospermic men had compromised fructose levels in comparison to the normal fertile cohort. In IUI treatments, men with higher fructose and TAC levels had significantly higher chances of pregnancies.
What is known already: Approximately 65–75% of the seminal fluid is provided by the seminal vesicles and one of the largest biochemical compounds found in this gland is fructose, the main carbohydrate energy source of spermatozoa. Seminal vesicles are also considered a major contributor of antioxidants that protect spermatozoa from oxidative insults and sustain their function and integrity. Fructose and TAC may elucidate genital tract patency and proper function of the male gamete, especially in men with severely compromised sperm parameters.
Study design, size, duration: A total of 80 men were included in this 5-month prospective study. Fructose and TAC were plotted against semen parameters in patients undergoing IUI or IVF.
Participants/materials, setting, methods: Specimens were collected at our center from consenting patients undergoing routine semen analysis. After liquefaction, semen parameters were assessed according to the WHO criteria (2010). The specimens were centrifuged and the cell-void seminal plasma was assessed for fructose and TAC concentrations using colormetric assays on an automated microplate reader ($\Delta OD_{405/570}$).
Main results and the role of chance: In 80 men (39.6 ± 8 years), sperm concentration was 26.7 ± 27 × 10^6/ml and motility 42.7 ± 14%. TAC inversely correlated to abstinence ($P = 0.01$) while positively with semen parameters ($P < 0.05$). TAC increased with fructose ($P < 0.001$) and both decreased with age ($P < 0.05$). Normozoospermic men ($n = 34$) exhibited a fructose (2.1 ± 0.9 µg/mL) higher than oligozoospermic ($n = 18$; 1.6 ± 0.9 µg/mL) and oligoasthenozoospermic ($n = 12$; 1.4 ± 1.2 µg/mL). NOA ($n = 8$; 2.5 ± 0.9 µg/mL) had the highest level of fructose ($P < 0.05$). In 22 men undergoing 41 IUI cycles with their female partner (35.5 ± 5 years), a clinical pregnancy of 17.1%, fructose of 2.8 ± 0.7 µg/mL, and TAC 2001.0 ± 133 nmol/ml, higher than the non-pregnant group (1.8 ± 1.3 µg/mL and 1.783.0 ± 195.6 nmol/ml) ($P < 0.05$). Five couples that failed IUI along with 20 others (maternal age 37.7 ± 5 years) underwent 35 ART cycles and achieved clinical pregnancies of 31.4% with a fructose level of 1.7 ± 1.1 mg and TAC of 1764.4 ± 261.5 nmol/ml.

Limitations, reason for caution: We have two identified seminal biomarkers that may be utilized in addition to a standard semen analysis to assess a man's fertility. While these results are promising, these are still preliminary data and need farther validation.
Wider implications of the findings: The inadequacy of a standard semen analysis to provide information on spermatogenesis has been widely recognized. Due to the concurrent dependence of seminal vesicle function and spermatozoa production under gonadotropic control, fructose assessment may provide insight on the germinal epithelium integrity**.** The interpretation of semen biomarkers such as the assessment of fructose and TAC may be particularly useful in diagnosing infertile men and to select appropriate insemination methods.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: NA.
Keywords: total antioxidant capacity TAC, fructose, seminal plasma, ICSI, IUI

P-055 Seminal fluid ROS-buffering capacity relates to sperm parameters, chromatin integrity and embryo developmental competence

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Study question: We question whether seminal plasma's total antioxidant capacity (TAC) has any effect on spermatozoa parameters, predicts male genome integrity, or impacts the ability of the male gamete to participate in embryo development. Therefore we wonder whether TAC measurement may aid in the prognostication of male factor infertility.
Summary answer: TAC decreases by lengthening the abstinence period, but increases with sperm concentration, motility, and morphology. TAC protects the sperm's chromatin's, as inferred by mean sperm DFI. Most importantly, a compromised TAC in the inseminating specimen was accompanied by a lower chance of pregnancy.
What is known already: The use of seminal biomarkers to predict gamete health and embryo developmental competence is under investigation. During spermiogenesis and throughout the genital tract, reactive oxidative species (ROS) are generated from developing, maturing, and decaying spermatozoa. The natural buffering capacity of seminal plasma reduces the effect of oxidative insults and sustains spermatozoa motility, preserving their competence. Direct and indirect assays to measure ROS are currently being validated to screen male factor infertility.
Study design, size, duration: Prospectively during the last 5 months, we assessed TAC in ejaculates of 65 men. We plotted TAC against semen parameters and sperm DNA fragmentation index (DFI). For subjects that underwent IUI and ART with their female partners, the relationship between TAC and clinical outcome was investigated.
Participants/materials, setting, methods: TAC, in Trolox equivalents was assessed by a colorimetric assay on an automated microplate reader (ΔOD_{405}). Direct ROS damage to sperm DNA was evaluated by TUNEL. For most of these men, their reproductive outcomes with IUI and ART were evaluated in relation to the TAC of the inseminating specimen.
Main results and the role of chance: In 65 men (39.7 ± 8 years), sperm concentration was 35.9 ± 25 × 10^6/ml, motility 42.7 ± 14%, and morphology 2.4 ± 1%. The TAC for normozoospermic men (n = 34) was 1965.9 ± 212 nmol/ml and for oligoasthenozoospermic (n = 12), 1720.2 ± 264.3 nmol/ml (P = 0.01). TAC inversely correlated to abstinence (P = 0.01) while positively with semen concentration, motility, and morphology (P < 0.05). In 13 subjects, a lower TAC was associated with compromised sperm DFI (P < 0.001). In 22 couples (maternal age 35.5 ± 5 years) treated in 41 IUI cycles, clinical pregnancies of 17.1% (n = 7) were accompanied by a TAC of 2001.0 ± 133 nmol/ml. This was higher than in couples whose treatments did not result in pregnancy (n = 15; 1783.0 ± 195.6 nmol/ml) (P < 0.01). Five couples (maternal age 37.7 ± 5 years) who failed IUI, along with 17 others with a suboptimal TAC (1,741.6 ± 296 nmol/ml) were treated in 32 ICSI cycles, resulting in a clinical pregnancy rate of 25.0%.
Limitations, reason for caution: Even though the benefit of a higher antioxidant capacity exerted by the seminal plasma has an undisputed benefit on the health and performance of the male gamete, this biomarker is not yet validated as a valuable screening assay for male gamete performance.
Wider implications of the findings: The limitations of the semen analysis to screen for male factor infertility are apparent and additional biomarkers are needed to acquire further insight on spermatozoa function and its structural integrity. Seminal TAC, an indirect measurement of ROS, provides information on sperm DNA integrity and may help predict IUI outcomes. TAC level in the ejaculate improves male factor infertility screening and helps steer the infertile couple toward the proper insemination method.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, WCMC.
Trial registration number: NA.
Keywords: reactive oxygen species ROS, total antioxidant capacity TAC, sperm DNA integrity, spermiogenesis, ICSI

P-056 Seminal biomarkers to guide sperm sourcing

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Study question: Insight on the protective effect of seminal antioxidant capacity on sperm DNA integrity and its effect on gamete characteristics and embryo developmental competence has guided us to perform this experiment. We question whether sampling the male gamete proximal to the germinal epithelium yields spermatozoa with a healthier genome.
Summary answer: DNA fragmentation index originates during spermiogenesis and progressively increases as the sperm traverses the male genital tract. When compounded by an impaired level of total antioxidant capacity (TAC) in the ejaculate, elevated DFI may justify offering alternative sperm sourcing to aid positive IVF outcomes.
What is known already: During the later stages of spermiogenesis DNA breakage is physiologically induced to allow tight chromatin compaction. While most spermatozoa undergo DNA repair, oxygen free radicals are the main cause of DNA injury. The buffering capacity of seminal antioxidants is the only agent protecting the DNA integrity of spermatozoa in the ejaculate. Therefore, testing the sperm's DFI and concurrent seminal TAC is essential to assess gamete competence.
Study design, size, duration: Over 15 months, men with extremely high DFI in their ejaculates (n = 36) received a TAC analysis on seminal fluid. Following counseling, men underwent surgical sampling, often bilateral, from vas deferens, epididymis, and testis. DFI and clinical outcome were recorded and compared for each individual sperm sourcing for men undergoing ICSI.
Participants/materials, setting, methods: Ejaculates processed in standard fashion were assessed for DFI and TAC. Surgical samples minced and smeared for DFI evaluation were cryopreserved for later use in ICSI. DNA fragmentation was measured by TUNEL on specimens isolated from all sites. TAC was determined by a colorimetric assay on an automated microplate reader.
Main results and the role of chance: In 51 ejaculates the average DFI was 49.8 ± 25.2% (range 26.0–96.0). In some men (n = 13), the DFI had a clear inverse correlation with TAC (P = 0.001). In 4 men aspiration of the vas deferens resulted in 19.3 ± 4.8% DFI (range 15.7–30.0) while in 16 men epididymal sampling yielded 19.0 ± 7.0% DFI (range 8.4–33.8) and in 33 the DFI on testicular spermatozoa was 13.2 ± 6.7% (range 2.0–27.0). The DFI progressively decreased as we retrieved proximally toward the vas deferens (P = 0.005), the epididymis (P = 0.0001), and testis (P = 0.0001). ART outcome achieved by ICSI treatment using these surgical sources yielded a clinical pregnancy of 34.3%, higher than with the ejaculate at 20.0%.
Limitations, reason for caution: Patients need to be informed of risks regarding surgery, anesthesia, and be aware that even with surgical spermatozoa a pregnancy may not occur. Thus, counseling should be conducted since many of these men have spermatozoa in their ejaculate. These data are still preliminary that require an evidence-based consensus.
Wider implications of the findings: The topographic sourcing of spermatozoa from different levels of the male genital tract indicates that disruption of DNA integrity, although starting during spermiogenesis, may actually suffer the additional effect of oxidative stressors that combined with a compromised TAC yield higher DFI in the ejaculate. Couples with recurrent pregnancy failures and ejaculated spermatozoa with high DNA fragmentation may benefit from undergoing surgical sampling for diagnostic and therapeutic purposes.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: NA.
Keywords: sperm DNA integrity, TESE, surgically retrieved spermatozoa, ICSI, total antioxidant capacity TAC

P-057 A seminal biomarker of germinative epithelium integrity

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Study question: To characterize the origin and meaning of round cells (RC) in the human ejaculate. We question the relationship of this sporadic marker with male gamete characteristics. We also wonder about the embryo developmental competence of spermatozoa presenting with RC.
Summary answer: RC are not related to infectious agents but are almost exclusively comprised of multiple, haploid nuclei generated during an offset spermatogenesis. RC may serve as an indicator of an injured germinal epithelium caused by a seasonal ailment affecting sperm production, chromatin integrity and sperm aneuploidy.
What is known already: The source of round cells in the ejaculate has always been a puzzling conundrum. The lack of an association of round cells with infectious agents together with their apparent beneficial effect on the competence of the male gamete's contribution to embryo developmental has further contributed to this equivocal interpretation.
Study design, size, duration: A total of 4,810 men undergoing male infertility screening over a 24-month period were included in this prospective study. Specimens utilized for ART were grouped according to [RC] 1–1.9 and ≥2 × 10^6/mL and compared to a control. LeucoScreen™, cytoplasmic markers, ploidy assessment, and TUNEL assay were used to typify RC.
Participants/materials, setting, methods: Ejaculates were grouped in relation to RC amount and evaluated for ART outcome. In men with repetitive observations, semen parameters were assessed in relation to the RC occurrences throughout the calendar year. To assess their spermiogenic stage, round cells were stained for ploidy, DFI, vimentin, inhibin, and protamine.
Main results and the role of chance: Prevalence of samples with RC was 5.4% (n = 261) with normal semen parameters. RC were comprised mostly of immature germ cells (IGC)(range 0.79–24.9 million) with the remainder being WBC at 26.7%. Most specimens (97%) were negative for uropathogens. IGC were composed of single or multiple haploid nuclei similar to that of spermatozoa, but decondensed with lower protamine content. Spermatozoa with IGC had a higher DFI (P = 0.0001) and aneuploidy (P = 0.0001). The DFI of IGC itself was higher than the accompanying spermatozoa (P = 0.0001). Men (n = 27) (42.3 ± 8 years) that underwent 33 ICSI cycles with their female partner (38.0 ± 4 years) reported decreased number of ongoing pregnancies and deliveries, but yielded higher pregnancy losses than a control (P < 0.05). Manifestation of RC had a biannual distribution overlapping the influenza profile of New York State.
Limitations, reason for caution: Seminal round cells are linked to the integrity of spermatogenesis and may bring new insights into the mechanism of recovery of the germinal epithelium following ordinary insult. However, ART outcome's correlation with the presence of this marker needs farther evaluation.
Wider implications of the findings: Genetic and epigenetic assessments of RC portray them as abnormal spermiogenic products engulfed in sloughed sertolicell cytoplasm. This suggests that the appearance of RC represent an effort by the germinal epithelium to restore spermatozoa production following an insult, as proven by the subsequent increase in sperm concentration. This accelerated turnover yields spermatozoa with increased DFI and aneuploidy. The apparent relation to seasonal illness may widen our understanding of the fluctuations of male fertility.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: NA.
Keywords: ICSI, TUNEL, FISH, round cells, spermiogenesis

P-058 The total number of normal morphology and progressively motile sperm: a novel semen parameter as a predictor of *in vitro* fertilization

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Study question: At present, it is still not clear which patients with male factor subfertility would benefit from conventional *in vitro* fertilization (IVF) and which patients need intra-cytoplasmic sperm injection (ICSI). No single semen parameter can be used conclusively to predict IVF outcome.
Summary answer: We proposed a novel combined parameter, the total number of normal morphology and progressively motile sperm (TNPS), to predict the outcome of *in vitro* fertilization.
What is known already: In many IVF centers, post-wash total motile sperm count is assessed in male factor subfertility to predict pregnancy during intrauterine insemination. However, recent evidence suggests that strict criteria sperm morphology is more closely relate to fertilization outcome. But no studies have provided a clear rule to differentiate between patients who do or do not need ICSI for fertilization.
Study design, size, duration: This retrospective study contains 1831 couples who performed their first IVF cycle between January 2011 and December 2013 in our center. The average TNPS during three months before IVF were calculated and the effects of TNPS on the fertilization outcome were assessed.
Participants/materials, setting, methods: TNPS in the ejaculate is calculated by multiplying the semen volume by the sperm concentration by the percentage of progressively motile sperm by the percentage of normal morphology. We divided five groups according to TNPS percentile and compared fertilization rate, cleavage rate, good embryo rate and pregnancy rate between groups.
Main results and the role of chance: The median value of TNPS is 11.2 × 10^6 and the 5th percentile was 1.5 × 10^6. In the group of TNPS < 1.5 × 10^6 (Group A), the fertilization rate was significantly lower (72.9%) than in any of the subgroups with TNPS ≥1.5 × 10^6. With the TNPS increased, the fertilization rate was significantly improved. Besides, the cleavage rate, good embryo rate and pregnancy rate in group A were also less than other groups, although not significantly. Furthermore, when TNPS < 1.5 × 10^6, the incidence of poor fertilization (IVF fertilization rate ≤ 50%) increased significantly (12.6%), higher than in any other groups. In this case, ICSI should be admitted to avoid IVF failure.
Limitations, reason for caution: The cutoff value was set at the 5th percentile of TNPS (1.5 × 10^6) according to a retrospective analysis in this study. A prospective study with larger samples should be performed and receiver operating characteristic curve (ROC) could be used to get a more objective cutoff value.
Wider implications of the findings: TNPS could be considered as an evaluating indication of semen quality in IVF therapy, in order to avoid unpredictable fertilization failure and meanwhile avoid overuse of more invasive ICSI procedure. For those patients with TNPS <1.5 × 10^6, half-ICSI or ICSI could be suggested.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by Shenzhen committee of innovation of science and technology (no. JCYJ20140415114532535 and no. JCYJ20130401092000370).
Trial registration number: NA.
Keywords: TNPS, total sperm count, fertilization rate, pregnancy rate, semen parameters

P-059 Sperm DNA fragmentation index is significantly associated with the total volume of vacuoles in the sperm head: a prospective study

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Study question: Is there an association between the percentage of DNA Fragmentation Index (DFI) in a sperm sample and the distribution of the total volume of vacuoles (TVOV) present in the sperm head?
Summary answer: The finding of this prospective study shows that, in a sperm sample, DFI groups (0–14.9%, 15.0–29.9%, and ≥30%) are associated with the percentage of sperm with either a small (0–4.99% of the total sperm head volume) or a large (≥11% of the total sperm head volume) total volume of vacuoles.
What is known already: Previous studies have demonstrated that the presence of vacuoles in a sperm head may affect the outcome of any resulting embryos leading to implantation failure and miscarriage. Intracytoplasmic morphologically selected sperm injection (IMSI) has been proposed as a suitable intervention for such cases. It is hypothesized that the presence of these vacuoles is associated with DNA damage and thus determining DFI in a sperm sample will aid in identifying patients that would benefit from IMSI.
Study design, size, duration: This was a prospective study of 311 sperm samples (with ≥5% motility and concentration >2 million/ml) from men attending

an IVF clinic. DFI was determined and 400 motile sperm from each sample were assessed for the presence of vacuoles and the total volume of these vacuoles was measured.

Participants/materials, setting, methods: The DFI of sperm samples was analysed using the Sperm Chromatin Structure Assay (SCSA), with the high magnification assessment of sperm being carried out independently using 'IMSI Strict' (Hamilton Thorne) software program. Total volume of vacuoles was determined as 0–4.9%, 5–10.9%, and ≥11% in size.

Main results and the role of chance: 221 participants had a low-range (0–14.9%), 73 a mid-range (15.0–29.9%), and 17 a high-range DFI (≥30%). The percentage of sperm with 0–4.9 or ≥11% TVOV differed significantly between the three DFI groups ($p = < 0.001$). Using regression analysis, it was shown that the percentage of sperm with 0–4.9 or ≥11% TVOV was associated with the DFI group of a sample ($p = < 0.001$). For every unit of increase in the percentage of sperm with ≥11% TVOV the odds of a DFI ≥30% were increased by 5% [odds ratio (OR): 1.05, 95% CI: 1.03–1.08] while the odds of a DFI < 15% were decreased by 4% (OR: 0.96, 95% CI: 0.94–0.98). The percentage of sperm with ≥11% TVOV could discriminate between samples with a DFI ≥30% or < 30% (AUC: 0.903, 95% CI: 0.864–0.933).

Limitations, reason for caution: Patients with very poor sperm parameters (motility and concentration) were not included in this study. Although DFI cannot be determined in these patients, it cannot be excluded that, they might also have a high percentage of ≥11% TVOV and thus might benefit from IMSI.

Wider implications of the findings: This study shows that TVOV is associated with DFI and whilst diagnostic assessment of DFI and TVOV will be useful in the pre-treatment evaluation of subfertile couples, the use of IMSI in couples where the male has a high DFI might be of value to prevent highly vacuolated sperm being used clinically and thus create embryos that have a better developmental potential.

Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Australia.

Trial registration number: NA.

Keywords: sperm, vacuoles, DFI, IMSI

P-060 Sperm DNA damage should be considered in infertile couples due to production of high inflammatory cytokines via toll-like receptors

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Study question: What is the relationship between sperm DNA damage and Toll like Receptors (TLRs) expression, their signalling pathways, as well as inflammatory cytokine production in human fallopian tube cells?

Summary answer: Sperm with high DNA fragmentation increase TLRs and their signalling pathways gene expression as well as inflammatory cytokine production in human fallopian tube cells.

What is known already: Sperm DNA damage is a useful biomarker for male infertility which is associated with reduced embryo quality, fertility and pregnancy rate. TLRs are the major compartment of innate immune system. It is well established that microbial PAMPs are ligand for TLRs. However, it is becoming clearer that certain locally produced endogenous substances can also stimulate TLRs like reactive oxygen species (ROS). In addition, ROS and apoptosis are the most discussed causes of DNA damage.

Study design, size, duration: Ten Fresh semen samples were obtained from unexplained infertile couple with DNA fragmentation more than 20% (by TUNEL assay) and without infection. Ten normozoospermic healthy donors with DNA fragmentation less than 3% were selected as a control. All these semen samples, after washing were co-incubated with human fallopian tube cell line (OE-E6/E7) in triplicate for 24 h.

Participants/materials, setting, methods: TLRs genes and protein expression in OE-E6/E7 cell line was investigated By RT-PCR and Immunostaining respectively and compared with human fallopian tube tissue. After co-incubation of high and low DNA fragmented sperm with fallopian tube cell line, Toll like receptors 1–6 and their adaptor molecules were evaluated by quantitative PCR. Supernatant were used for the measurement of Interleukin 6 (IL6), Interleukin 8 (IL8), Tumor Necrosis factor alpha (TNFα) by ELISA.

Main results and the role of chance: TLR1–6 gene and protein were expressed in OE-E6/E7, like fallopian tube tissue. The mean relative expression of TLR genes 1–6 were higher significantly in response to sperm with high DNA fragmentation in compared to sperm with low DNA fragmentation ($P < 0.05$). Also, The mean relative expression of MyD88, TIRAP and TRIF(TICAM 1), were significantly increased in case group than control. However, in TRAM (TICAM2) pathway the result was not significant($P < 0.05$). Measurement of IL-6, IL-8 and TNFα by ELISA showed an elevation of IL-6 and IL-8 in response to sperm with high DNA damage than Low one ($P < 0.05$). TNFα in both groups were below the lower detection limit of the kit.

Limitations, reason for caution: The results need to be confirmed in more cases.

Wider implications of the findings: Sperm DNA damage plays an important role in immunological interaction of sperm with female reproductive tract. Excessive ROS production causes lipid peroxidation and oxidative DNA damage, which leads to DNA fragmentation. Besides, fatty acids and reactive oxygen species (ROS) are the endogenous ligands of TLRs. Maybe, by this mechanism, DNA fragmentation can increase TLR expression and more production of inflammatory cytokine as well as causes infertility in high DFI unexplained infertile men. So, evaluation of DNA damage should be considered in treatment of these patients due to exessive Inflammatory cytokine production.

Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s) – Iran University of Medical Sciences, Royan Institute.

Trial registration number: NA.

Keywords: sperm DNA damage, toll-like receptors, fallopian tube, innate immunity

P-061 The relationship between occupational exposures and semen quality among South Tunisian infertile men: a questionnaire study

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Study question: The aim of this study was to investigate the association between semen quality and self-reported occupational exposure using a questionnaire that was developed for use in routine consultation.

Summary answer: Self-reported exposure to pesticides and cement was strongly associated with impaired semen quality. Exposure to physical risk factors, such as mechanical vibrations and excess heat, was not associated with sperm anomalies.

What is known already: The decline in sperm quality reported over the last decades have raised concerns about the detrimental effect of occupational exposures on male fertility. Assessing effects of occupational risk factors on fertility is difficult given the wide variety and the limitations of epidemiologic methods used for this purpose (Job Exposure Matrices, quantitative measurements, questionnaires).

Study design, size, duration: We conducted a retrospective cross-sectional study among 2122 men who have consulted for couple infertility during the past 16 years. Subjects aged under 20 or above 55 years or with a known male infertility factor that wouldn't be linked to occupational exposures were excluded.

Participants/materials, setting, methods: All patients data were collected through a questionnaire designed to be used in routine consultation and to assess the most common occupational risks of male fertility impairment according to the literature. Data obtained were used to classify subjects into exposed and unexposed groups. Exposure effects on semen parameters were investigated.

Main results and the role of chance: An association between pesticides exposure and decreased semen volume, motility and vitality was found. Exposure to

pesticides was also associated with significantly higher risk of asthenospermia (adjusted odds ratio [OR] – 1.6; 95% confidence interval [CI], 1.0?2.4) and necrospermia (OR – 2.6; 95% CI, 1.4?4.7). Exposure to cement was found to be correlated to a higher risk of oligospermia (OR – 1.1; 95% CI, 0.9?1.4). Exposure to solvents, mechanical vibrations and excess heat was not associated with semen impairment.

Limitations, reason for caution: The retrospective collection of data and the lack of information on the intensity and duration of occupational exposures were the major limitations of this study.

Wider implications of the findings: There are few data in the literature about the relationship between cement exposure and semen quality. Further work is needed in the future in order to elucidate the mechanisms through which cement components could affect semen parameters. The present study validates the usefulness of questionnaires as a tool for occupational exposure survey. Thus, its use in routine consultation could be helpful for the prevention and management of occupational exposure in infertile men.

Study funding/competing interest(s): Funding by University(ies) – Medical school of Sfax, University of Sfax, Tunisia.

Trial registration number: NA.

Keywords: semen, occupational exposure, male infertility, questionnaire

P-062 Prevalence and characteristics of isolated LH and isolated FSH deficiencies in white-European men presenting for primary couple's infertility – results of a cross-sectional survey

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Study question: The prevalence of isolated Luteinizing Hormone (LH) and isolated Follicle Stimulating Hormone (FSH) deficiency is little known in the male population. We assessed prevalence, clinical and seminal characteristics of single LH and FSH deficiency in a cohort of white-European men presenting for primary couple's infertility.

Summary answer: Isolated deficit of LH and FSH account for roughly 2% each in men presenting for primary couple's infertility. Infertile men with isolated LH deficiency have an overall underactive endocrine testicular compartment. Both FSH and LH isolated deficiency are associated with higher prevalence of comorbidities and a lower left testicular volume.

What is known already: Isolated deficiencies of LH or FSH are thought to be extremely rare, but few reported data exists at this regard. Significant association with alterations in sperm parameters was found when considering normal sperm morphology and motility. No significant correlation with age or semen volume has been reported. Isolated LH deficiency has been related to decreased virilization, eunuchoidal proportions and hypogonadal testosterone levels despite normal testicular size and preserved spermatogenesis.

Study design, size, duration: A cross-sectional survey of 2100 white-European men seeking consult for infertility issues in the last ten years was performed. Prevalence of isolated deficit of LH (defined as LH <1.5 mUI/mL) and of FSH (defined as FSH <1.5 mUI/mL) were defined respectively.

Participants/materials, setting, methods: Health-significant comorbidities were scored with Charlson Comorbidity Index (CCI; categorized 0 vs. 1 vs. ≥2). Testicular volume was assessed with a Prader orchidometer. Semen analysis values were assessed basing on 2010 World Health Organization criteria. Descriptive statistics tested association between clinical characteristics /semen parameters and isolated LH or FSH deficiencies.

Main results and the role of chance: Isolated LH and FSH deficiencies were found in 55 (2.6%), and 46 (2.2%) men, respectively. Patients with isolated LH deficiency were older ($p = 0.02$), had higher mean BMI ($p = 0.02$), greater prevalence of obesity (NIH class ≥1) (Chi^2:9.51; $p < 0.01$) and more comorbidities, defined as CCI ≥1 (Chi^2: 20.2; $p < 0.001$), as compared with those with normal/high value of LH. Similarly, lower left testicular volume ($p = 0.025$), lower mean total testosterone and SHBG ($p = 0.002$ both) and higher prevalence of hypogonadism (Chi2:26.3; $p < 0.001$) were reported. On the other hand, patients with isolated FSH deficiency showed higher rate of comorbidities (Chi2:5.9; $p = 0.02$) and lower mean left testicular volume ($p = 0.04$) as compared with those with a normal/high value of FSH. No significant differences were observed in terms of seminal parameters in both cases.

Limitations, reason for caution: Major limitation is the cross-sectional design of our study.

Wider implications of the findings: We demonstrated that isolated LH or FSH deficiencies account together of almost 5% of patients in our population. Thus these conditions deserve consideration not only as infertility work-up issues but also as points of general health in the infertile male.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Ospedale San Raffaele, Milano.

Trial registration number: NA.

Keywords: LH, FSH, infertility, hypogonadism, comorbidities

P-063 Progressive stages of diabetes mellitus induce testosterone deficiency promoting a modulation of Sertoli cell metabolism

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Study question: How does Sertoli cell (SC) metabolism responds to the reduced testosterone (T) levels induced by progressive stages of diabetes mellitus (DM)?

Summary answer: T deficiency promoted by progressive stages of DM does not favor glycolytic flux in SCs. Glucose taken up by SCs is not as efficiently converted into lactate in T deficient conditions, being partly redirected to other metabolic pathways.

What is known already: DM is a metabolic disease that compromises male fertility through the induction of hormonal deregulation. Within seminiferous epithelium, androgen receptors are exclusively expressed in SCs and under culture conditions androgens induce a metabolic shift from a Warburg-like into an oxidative Krebs cycle metabolism in SCs. Under detrimental conditions, SCs use alternative substrates as a compensatory mechanism to ensure the adequate conditions for germ cell development and to counteract the deleterious effects of DM.

Study design, size, duration: SCs obtained from normal Wistar strain rats ($n = 6$) and from rodent models of prediabetes (PreD) ($n = 6$) and type 2 diabetic mellitus (T2DM) ($n = 6$) were cultured during 96 h with sex steroid concentrations within the physiologic range (T-CTR group), PreD conditions (T-PreD group) and T2DM conditions (T-T2DM group).

Participants/materials, setting, methods: Metabolite secretion/consumption profile of cultured SCs was evaluated by ^{1}H-NMR spectrometry. Protein expression levels were assessed by Western blot. Intracellular glycogen content was quantified by using specific kits. Lactate dehydrogenase activity was determined using a commercial assay kit. Alanine aminotransferase activity was determined by spectrophotometric methods.

Main results and the role of chance: Both glucose and pyruvate consumption were significantly decreased in PreD conditions, whereas T2DM conditions reversed this profile. Lactate production was not significantly altered at the end of the treatment, although the expression and activities of the lactate production-associated proteins were increasingly affected by progressive T-deficiency conditions. Alanine production was significantly increased in SCs of both groups, suggesting an alternative metabolic fuel. Notably, intracellular glycogen content was only increased in SCs of the T2DM group.

Limitations, reason for caution: Not applicable.

Wider implications of the findings: These results illustrate that gradually reduced T levels, induced by progressive stages of DM, impair glycolysis favoring glycogen metabolism, with the more pronounced effects being concurrent with lower T levels. Even in the T2DM conditions, SCs were able to adapt their metabolism to sustain lactate metabolism. This report highlights the physiologic significance of T in the regulation of the glycolytic profile of SCs metabolism, in particular when associated with the progression of T2DM.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the Portuguese 'Fundação para a Ciência e a Tecnologia' – FCT co-funded by FEDER via Programa Operacional Factores de Competitividade—COMPETE/QREN [PTDC/QUI-BIQ/121446/2010 and PEst-C/SAU/UI0709/2014]. L. Rato [SFRH/BD/72733/2010], M. G. Alves [SFRH/BPD/80451/2011] and A. I. Duarte

[SFRH/BPD/84473/2012] were financed by FCT. P. F. Oliveira was financed by FCT through FSE-POPH funds (Programa Ciência 2008).
Trial registration number: NA.
Keywords: prediabetes, type 2 diabetes mellitus, testosterone deficiency, sertoli cell, glucose metabolism

P-064 Serum micro-RNA-155 as a new biomarker of male fertility

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Study question: Are serum levels of micro RNAs miR-155 and miR-146a potential biomarkers of male fertility?
Summary answer: Serum levels of miR-155 but not miR-146a were associated with risk of male subfertility and combination of miR-155 and FSH analysis gave higher predictive value than any of the markers separately.
What is known already: Male subfertility has been associated with low grade systemic inflammation (LGSI) as well as with androgen deficiency. MiR-155 and miR-146a are central regulators of inflammation and their levels in cells and in the serum has been associated with several inflammatory conditions but their possible association with male fertility is, so far, unknown..
Study design, size, duration: Case control study, based on two independent groups of 60 men – an exploratory and a confirmatory cohort. The subjects were selected among those included in a larger study performed during the period 2007–2012.
Participants/materials, setting, methods: Both the exploratory and the confirmatory cohort included 60 men each; 40 from infertile couples, without known female factor, and 20 age-matched population-based controls. Total RNA was isolated from cell-free serum. As internal control, the synthetic miRNA UniSp6 was added to each sample. Micro RNAs were measured by real-time RT-PCR.
Main results and the role of chance: Serum levels of miR-155 were associated with levels of miR-146a, but only miR-155 was associated with subfertility (means: for subfertile group 1.88 U vs. 1.15, 95% CI 1.0–1.2 U in controls; $p = 0.001$). ROC analysis indicated that miR-155 with a cutoff value of 1.77 U had 47% sensitivity and 95% specificity for identifying subfertility and positive and negative predictive values of 95 and 47% respectively. When used in combination with FSH, sensitivity and specificity were 80 and 100% respectively while positive and negative predictive values were 100 and 71% respectively, those values being higher than for the FSH alone.

In order to exclude the risk of chance, the results obtained in the exploratory cohort were repeated in an independent confirmatory cohort.
Limitations, reason for caution: Although our primary results were confirmed in the new cohort, studies from other centers are needed to establish the role of miR-155 as a new biomarker of male fertility. Furthermore, the role of this marker in distinguishing between different groups of male subfertility is to be elucidated.
Wider implications of the findings: Association of the inflammatory miRNA miR-155 with fertility may contribute to our understanding of the pathophysiology of subfertility and suggests a novel biomarker. Serum miR-155 in combination with FSH has higher diagnostic specificity and sensitivity compared to FSH. Thus, this finding may have interesting biological and clinical implications in relation to understanding and managing fertility problems.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s) – Lund University, Skane University Hospital, Swedish Governmental Fund for Clinical Research, Skane county council's research and development foundation.
Trial registration number: NA.
Keywords: male subfertility, micro RNA, serum marker

P-065 Whole exome sequencing (WES) analysis in a patient with situs-inversus totalis

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Study question: Is Whole Exome Sequencing (WES) able to establish a genetic diagnosis of total sperm immotility in a patient with situs-inversus totalis?
Summary answer: WES analysis revealed a novel homozygous missense variant in *CCDC103* and a novel frame-shift variant in *INSL6*. These were considered the most plausible causes, respectively, of the absence of dynein arms and of the total sperm immotility in the patient with *situs inversus totalis*.
What is known already: Sperm immotility is one of the major causes of male infertility, but the genetic basis of sperm motility is not fully understood. In the present study, we analysed one patient with total sperm immotility and situs inversus totalis using WES, a technique not yet applied to the genetic screening of these patients.
Study design, size, duration: A genetic screening was performed in a patient with situs-inversus totalis in search for the cause of total sperm immotility due to complete absence of dynein arms and nexin links.
Participants/materials, setting, methods: The exome of a patient with situs inversus totalis and total sperm immotility due to complete absence of dynein arms and nexin links was sequenced using the AmpliSeq strategy on an Ion Proton next-generation sequencing platform using the optimized parameters for WES.
Main results and the role of chance: From more than 50,000 DNA sequence variants, with the GEMINI database framework, the Ion Reporter, bioinformatics and the BAM file, six were confirmed by Sanger sequencing as true variants. The novel homozygous variant c.104G > C at exon 2 of ***CCDC103*** (dynein arm attachment factor), is suggested as potentially pathogenic by three different bioinformatic softwares (SIFT, Polyphen-2, MutationTaster). The novel heterozygous deletion in the coding region (c.262_263del) of *INSL6* was also considered disease-causing. Two other heterozygous misssense variants were identified, in genes *DNAH10* and *DNAH6* (novel) that encode proteins of the dynein heavy chain. Although predicted to be damaging they need further studies. Finally, the two missense heterozygous variants were found in genes *GAS8* (novel) and *SPAG17* (novel), but no pathogenic impact was predicted.
Limitations, reason for caution: WES faces some limitations with a considerable rate of false-positive variants generated by misaligned reads or sequencing errors and consequently Sanger sequencing is still needed to confirm the results. Further, WES only covers the exonic regions, meaning that non-coding variants that may be associated with the disease are not detected.
Wider implications of the findings: WES appeared the most effective technical approach to study cases with total sperm immotility. With this work we expect to increase the knowledge on the genetics of sperm immotility to allow future identification of potential genetic biomarkers and treatments.
Study funding/competing interest(s): Funding by University(ies) – This work was financed by the Institutions of the authors and in part by UMIB, which is funded by National Funds through FCT-Foundation for Science and Technology, under the Pest-OE/SAU/UI0215/2014.
Trial registration number: NA.
Keywords: dysplasia of the fibrous sheath, genetic diagnosis, Sanger sequencing, sperm immotility, whole exome sequencing

P-066 Titanium dioxide nanoparticles reduce human sperm DNA stability

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Study question: In this study we investigated the damage to chromatin integrity and the consequent DNA fragmentation in human sperm exposed *in vitro* to two concentrations of nanosized titanium dioxide (n-TiO_2) (1 and 10 μg/L) for three different times.

Summary answer: Exposure to *n*-TiO_2 induced DNA damage in human sperm. The *n*-TiO_2 induced a loss of sperm DNA integrity and a high decrease in genomic stability, but did not increase sperm DNA fragmentation. The DNA damage was greatest at the highest concentration of *n*-TiO_2.

What is known already: The impact on the human fertility of the *n*-TiO_2 use is not well defined or unknowns yet. To date there are no data in the literature on the effects on human sperm DNA induced *in vitro* by TiO_2 nanoparticles. However it is known that the nanoparticles can penetrate the blood-testis barrier, due to their nanosize, acting at various biological levels and so could contribute to alter reproductive functions.

Study design, size, duration: Human semen ejaculates from 76 men between 25 and 35 years old were collected from April to November 2014. The seminal fluids with normal parameters according to WHO (2010) were selected for the study.

Participants/materials, setting, methods: The samples, after selection with Percoll gradient, were exposed to *n*-TiO_2 for 15, 30 and 45 min. One aliquot was treated with benzene (positive control) and a untreated aliquot was used as negative control. The genotoxicity was assessed by Comet Assay, TUNEL technique and RAPD-PCR technique.

Main results and the role of chance: The Comet Assay showed a statistically significant loss (p-value ≤ 0.05) of sperm DNA integrity already after 15 min of exposure for both concentrations tested. The DNA damage was greatest at the highest concentration of *n*-TiO_2.The results of the TUNEL test showed no increase in sperm DNA fragmentation. The RAPD-PCR analysis showed a variation of the polymorphic profiles of the sperm DNA exposed to *n*-TiO_2 respect to the DNA of the not-treated sperms. The genome template stability (GTS%) of sample treated with 1 µg/L *n*-TiO_2 was reduced of 18% after 15 and 30 min and 27% after 45 min, while the treatment with 10 µg/L *n*-TiO_2 reduced GTS% of 45% after 15 min, 54% after 30 min and 63% after 45 min.

Limitations, reason for caution: No limitations.

Wider implications of the findings: This research provides the first data on the evaluation of the potential genotoxicity of *n*-TiO_2 on human seminal liquid. Thanks to the qualitative analysis of the RAPD profiles, we hypothesized that the damage to sperm DNA induced by *n*-TiO_2 occurs through the production of reactive oxygen species (ROS). The data provide a starting point for investigations on the possible effects that other nanomaterial could have on sperm DNA and consequent infertility rate.

Study funding/competing interest(s): Funding by University(ies) – Second University of Naples (Italy).

Trial registration number: No

Keywords: titanium dioxide nanoparticles, genotoxicity, human sperm, male infertility

P-067 Low values of *n*-3 polyunsaturated fatty acids in sperm cells are associated to lower fertilization rates in IVF cycles

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Study question: Are sperm *n*-3 polyunsaturated fatty acids (PUFA) levels, especially docosahexaenoic acid (22:6 *n*-3; DHA), related to semen quality and *in vitro* fertilization (IVF) results in terms of fertilization and pregnancy rate?

Summary answer: High sperm *n*-3 PUFA levels are related with optimal semen quality and an accurate fertilization process.

What is known already: The lipid composition of spermatozoa plays an important role for the viability, maturity and functional characteristics of those cells. Long-chain PUFAs have been detected at high concentration in human spermatozoa and has been suggested that the proportion of PUFAs is closely correlated with sperm membrane fluidity and flexibility, being essential for fertilization process. However, there is a lack of information about the importance of PUFA composition of the sperm membranes in the outcome of IVF.

Study design, size, duration: Prospective study. Semen samples were obtained from 340 consecutive males from infertile couples participating in the FIV/ICSI programme of the Human Reproduction Unit at Cruces Hospital, during 2010. For the analysis of IVF results, only samples of subjects with a lack of detectable abnormalities in the female partner are included (n = 230).

Participants/materials, setting, methods: Seminal samples were classified according to WHO criteria in normal (≥15 millions/ml; ≥32% progressive motility and ≥4% normal forms) and pathological samples. Sperm preparation was performed by swim up. Sperm fatty acids were analysed by capillary gas-liquid chromatography. Results were expressed as nmole percentages of total fatty acids.

Main results and the role of chance: PUFA, *n*-3 PUFA and DHA sperm levels were significantly lower in samples assessed as pathological by 2010 WHO criteria (26.2%; 13.09 and 12.2% respectively, $p < 0.001$) than in normozoospermic samples (29.1%; 16.4 and 15.5% respectively). On the other hand, the levels of saturated fatty acids (SFA) were significantly higher in pathological samples (52.07%; $p < 0.001$) compared with normal samples (50.0%). PUFA, *n*-3 PUFA and DHA content showed a positive and significant correlation with sperm concentration, progressive motility and the percentage of normal forms ($p < 0.001$). Nevertheless, an inverse relationship was found for SFA and n6/n3 ratio ($p < 0.05$) with the seminal parameters.

Regarding IVF results, we found a positive and significant correlation between *n*-3 PUFA and DHA content of post-swim up spermatozoa and fertilization rate ($p < 0.05$), while the correlation was negative for SFA and n6/n3 ratio ($p < 0.05$). However, we did not detect any relationship between sperm fatty acids composition and pregnancy rates.

Limitations, reason for caution: This study did not involve proven fertile subjects; the results are limited to a population of patients from infertile couples with either male or female infertility factor. Our results show a correlation with fertilization rates, but not with the truly clinically relevant outcome (pregnancy and newborn rates). More studies are needs to ascertain if the lack of differences is due to the relatively small sample size

Wider implications of the findings: High *n*-3 PUFA content, especially DHA, is associated with optimal semen quality required for proper fertilization and with higher fertilization rates in IVF cycles. This is the first study, in our knowledge, to report a significant relationship between the sperm composition of *n*-3 PUFA and the IVF fertilization rates. The present findings suggest that measurement of *n*-3 PUFA levels of spermatozoa can be regarded as a useful tool for predicting sperm functionality.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital de Cruces.

Trial registration number: NA.

Keywords: polyunsaturated fatty acids, semen quality, fertilization rate, pregnancy rate

P-068 The pregnancy and abortion rates of couples with high levels of sperm DNA damage is markedly improved following MACs treatment

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Study question: This study was conducted to assess the impact of reducing sperm DNA fragmentation (SDF) using a commercial Magnetic Cell Sorting system (MACs) on corresponding pregnancy and abortion rates

Summary answer: The use of the MACs procedure prior to ICSI reduced sperm DNA damage observed in neat ejaculate, resulting in an increase in pregnancy rate and a marked reduction in the incidence of abortion in patients with this predisposition

What is known already: High sperm DNA quality and homeostasis are essential for effective transmission of genetic information to the offspring. Evidence based medicine has now shown that abnormal sperm chromatin or damaged DNA can adversely affect fertility and contribute to abortion. A certain proportion of spermatozoa in the ejaculate of most species contain abnormal sperm because of DNA or protein damage. Recently, MACs has been used to remove a portion of the damaged DNA contained in the ejaculate.

Study design, size, duration: Retrospective study of couples attending an ART clinic over two years. All males (n = 305) presented SDF values higher than 30%. Two cohorts where established: Standard-ICSI (S-ICSI) and Ovodonation-ICSI (O-ICSI). Within each of these cohort, two further subgroups were created (1) SDF ranging from 30 to 50% and (2) SDF higher than 50%.

Participants/materials, setting, methods: IVF clinics and Universities. Female averaged age: S-ICSI:32.3; O-ICSI;43.7. Distribution of patients within each subgroup: S-ICSI: SDF ranging from 30 to 50% (Control n = 144 and MACs n = 42). SDF ≥50% (Control n = 23 and MACs n = 7). O-ICSI: SDF ranging from 30 to 50% (Control n = 40 and MACs n = 29. SDF ≥50% (Control n = 11 and 9 MACs n = 9). Pregnancy and abortion rates were assessed within each subgroup.

Main results and the role of chance: The overall pregnancy rate of the S-ICSI group was lower than the O-ICSI group (P = 0.005). When S-ICSI and O-ICSI cohorts were compared, the pregnancy rate was higher (P = 0.005) for subgroups were the SDF was less than 50%. Abortion was not detected in those females receiving MACs treated sperm. Pregnancy and abortion rate for all groups are reported in Table 1

Table 1: Pregnancy and abortion data within each established group.

		SDF 30–50%	SDF 30–50%	SDF ≥50%	SDF ≥50%
		Control	MACs	Control	MACs
S-ICSI	Preg	54	54	35	42
	Ab	5	0	8	0
O-ICSI	Preg	60	70	46	55
	Ab	3	0	8	0

Limitations, reason for caution: While our results clearly revealed treatment of sperm with MACs procedure prior to ICSI, results in a marked improvement in pregnancy rate and cessation of the abortion rate in couples whose ejaculates initially had high levels of SDF, further observations are required to confirm the statistical robustness of our preliminary findings.

Wider implications of the findings: While our results clearly revealed treatment of sperm with MACs procedure prior to ICSI, results in a marked improvement in pregnancy rate and cessation of the abortion rate in couples whose ejaculates initially had high levels of SDF, further observations are required to confirm the statistical robustness of our preliminary findings.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Ginemed Clinic. None of the authors declare conflict of interest.

Trial registration number: This investigation is a part of the experimental protocols included in the research project BFU-2013-44290-R, as approved by the University in accordance with the participating IVF clinics.

Keywords: MACS, DNA damage, SCD, ICSI, pregnancy rate

P-069 The relationship between chromatin condensation assay (aniline-blue) on testicular spermatozoa of non-obstructive azoospermia (NOA) patients and the testicular histology in ICSI cycles. A pilot study

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Study question: The aim of the study was to investigate the correlation between testicular spermatozoa stained with Aniline Blue (AB) and the etiology of testicular patients with NOA. Besides, we wanted to study the relationship between the success rate of *in vitro* fertilization and AB staining

Summary answer: Our results show that NOA patients with mild testicular damage show a higher percentage of AB negative spermatozoa compared to those with severe damage, and that mild testicular damage in NOA patients is associated with a higher likelihood of finding mature spermatozoa in the testis and a higher IVF success.

What is known already: During spermiogenesis, the somatic histones are substituted by protamines for a normal nuclear condensation. A significant association between male infertility, imperfect spermiation and abnormal chromatin condensation has been reported. Aniline-Blue selectively targets lysine-rich histones showing completely or partially blue nuclei while protamine-rich nuclei of mature spermatozoa remain unstained.

Study design, size, duration: Thirty-four NOA patients performed Testicular Sperm Extraction between June/2013 and December/2014. Spermatozoa were retrieved in 27 patients (27/34 = 80%). These patients were included in the AB study ad were divided in two groups: group-a) mild hypospermatogenesis (n = 19); and group-b) severe hypospermatogenesis and Klinefelter-Syndrome (n = 8). AB-staining results were confronted with histological analysis.

Participants/materials, setting, methods: Mean male and female age were similar in both groups. Testis samples were smeared, fixed in 5% paraformaldehyde, stained with AB solution for 5 min, rinsed with water and observed at 100× under oil. One-hundred spermatozoa were evaluated per patient. All histological examinations were assessed by the same pathologist.

Main results and the role of chance: The average percentage of AB positive spermatozoa in group-a and b were respectively 34.6% ± 28.7 and 63.7% ± 29.4 (p = 0.03, t-test). Significance was set at $p \leq 0.05$. Levels of serum FSH and LH were respectively 5.2 ± 8.7 and 4.0 ± 2 in group-a and 18.6 ± 8.7, 7.1 ± 2.6 in group-b (P = 0.0012 and P = 0.025, t-test). In group-a, fertilization rate, day-3 cleavage rate and day-3–5 embryo degeneration were respectively 70, 65.4 and 37.2% while in group-b were 57.5, 76.1 and 71.4% (P = 0.08; P = 0.2; P = 0.0007, Fisher's exact test). Five babies and six clinical pregnancies have been obtained to date in group-a (14 embryo transfers). In group-b no clinical pregnancy has been obtained (7 patients). Our data show a positive correlation between the AB positive sperm number and the testicular etiology obtained by histological exam.

Limitations, reason for caution: Additional data are necessary. We were unable to analyze a higher number of spermatozoa due to the difficulty of the testicular sperm retrieval.

Wider implications of the findings: This study provides a novel insight into how abnormal protamination can affect testicular sperm. The Aniline-blue threshold for ejaculated spermatozoa is ≤30% in our laboratory. These value seems less important when considering testicular sperm. All NOA patients display AB staining higher than 30%, however clinical outcomes are good provided that the patients have mild testicular damage. Our results will contribute to a deeper understanding of the benefits and limitations of spermatozoa selection for ICSI.

Study funding/competing interest(s): Funding by hospital/clinic(s) – European Hospital, Medicina della Riproduzione.

Trial registration number: NA.

Keywords: azoospermia, aniline-blue staining, testicular sperm, male infertility

P-070 Single nucleotide polymorphism rs 175080 in the MLH3 gene and its relation to male infertility

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Study question: To investigate the association between the single nucleotide polymorphism (SNP) rs 175080 in the MLH3 gene with male infertility in a Caucasian population.

Summary answer: The studied SNP in the MLH3 gene may be linked to oligozoospermia in Caucasian men.

What is known already: Male infertility accounts for approximately 50% of couple infertility. Studying the genetic basis of impaired spermatogenesis and male infertility is crucial for diagnosis and treatment. There is cumulative evidence associating different polymorphisms in genes involved in spermatogenesis with male infertility. MLH3 is a MutL homolog protein in mammals playing a role in DNA mismatch repair, but also associated to spermatogenesis and male infertility. There are scanty data regarding the association between certain MLH3 polymorphisms and male infertility.

Study design, size, duration: Cohort study in three hundred men that were subjected to IVF/ICSI-ET treatments between 2011 and 2013.

Participants/materials, setting, methods: Genomic DNA was extracted from 300 peripheral blood samples and conventional quantitative real time PCR was performed for genotyping 122 controls and 178 cases (men with sperm

concentrations above and below 15 million/ml, respectively). Serum concentrations of FSH, LH, estradiol, testosterone and prolactin, and the sperm parameters were compared between the three groups of genotypes (GG, GA and AA). Furthermore, the frequencies of these three genotypes were compared between cases and controls.

Main results and the role of chance: This is the first study that investigated the SNP rs 175080 in Caucasian men. There were no significant differences in anthropometric parameters and hormonal values between the three groups of genotypes. A significantly lower sperm concentration was found in men with the AA genotype as compared to men with the GG and GA genotypes ($p < 0.001$). The group with the AA genotype had the lower progressive motility values as compared to the two other groups ($p < 0.05$). Also, there was a significantly different distribution of the frequencies of the three genotypes between cases and controls ($p < 0.001$).

Limitations, reason for caution: The relatively small sample size of the present study does not allow drawing solid conclusions. Besides, our results refer to Caucasians, therefore, these conclusions should not be expanded.

Wider implications of the findings: Polymorphisms may be widely used for the investigation of male infertility. The present study provides data for further research in the context of larger studies.

Study funding/competing interest(s): Funding by University(ies) – University of Thessaly, Department of Obstetrics and Gynaecology.

Trial registration number: NA.

Keywords: MLH3, polymorphism, male infertility

P-071 A combination of eight micronutrients is superior to a mono preparation comparing improvement of variant groups of impaired sperm motility

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Study question: The objective of this retrospective study was to compare the effect of a combination of 8 active compounds (l-carnitine 440 mg, l-arginine 250 mg, zinc 40 mg, vitamin E 120 mg, glutathione 80 mg, selenium 60 µg, coenzyme Q10 15 mg, folic acid 800 µg) with a single active compound (l-carnitine 1000 mg) on subgroups of impaired semen motility.

Summary answer: Both therapies increased semen motility to a significant level. A low previous fraction of fast and slowly progressive sperm results in a higher improved by means of micronutritient supplementation. The combination supplement was significantly superior in improvement of progressive and total motility.

What is known already: Approximately 50% of all infertile couples' cases are related to an impaired semen quality which is caused in 30–80% by oxidative stress. Oral intake of micronutrients can ameliorate sperm motility

Study design, size, duration: From 2006 to 2014 261 patients of the IMI Fertility Center, Vienna and the Med19 Study Center, Vienna were enrolled in the study. For 3 months patients took either the mono preparation or the combination treatment. Semen analysis was performed before and after intervention. Motility subgroups where statistically.

Participants/materials, setting, methods: Patients were 18–60 years old, suffered from subfertility over 1 year, had one or more recent pathologic semen analysis according WHO 2010 and didn't meet any exclusion criteria. 144 subfertile men were treated with a mono preparation and 127 patients received the combined preparation.

Main results and the role of chance: Within both therapy groups, treatment regimes increased semen motility to a highly significant level ($p < 0{,}001$). Subgroup analysis revealed that the lower the previous fraction of fast and slowly progressive sperm has been, the greater the improvement was. Motility counts of 40% progressive sperms and more couldn't be increased significantly any further. The combined therapy increased rapidly progressive motile sperms highly significantly ($p = 0{,}004$) and overall progressive sperm count significantly ($p = 0{,}01$) compared to the mono-substance group.

Limitations, reason for caution: –

Wider implications of the findings: –

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Lenus Pharma, Seeböckgasse 59, 1160 Vienna, Austria.

Trial registration number: NA.

Keywords: sperm, oxidative stress, motility

P-072 Mycoplasmas and ureaplasmas infection and male infertility: a systematic review and meta-analysis

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Study question: The goals of this study were to evaluate the association between genital Ureaplasma (U. urealyticum and U. parvum), Mycoplasmas (M. genitalium and M. hominis) and risk of male infertility, and to compare the prevalence of genital Ureaplasma and Mycoplasmas infection in China relative to the world average

Summary answer: Our analysis supports that U. Urealyticum and M. Hominis, but not U. Parvum and M. genitalium is an etiological agent in male infertility

What is known already: The correlationship between Mycoplasmas, Ureaplasmas infection and male infertility has been studied widely. However, The role that U. urealyticum and M. hominis infections play in male infertility is controversial. Hitherto, M. genitalium and U. parvum have seldom been investigated in infertile men.

Study design, size, duration: There were 20 studies in this research between January 2000 and December 2014.Nineteen studies with 3975 case and 2249 controls were concerning U. urealyticum infection and 9 studies with 2410 cases and 1223 controls were about M. hominis infection. Other two infection (U. parvum and M. genitalium) were studied in 5 and 3 studies, respectively.

Participants/materials, setting, methods: The major criteria were as follows: (a) case-control studies about the associations of genital Ureaplasma or Mycoplasmas with male infertility; (b) the patient group was men who were diagnosed with infertile. The major exclusion criteria were as follows: (a) duplicate data; (b) abstract, comment, review and editorial.

Main results and the role of chance: This meta-analysis indicated that the U. parvum and M. genitalium might be not associated with the risk of male infertility. However, Ureaplasma urealyticum and Mycoplasma hominis was significantly associated with increased risk of male infertility[ORs were 3.81 (2.48–5.85) $P < 0.00001$; 1.84 (0.93–3.64) $P = 0.025$].Compared to the world average, a significantly higher positive rate of Ureaplasma urealyticum was observed in both the infetile and control groups in China. In contrast, a significantly lower positive rate of Mycoplasma hominis was observed in both the infertile and control groups in China.

Limitations, reason for caution: First, the sample size was small, which might potentially influence the combined results. Second, other environmental factors, such as smoking and drinking, were not considered in our meta-analysis due to data deficiency. Third, this meta-analysis was conducted based on case-control study that has risk of recall bias.

Wider implications of the findings: More detailed studies of these four species in China and the world could contribute to a better understanding of the epidemiology and pathogenesis, and facilitate the development of better strategies for treatment and prevention of male infertility.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by Changsha City Science and Technology Project (K1106001-31).

Trial registration number: NA.

Keywords: *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, male infertility

P-073 Short-term hypothermic storage of human spermatozoa in electrolyte free medium (EFM): outcomes of 96 IVF cycles

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Study question: To determine whether short-term hypothermic storage of human spermatozoa in EFM is effective in routine practice for IVF/ICSI.

Summary answer: Short-term hypothermic storage of human spermatozoa in EFM for up to 2 weeks is safe and effective, and may be used in IVF programs.

What is known already: Storage of human sperm at + 4°C in EFM composed of glucose and bovine serum albumin allows sperm preservation for at least 2 weeks (Saito et al., 1996; Kanno et al., 1998). This method was used to generate healthy murine offspring and was shown to be genetically safe (Riel et al., 2007, 2011). Short-term hypothermic storage of human spermatozoa in EFM has not been utilized in clinical practice for infertility treatment with IVF.
Study design, size, duration: This study included 96 couples who underwent IVF treatment between September 2010 and December 2013. Normospermia was the main requirement for participation. After 2-week hypothermic storage in EFM, the spermatozoa were used for fertilization by ICSI.
Participants/materials, setting, methods: Sperm was stored in EFM at +4°C and further processed according to standard protocol. Sperm motility and sperm DNA fragmentation were evaluated before and after hypothermic storage in EFM. Each newborn's physical status was evaluated by questionnaires sent to physicians and parents at conception and delivery.
Main results and the role of chance: After 2-week hypothermic storage in EFM, 56.2 ± 5.0% of spermatozoa regained motility. The sperm DNA fragmentation rate was slightly higher after than before storage (11.2 ± 3.1 vs. 8.5 ± 2.5%, $p = 0.11$), but the difference was not statistically significant. The fertilization rate was 78%, and the clinical pregnancy rate was 34.4% (33 of 96), with 26 pregnancies resulting in the successful delivery of 34 babies. Delivery date and birth length and weight complied on average with standards.
Limitations, reason for caution: The method was effective for ejaculated sperm with normal parameters stored for a period of up to 2 weeks.
Wider implications of the findings: Hypothermic storage of human spermatozoa in EFM is a simple and cost-effective option, if ejaculated sperm cannot be retrieved on the day of ovarian puncture. The method guarantees that the spermatozoa are safely stored for at least 2 weeks, and then regain their motility and viability, with preservation of DNA integrity. Hypothermic storage of human spermatozoa in our IVF/ICSI programs resulted in a high pregnancy rate and high physical status scores in the resulting newborns.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for reproductive medicine MAMA, Moscow, Russia C.I.S.
Trial registration number: NA.
Keywords: sperm, hypothermic storage

P-074 Who is the most suitable candidate for varicocele ligation? – Findings from a cross-sectional survey in a cohort of infertile Caucasian-European patients

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Study question: We assessed i) the impact of health-significant comorbidities and hormonal milieu on semen parameters; and, ii) the main predictors of oligospermia in order to identify patients who will not significantly benefit from varicocele ligation.
Summary answer: Current findings demonstrate that almost half of patients presenting for couple's infertility had a clinically-significant varicocele. Our data would suggest not to submit infertile men with high serum levels of FSH to varicocele ligation.
What is known already: According to current EAU guidelines, clinical varicocele should be repaired in infertile patients with oligospermia and an infertility duration of ≥2 years. Indeed, patients' comorbidity profile and hormonal milieu seems to have no importance for therapeutic decision making.
Study design, size, duration: Complete data from 2100 consecutive infertile men were analyzed in a retrospective fashion.
Participants/materials, setting, methods: Comorbidities were scored with the Charlson Comorbidity Index (CCI; categorized 0 vs ≥1). Testicular volume was assessed with a Prader orchidometer. Color-Doppler US was used to detect spermatic vein reflux and to classify the grade of varicocele. Semen analysis values were assessed based on the 2010 WHO reference criteria. Descriptive statistics detailed the association between varicocele, clinical comorbidities and seminal parameters. Logistic regression models tested the association between clinical predictors and oligospermia. Serum FSH was included in the model as both a continuous and a categorized variable (according to the most informative cut-off: 11.1 mUI/ml).
Main results and the role of chance: Overall, varicocele was found in 982 (46.8%) patients. Patients with varicocele had a mean (SD) age, BMI and Prader of 42.5 (6.7), 25.46 (3.3) kg/m^2 and 16.48 (5.9), respectively. Patients with varicocele and CCI ≥1 presented a higher prevalence of oligospermia (x2: 10.8; $p < 0.001$); conversely, no differences were found in terms of rates of either asteno- or teratozoospermia. Patients with serum FSH > 11.1 mUI/ml more frequently had a higher BMI ($p = 0.04$), lower testicular volume ($p < 0.001$), lower serum inhibin B ($p < 0.001$) and lower AMH ($p < 0.001$), and testosterone levels ($p < 0.001$). At MVA, FSH was significantly associated with oligospermia as either a continuous (OR: 1.13; $p < 0.001$) or a categorical predictor (OR: 3.8; $p < 0.001$), after accounting for other variables. Likewise, CCI ≥1 (OR: 3.3; $p = 0.001$), duration of infertility (OR:1.1; $p = 0.03$) and testicular volume (OR:0.9; $p < 0.001$) achieved independent predictor status for oligospermia. Conversely, patient age and varicocele were not significantly associated with oligospermia.
Limitations, reason for caution: Cross-sectional, retrospective analyses.
Wider implications of the findings: A general consensus regarding indications for varicocele ligation still lacks. A better selection of patients would allow to identify who will not significantly benefit from surgery.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: varicocele ligation, oligospermia

P-075 Routine determination of sperm DNA fragmentation incorporating the sperm degradation index (SDi) is a useful noninvasive biomarker to identify patients with varicocele in seminograms

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Study question: The study attempted to establish the incidence of sperm with degraded nuclei in a large population of semen samples obtained from donors and patients of varying fertility to explore the value of using this parameter as a noninvasive marker of varicocele.
Summary answer: Although not pathognomonic, if a neat ejaculate shows at least 1 in 3 spermatozoa with degraded nucleus, then there is high probability that the individual has varicocele.
What is known already: Sperm with degraded nucleus are a specific subpopulation of spermatozoa identified by means of the Sperm Chromatin Dispersion test (SCDt) that are characterized by massive levels of single and double strand DNA breaks and alteration of nuclear proteins. A high proportion of these sperm with degraded nucleus has been repeatedly observed in varicocele patients but its relative presence has not been systematically analyzed in patients exhibiting different sperm pathologies.
Study design, size, duration: Retrospective international multicenter study consisting of 1528 semen samples obtained from (1) Non-varicocele subjects: including fertile donors, patients with leukocytospermia, cancer or idiopathic infertility, and (2) Varicocele patients exhibiting clinical and subclinical varicocele.
Participants/materials, setting, methods: Sperm with degraded nucleus were identified using the Sperm Chromatin Dispersion test (Halotech DNA, Madrid), protein staining and 2-dimensional neutral and alkaline comets. The proportion of sperm with degraded nuclei in the total population with fragmented DNA was established as the Sperm Degradation index (SDi).
Main results and the role of chance: SDi was significantly higher in individuals diagnosed with varicocele (mean = 0.54; SD = 0.16; Range = 0.227 – 0.952) compared with non-varicocele. ROC curve analysis revealed that a SDi value of greater than 0.32 was able to identify varicocele patients with a sensitivity of 93%, a specificity of 90%, a negative predictive value of 99.5% and an area

under the curve (AUC) of 0.942 ($p < 0.001$); this high level of SDi was observed in 89.6% of the patients diagnosed with varicocele. While the frequency of sperm with fragmented DNA was statistically different among the different groups established in this protocol, this parameter on its own (not segregating degraded nuclei) was not as good a predictor of the presence of varicocele (AUC = 0.612; $p < 0.001$).

Limitations, reason for caution: Idiopathic infertility is a complex heterogeneous group of unknown etiology. Similar values for SDi as those observed in varicocele patients may occur in unidentified subsets of infertile patients.

Wider implications of the findings: Routine determination of sperm DNA fragmentation with the incorporation of SDi would be a useful noninvasive predictor of varicocele. SDi could be potentially used to identify patients more likely to have varicocele in seminograms when the SDi is >0.32.

Study funding/competing interest(s): Funding by national/international organization(s) – MINECO.

Trial registration number: This investigation is a part of the experimental protocols included in the research project BFU-2013-44290-R.

Keywords: varicocele, sperm DNA fragmentation, sperm chromatin dispersion test, degraded sperm nucleus

P-076 Genetic variability in bitter/sweet taste related genes and male infertility

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Study question: The aim of this study was to determine whether the genetic variability of the taste receptors genes (TAS2Rs/TAS1R) and of the guanine nucleotide binding protein, alpha transducing 3 (GNAT3) was associated with male infertility.

Summary answer: Our preliminary results suggest a possible associations between the genetic variants in taste related genes and specific sperm parameters.

What is known already: Recent studies have demonstrated taste receptor expression also in testis and in sperms, highlighting their possible role in sperm maturation as well as in sperm behavior and fertilization by sensing chemicals in the milieu. The high frequency of human genetic variations of genes involved in taste perception results in a large variability from super-taster subjects to totally "non-taster" patients, that might be influence sperm functionality too.

Study design, size, duration: In this epidemiological study 163 male patients were enrolled and their DNA was isolated from buccal swab.

Participants/materials, setting, methods: The patients enrolled at the Centre of Couple Sterility, Siena University Hospital (Italy), were characterized for main sperm parameters, according to WHO (2010) guidelines: concentration, morphology, progressive and total motility. All subjects were then genotyped for 14 single nucleotide polymorphisms (SNPs) in TAS2Rs/TAS1R and GNAT3 genes. The genotyping was performed using the KASPar SNP genotyping system.

Main results and the role of chance: Association between the SNPs and the main sperm parameters was tested through chi^2 test. We found a statistically significant association between sperm concentration and the TAS1R2 rs35874116 SNP ($p = 0.049$) and with the TAS2R49 rs7135018 SNP ($p = 2.7 \times 10^{-6}$). Additionally, we observed a statistically significant association between progressive motility and the TAS2R14 rs11610105 variant ($p = 0.036$). Using the Bonferroni method in order to correct for multiple testing, only TAS2R49 rs7135018 SNP remains significant.

Limitations, reason for caution: The TAS2R49 rs7135018 SNP has a low frequency in the population and therefore a larger study needs to be carried out in order to validate the findings.

Wider implications of the findings: Our study, if replicated in a larger population, might contribute in further understanding the genetic components of male infertility.

Study funding/competing interest(s): Funding by University(ies) – University of Siena.

Trial registration number: NA.

Keywords: taste genes, spermatogenesis, SNPs, male infertility

P-077 In male factor infertility, the sperm aneuploidies of chromosomes 16 and 18 correlate with polymorphism of follicle-stimulating hormone receptor

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Study question: Does the chance to observe a chromosomal aneuploidy in sperm correlate with the presence of genetic polymorphism Thr307Ala and Asp680Ser of follicle-stimulating hormone receptor (FSHR)?

Summary answer: A statistically significant correlation between the presence of alternative alleles of *FSHR* gene and the occurrence of sperm aneuploidy is observed for chromosomes 16 and 18 ($p < 0.01$), but is absent for chromosomes 13, 21, X and Y.

What is known already: The influence of alternative variants of FSH receptor (FSHR) on male infertility is not completely understood. In males, FSH is responsible for Sertoli cell's function and, by means of specific receptor FSHR, participates in induction and maintenance of spermatogenesis. However, any results regarding possible correlation of some specific type of semen chromosomal aneuploidy (that may cause male infertility) with FSHR genotype's particular abnormality have not been reported to date.

Study design, size, duration: The study was un-blindly performed for a group of 32 infertile men of age 32.33 ± 5.71 years old. The sperm aneuploidies in chromosomes 13, 16, 18, 21, X any Y and the genotypes of FSHR gene Thr307Ala and Asp680Ser were studied in the mentioned group. The karyotype abnormalities were also studied.

Participants/materials, setting, methods: The genotype distribution and allele frequency of FSHR Thr307Ala and FSHR Asp680Ser polymorphisms were analyzed by Taqman assays on the ABI PRISM 7500 real-time PCR system. The sperm aneuploidies in chromosomes 13, 16, 18, 21, X any Y were analyzed exploiting the method of fluorescence in situ hybridization (FISH).

Main results and the role of chance: The average level of sperm aneuploidy for chromosomes 16 and 18 was significantly higher in carriers of FSHR polymorphisms, compare with in normal-genotype patients ($p < 0.01$). A statistically significant positive correlation between number of alternative alleles 680Ser of *FSHR* gene in genotype and the level of sperm aneuploidies for chromosomes 16 and 18 was proved ($\mathbf{r_s = 0.694}$, $p < 0.01$; $\mathbf{r_s = 0.802}$, $p < 0.01$). Also a statistically significant positive correlation between number of alternative alleles 307Thr of *FSHR* gene in genotype and the level of sperm aneuploidies for chromosomes 16 and 18 was proved ($\mathbf{r_s = 0.784}$, $p < 0.01$; $\mathbf{r_s = 0.713}$, $p < 0.01$). There was no correlation between the presence of alternative alleles of *FSHR* gene in genotype and the sperm aneuploidy for chromosomes 13, 21, X, Y.

Limitations, reason for caution: Present technique can evaluate the DNA fragmentation level in cases, when the sperm count excides 2 mln/mL.

Wider implications of the findings: Because semen chromosomal aneuploidy is a possible source of male infertility, our findings allow us to speculate that genetic abnormalities of FSHR (Thr307Ala, Asp680Ser) might have some negative impact on the process of meiosis in spermatogenesis. Sperm aneuploidy level of chromosomes 16 and 18 is worse among patients with these FSHR polymorphic genotypes, compare with normal-genotype patients. The correlation is observed with certain statistical significance in a general comparison "normal genotype vs. all polymorphic ones".

Study funding/competing interest(s): Funding by hospital/clinic(s) – Center of Human Reproduction "Sana-Med Ltd" (Kharkov, Ukraine). No conflict of interests exist.

Trial registration number: NA.

Keywords: FSHR Thr307Ala, FSHR Asp680Ser, sperm aneuploidy

P-078 Anatomic vascular variations in sub-inguinal varicocelectomy

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Study question: Is Knowing Subinguinal anatomy reduce preoperative and post operative complication?

Summary answer: Microsurgical subinguinal varicocelectomy is an effective method in the treatment of varicocele. However, the branching ratios of arterial and venous structures in this region are higher according to the inguinal and

high inguinal regions. Therefore it is very important to carry out a better dissection as it may lead to relapses in unconnected veins and may negatively effect the connection of functions of the testicular arteries.
What is known already: Microsurgical vaicocelectomy is a widely applied open surgical technique in the treatment of varicocele.
Study design, size, duration: During three years a retrospective study
Participants/materials, setting, methods: A total of 65 patients who underwent microsurgical subinguinal varicoselectomy and whom were contacted data were retrospectively assessed according to number and adjuvant conditions of perioperative artery and vein.
Main results and the role of chance: Per-operative single artery in 32% (21/65) patients, double arteries in 46% (30/65) patients and three or more arteries in 15% (10/65) patients was determined. The mean number of veins connected during the operation was found 9,76 ± 0,96 (3–14). The artery vein neighborhood was determined as a vein package in 91% (59/65) patients. However an artery was determined at a single vein neighborhood in 3% (2/65) of the patients where the artery was separated from the vein package. Arterial structures were not determined clearly in 6% (4/65) of the cases.
Limitations, reason for caution: Size of the study, retrospective study.
Wider implications of the findings: Microsurgical varicocelectomies requires detailed dissection.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Firat University Hospital.
Trial registration number: NA.
Keywords: varicocele, microsurgical varicocelectomy, infertility.

P-079 Relationship between urokinase-type plasminogen activator (uPA) in seminal plasma and spermatozoa with human sperm parameters

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Study question: Urokinase type plasminogen activator (uPa), is together with tissue-type plasminogen activator (tPA) one of the activators for plasminogen-plasmin system. The aim of this research was evaluated the relationship between total and active uPA concentration in seminal plasma and spermatozoa with human sperm parameters
Summary answer: Active uPA in seminal plasma was directly and significantly related with the volume of the ejaculate, total number of spermatozoa in the ejaculate (x10^6) and motility.
What is known already: The plasminogen/plasmin system has been related to different reproductive process as spermatogenesis, sperm capacitation, and fertilization. Some authors have previously detected the presence of plasminogen activators in human seminal plasma and spermatozoa. Lison *et al*, 1993, correlated significantly plasminogens activators with seminal parameters. Huang et al. 1997. found that uPA activity was higher in fertile than astenozoospermic men. However, the specific role and importance of uPA for the male reproductive function is not well known.
Study design, size, duration: Semen samples from 182 patients attending IVI-Murcia clinic for infertility screening were enrolled in this Cross sectional study between 2012 and 2014
Participants/materials, setting, methods: Semen samples were examined for volume, sperm concentration, morphology and motility according to WHO 2010. Aliquots of each sample were centrifuged and seminal plasma and spermatozoa were stored at -80°C until uPA evaluation. Total and active uPA were determined by ELISA KIT and confirmed by Wester blotting
Main results and the role of chance: Total uPA contents neither seminal plasma nor spermatozoa was not related to the seminal parameters ($p > 0.05$). On the other hand, active uPA in seminal plasma was directly and significantly related to volume of the ejaculate, total number of spermatozoa in the ejaculate (x10^6) and motility($p < 0.05$). Total and active uPA in seminal plasma from the samples analyzed were not significantly related (Pearson correlation rate: 0.13, $p > 0.05$. The active uPA represented less than 10% of the total UPA in most of the cases (mean value 8.27%)
Limitations, reason for caution: 182 patients were included in this research although we could not analyze all parameters in every ejaculated. Regarding seminal plasma we analyzed 148 cases for active uPA and we evaluated 64 samples for uPA total. Whereas in spermatozoa we evaluated 68 samples for total uPA
Wider implications of the findings: Results of this research showed that active Upa play an important role in different semen parameters. Further research will be necessary in order to study whether the addition of UPA to ejaculated improve seminal characteristics like sperm motility.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s) – IVI MURCIA S. L, Murcia University, Spanish Ministry of Economy and European Regional Development Fund (Feder). CDTI ID-2012053.
Trial registration number: NA.
Keywords: plasminogen/plasmin system, uPA

P-080 Semi-quantitative assessment of superoxide anions in neat semen using OxiSperm®: a survey to compare visual, spectrophotometric and image analysis results

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Study question: This investigation examined the efficiency of three colorimetric and semi-quantitative methods to assess the level of superoxide oxide (O^{-2}: SOx) in the neat human ejaculate using a Nitro Blue Tetrazolium (NBT) based protocol.
Summary answer: Discrimination amongst the different levels of SOx in the neat ejaculate was possible with a margin of error of <10% irrespective of whether colorimetric assessment was conducted visually, spectrophotometrically or via image analysis.
What is known already: An ability to assess oxidative stress in semen rapidly following collection is highly beneficial for the clinical andrologist as the balanced presence of SOx in neat semen is commensurate with high sperm quality. Unfortunately, the presence of these molecules has been typically difficult and/or expensive to quantify in neat semen.
Study design, size, duration: Prospective study consisting of 230 men aged between 25 to 53 years and attending their first visit to the fertility clinic. Sample was recruited over one year.
Participants/materials, setting, methods: IVF center and two public Universities. Two hundred and thirty ejaculates were assayed for SOx imbalance using OxiSperm® which uses NBT to produce a colorimetric reaction. Concentrations of NBT in the semen samples were assessed and compared using visual assessment (4 pre-established levels from L1 –low- to L4 –high-), spectrophotometry (4 levels) and image analysis of direct scanned digital images (4 levels).
Main results and the role of chance: The results show that a color change associated with the reaction of SOx with NBT in a reactive gel using OxiSperm® could be used to readily discriminate formazan precipitates and thereby successfully be used as semi-quantitative methodology for the assay of SOx in the human ejaculate. All three semi-quantitative techniques used here allowed discrimination amongst the different individuals with a margin of error between each methodology of < 10%. Of the semen samples analysed in this study using the OxiSperm® procedure, 15% showed a high level of SOx on the base of the threshold levels established for each detection system.
Limitations, reason for caution: The OxiSperm® assessment procedures tested in this study can all be easily incorporated into the routine andrology laboratory procedure but will need customized calibration for in house standardization in the case of image analysis assessment. Once this calibration is performed, image analysis using digital information provided by a standard digital scanner will be the cheapest and most reliable system for comparison.
Wider implications of the findings: Semi-quantitative assessment of SOx in the neat ejaculate can be used to confirm the efficacy of antioxidant treatments.
Study funding/competing interest(s): Funding by University(ies) – None of the authors declare conflict of interest.
Trial registration number: This investigation is a part of the experimental protocols included in the research project BFU-2013-44290-R, as approved by the University in accordance with the participating IVF clinics.
Keywords: superoxide anions, formazan

P-081 The emerging epigenetic role of altered chromatin condensing factors in male (in)fertility

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Study question: What is the potential epigenetic role of chromatin condensing factors (TNPs, PRMs, JMJD1A, CDY1 and CREM) in spermatogenesis impairment and male infertility?
Summary answer: Decreased expression of *TNPs*, *PRMs, JMJD1A,CDY1* and *CREM* also decreased incorporation of JMJD1A, CDY1 and CREM into regulatory regions of *TNPs* and *PRMs* genes were correlated with spermatogenesis impairment.
What is known already: Successful spermatogenesis requires a series of tightly controlled epigenetic events leading to condensation of sperm chromatin. Animal model based studies showed any failure in this process leads to impaired spermatogenesis and infertility. Many genes are involved in compaction of sperm chromatin including transition proteins (*TNPs*) and protamines (*PRMs*) which are replacements of histones, also epigenetic regulatory factors such as histone demethylases (e.g., JMJD1A) and actyle transferases (e.g., CDY1) and key master genes like *CREM.*
Study design, size, duration: Consent was obtained from azoospermic infertile men referred to Royan institute, according local ethical approval, then though ART procedure and based on pathological features, testes tissue samples were collected from three groups including complete maturation arrest, Sertoli cell only syndrome, and hypospermatogenesis as positive control (at least 30 samples in each group).
Participants/materials, setting, methods: Expression of *TNPs, PRMs, JMJD1A*, *CDY1* and *CREM* were evaluated by qRT-PCR. Also, ChIP-real time PCR was performed to evaluate the incorporation of JMJD1A, CDY1 and CREM regulatory factors into re-gulatory regions of *TNPs* and *PRMs* genes.
Main results and the role of chance: Relative expression profile of chromatin condensing genes of *TNPs* and *PRMs* showed significant decrease in complete maturation arrest and Sertoli cell only syndrome groups compared to hypospermatogenesis group ($p < 0.05$). Also expression of *JMJD1A, CDY1* and *CREM* as up-regulating factors of *TNPs* and *PRMs* showed significant decrease in two mentioned groups vs. positive control ($p < 0.05$). These findings also confirmed by ChIP data revealed decreased incorporation of histone modifying enzymes of JMJD1A and CDY1 as well as key regulator of spermiogenesis, CREM, into regulatory regions of *TNPs* and *PRMs* in both groups with spermatogenesis impairment vs. positive control ($p < 0.05$).
Limitations, reason for caution: The study population could be expanded and the interaction between other epigenetic factors such as activator of CREM (ACT) and bromodomain testis-specific protein (BRDT) could be investigated.
Wider implications of the findings: The findings implies significant association between deregulation of epigenetic sperm chromatin condensing factors with impairment of spermatogenesis and male infertility.
Study funding/competing interest(s): Funding by national/international organization(s) – Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Trial registration number: NA.
Keywords: male infertility, spermatogenesis, epigenetic, chromatin

P-082 Analysis of nuclear spermatic quality: sperm DNA integrity, chromatin condensation and aneuploidy in men with total non syndromic teratozoospermia

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Study question: Can we assess the impact of a nuclear spermatic quality analysis in infertile patients with a total non syndromic teratozoospermia on the chances of success in assisted reproductive technology (ART)?
Summary answer: Men with non syndromic teratozoospermia have an impaired nuclear sperm quality as well as chromosomal aberrations focusing mainly on gonosomes which obviously affects the fertilizing sperm power. Analysis of sperm nucleus in these cases is very useful before assisted reproductive techniques.
What is known already: Male infertility is often associated with abnormal spermatogenesis resulting in abnormal sperm count, mobility or morphology qualified as teratozoospermia. Several studies have shown that patients with a syndromic teratozoospermia manifest various nuclear alterations such as abnormal chromatin structure, DNA fragmentation and aneuploidy. Whereas, the impact of a total non syndromic teratospermia prevailing on head abnormalities still not clearly known.
Study design, size, duration: This is a case control study carried out on 59 men: 34 patients with non syndromic total teratozoospermia and 25 fertile men with normal semen profiles who acted as controls. Patients are recruited between March 2014 and August 2014.
Participants/materials, setting, methods: Semen samples were analyzed according to the World Health Organization criteria. Chromatin condensation was assessed by aniline-blue Staining. Sperm DNA fragmentation was evaluated by terminal desoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) assay and chromosome abnormalities by fluorescence *in situ* hybridization (FISH) for chromosomes X, Y and 18.
Main results and the role of chance: Semen morphology analysis performed more than one time shows a total teratozoospermia with a predominance of head abnormalities (acrosome abnormalities, microcephaly...) and no specific syndrome was characterized. The rate of aniline blue-reacted spermatozoa was significantly higher in patients compared to the control group (51,08 ± 17,58 vs 12,70 ± 6,02%; $p < 0,001$). The mean DNA fragmentation index (DFI) was also higher in patients compared to controls (27,25 ± 12,06 vs 10,25 ± 3,83%; $p < 0,001$). The results of aneuploidy frequencies showed a significant difference between both groups (1.52 ± 0.24 vs 8,31 ± 8,42%; $p < 0,001$). We found a significant increase in the mean disomy rate for sex chromosomes (1.05 ± 0.56 vs 5,2 ± 3,94%; $p < 0,001$) and chromosome 18 (0.15 ± 0.07 vs 2,76 ± 0,15%; $p < 0,001$).
Limitations, reason for caution: A limitation of this study is the low number of teratozoospermic patients considering the short duration of study.
Wider implications of the findings: Despite the small sample our results confirms the importance of morphologic sperm evaluation, study of sperm chromosomal aneuploidy, chromatin condensation and DNA fragmentation before ART for non syndromic teratozoopsermic patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: male infertility, total teratozoospermia, sperm aneuploidies, DNA fragmentation, chromatin decondensation

P-083 Effect of positive bacteriospermia on IVF results

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Study question: Is there a detrimental effect of positive bacteriospermia detected the day of oocyte retrieval on IVF results?
Summary answer: Polymicrobian contaminations of sperm affect significantly quality of sperm and the embryonic cleavage rate.
What is known already: French guidelines recommend the practice of a culture of semen at least six months before each IVF attempts. While we respect this recommendation, we hypothesize that some sperm could be infected, although culture was negative six months earlier. The effects of bacteriospermia are supposed to be negative on the results of IVF, but the literature is controversial.
Study design, size, duration: This prospective study was conducted between September 2011 and April 2012, for its first part and between January 2014 and Mars 2014 for its second part, in Antoine Béclère hospital (Clamart – France). The day of oocytes retrieval, 133 and 104 semen culture were analyzed respectively in the first and second part of the study.
Participants/materials, setting, methods: In the first part of this study a semen culture analysis was performed on raw sperm the day of oocyte retrieval

while in the second part,semen cultures were performed in same conditions but with enhanced hygiene recommendations given to patients associated with a water intake. Three groups have been defined according the result of semen cultures *ie*: negative, polymicrobial or monomicrobial. Semen parameters and IVF results were analysed for each group.

Main results and the role of chance: In the first part of this study, out of 133 semen culture analysed, 94 (71%) were negative, 28 (21%) were positive polymicrobial and 11 (8%) were positive monomicrobial. The initial sperm count and progressive motility after preparation were significantly lower in polymicrobial group but no impact on IVF results was observed. In the second part, 104 semen cultures were analysed after enhancement of hygiene instructions and water intake. Fifty one (49%) semen cultures were positive with a bacteriospermia significantly higher in the second part of our study (45 polymicrobial and 6 monomicrobial). The analysis of combined data showed a significant decrease of the initial sperm count as also the embryos' cleavage rate (number of embryos obtained divided by the number of inseminated oocytes) in polymicrobial group. Embryos' morphology as well as the clinical pregnancy rates were comparable between the three groups. However, the miscarriage rates seem to be increased in the polymicrobial group compared to the negative group (8/18 (44%) vs 10/47 (21%)).

Limitations, reason for caution: The size of the cohort has to be increased to reach the power required. The increased miscarriage observed in this study remain under caution.

Wider implications of the findings: We showed in this study that polymicrobian contaminations of sperm affect significantly quality of sperm and the embryonic cleavage rate. Unexpectedly a water intake had no beneficial effect on bacteriospermia's incidence.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Assistance Publique des hôpitaux de Paris.

Trial registration number: NA.

Keywords: asymptomatic bacteriospermia, semen parameters

P-084 Correlation between sperm chromatin decondensation, density, aneuploidy and hyaluronic binding capacity

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Study question: Whether there is relation between sperm chromatin decondensation, concentration, hialuronic acid (HA) binding capacity and aneuploidy of semen samples of fertile, normozoospermic and infertile, oligozoospermic men?

Summary answer: There are correlations between sperm condensation and the examined biomarkers, irrespective to fertility status. The sperm condensation rate, HA bindig capacity related fertility potential and sperm density are strongly related. Also, there is relation between sperm condensation and aneuploidy rate.

What is known already: Importance of sperm biomarkers are increasing in male infertility. Beside genetic integrity and fertilization potential tests, epigenetic studies came to the front. Chromatin decondensation is related to persistent histones detected by aniline blue, while HA binding capacity reflects genetic integrity and maturity related fertilization potential. Paralel evaluation of test results improves the understanding the regulation mechanisms of spermatogenesis and fertilization, may help in choosing the most effective therapeutic approach and sperm selection for assisted reproduction.

Study design, size, duration: This is a cross-sectional study examining semen of 20 infertile, oligozoospermic and 20 fertile, normozoospermic men attending at the Andrology Center and the Sperm Cryopreservation Laboratory.

Participants/materials, setting, methods: The ejaculates were assessed as per WHO guidelines. Hyaluronic acid binding capacity test was the functional test. To determine the numerical chromosome aberrations we used multicolor fluorescent in situ hybridization (FISH) for chromosomes X, Y and 17. Aniline blue staining was performed as a nuclear decondensation marker test.

Main results and the role of chance: In normozoospermic samples mean sperm concentration was 73,4 M/mL, rate of decondensed sperm nuclei detected by aniline blue 16.2%, HA-binding capacity 82% and estimated aneuploidy rate 4.12%. In the oligozoospermic samples mean sperm concentration was 7.13 M/mL, rate of dencondensed nuclei 41.8%, HA binding capacity 47.5% and the estimated aneuploidy rate 6.17%. All examined parameters differ significantly, if samples below and over 20% decondensation compared. There are inverse correlations between sperm decondensation and sperm concentration (r = -0.876), as well as sperm decondensation and hyaluronic binding capacity (r = -0.876). Sperm decondensation rate is also related to aneuploidy rate (r = 0.559).

Limitations, reason for caution: Data on confounders influencing sperm characteristics such as smoking, occupational or environmental hazards, comorbidities were not collected.

Wider implications of the findings: Although chromatin condensation occurs in late phase of spermatogenesis, it shows correlation with cytogenetic abnormalities and membrane remodelling, irrespective to the fertility status. It can be explained with a common regulation mechanism, thus malfunction of it can result in various sperm defects on different levels. However, since examined biomarkers reflect different sperm defects, they cannot replace each other.

Study funding/competing interest(s): Funding by national/international organization(s) – This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/ 2-11/1-2012-0001

Trial registration number: NA.

Keywords: sperm function, sperm biomarkers, sperm decondensation, sperm aneuploidy, hyaluronic binding capacity

P-085 Role of plasma membrane Ca^{2+} ATPase 4 gene in sperm motility and male infertility – a preliminary study

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Study question: A project aims to explore the role of the Plasma Membrane Ca^{2+} ATPase (PMCA) 4 in sperm motility and male infertility.

Summary answer: Mutations in PMCA4 gene seem to be associated with decreased sperm motility, and male infertility.

What is known already: Experiments on mice have demonstrated that spermatozoa of the animals with deleted PMCA4 loose their motility ability in the environment that requires the hyperactivation of sperms (corresponding the environment of the upper female genital tract) due to their defect in Ca^{2+} homoeostasis. There are no data available at the moment whether functional status of the PMCA4 (polymorphisms, mutations) in humans has any impact on sperm motility function and, thereby, on male fertility.

Study design, size, duration: Cross-sectional study of 191 patients, and a control group of 180 men

Participants/materials, setting, methods: Patient group consists of 191 Estonian men from infertile couples, with low progressive sperm motility (<20%). Control group consists of 180 young men (age 18–25 years) from general population with normal semen quality.

Main results and the role of chance: Apart from different synonymous polymorphisms with no functional impact, a known polymorphism variant G > A that causes the replacement of Glu to Lys has been detected in 11 patients in heterozygote state in exon 11, calling for the A allele frequency of 0.029. To assess whether the detected variant has been shown also in other populations, the available data from the worldwide 1000 genome project was examined. It showed that rs147729934 A allele in heterozygote state has been found only in 3 Finnish women among 93 Finns analysed (allele frequency 0.016). No A allele has been detected in other European populations (comprising in total 379 Europeans) analysed for this mutation, decreasing the frequency of the mutation among Europeans to 0.004. It makes the incidence of the mutation detected in this study among the 191 infertile male patients 7 times higher than in the other European populations. Analysis of the control group is under the process, the results will be reported during the congress.

Limitations, reason for caution: Since mutation has been also shown in some Finnish women, it should be checked whether the mutation found is really a disease associated, or the ethnicity specific variant – Estonians and Finns belong to the Finno-Ugric group.

Wider implications of the findings: Infertile patients with mutated PMCA4 most likely cannot benefit from the IUI or conventional IVF treatment due to the defected ATP synthesis needed for sperm hyperactivation, supposedly, ICSI is the method of choice for these patients. It will be also checked whether Latvians, Lithuanians and Russians that do not belong to the Finno-Ugric ethnicity group carry this mutation. These findings can shed the light on population genetics in Baltic-Scandinavian-Russian region.
Study funding/competing interest(s): Funding by national/international organization(s) – Latvian Council of Sciences.
Trial registration number: 233/2012.
Keywords: sperm, motility, gene, ATP

P-086 Predictive factors influencing pregnancy rates after artificial insemination with donor semen: a prospective observational study of 935 cycles

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Study question: In this prospective observational study, we aimed to examine to which extent certain covariates such as female age, smoking/non-smoking, BMI, use of natural cycle, ovarian stimulation protocol, the insemination procedure itself, sperm quality parameters etc., can influence pregnancy rates after artificial insemination with donor semen (AID).
Summary answer: Taking all covariates into account, statistical analysis showed that the pregnancy rate significantly increases when the patient is young, stimulated with low dose gonadotrophins (hMG) or recombinant FSH (rec FSH) and when a high percentage of grade A sperm motility is observed.
What is known already: Many studies examining different factors predicting pregnancy outcome after AID treatment have been published before. The predictive value of covariates such as female age, smoking, BMI, ovarian stimulation protocols etc. was observed in many studies. However, in almost all studies these covariates were examined as independent of each other, which may result into misleading conclusions.
Study design, size, duration: During the period of July 2011 until November 2014, data from 935 AID cycles in 306 couples were collected prospectively in a tertiary referral infertility centre. Since the outcome results after AID cannot be analysed independently, statistical analysis was performed using a Generalized Estimating Equations (GEE) model.
Participants/materials, setting, methods: Covariates taken into account were female age, smoking/non-smoking, BMI, primary/secondary infertility, infertility diagnosis, cycle number, ovarian stimulation method, day 0 estradiol/progesterone levels, HCG-insemination interval, the insemination procedure itself, occurrence of blood loss after insemination, sperm quality parameters, inseminating motile count (IMC) and sperm washing procedure.
Main results and the role of chance: The clinical pregnancy rate, i.e., presence of fetal heart beat at 6–7 weeks of gestation, was 15,6% per cycle. A univariate statistical analysis of the dataset revealed the following parameters as predictive for a successful pregnancy outcome: age, primary/secondary infertility, stimulation method, estradiol levels at day 0, the percentage of grade A motility and the total motile sperm count (TMSC). By using the multivariate GEE analysis, we observed that only female age, the stimulation method and grade A motility were useful in predicting pregnancy after AID. Based on these predictive factors, we were able to build a Microsoft Excel sheet which can calculate pregnancy rates based on the values entered for female age, stimulation method and percentage grade A motility.
Limitations, reason for caution: It can be misleading to look at only one covariate at a time, because different observations are not independent of each other. Furthermore, GEE analysis has a low statistical power to detect statistically significant differences in groups with low success rates, such as observed in an AID programme.
Wider implications of the findings: According to our results, it seems that female age, whether or not using ovarian stimulation, type of ovarian stimulation and percentage grade A sperm motility are the most important factors influencing the success rate in a sperm donation programme. Ovarian stimulation with low dose protocols of hMG or rec FSH significantly improve the pregnancy rate per cycle, but one has to be careful for multiple pregnancies. Surprisingly, BMI and smoking didn't affect the outcome results significantly.
Study funding/competing interest(s): Funding by University(ies), funding by hospital/clinic(s), Funding by national/international organization(s) – This study is part of the 'Limburg Clinical Research Program (LCRP) UHasselt-ZOL-Jessa', supported by the foundation Limburg Sterk Merk, Province of Limburg, Flemish government, Hasselt University, Ziekenhuis Oost-Limburg and Jessa Hospital, Belgium.
Trial registration number: NA.
Keywords: artificial insemination, donor semen, predictive factors, pregnancy rate

P-087 Mission impossible? Improving ART outcome following unexplained total failed fertilisation

Abstract withdrawn by the author

P-088 Impact of clinical, hormonal and histopathological findings on sperm retrieval and pregnancy rates in azoospermic patients

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Study question: Is it possible to predict sperm retrieval, fertilization, clinical pregnancy and live birth by clinical and/or hormonal factors in azoospermic patients with different testicular histolopathologies undergoing testicular sperm extraction (TESE) procedure after an ICSI cycle?
Summary answer: There was no significant variable to predict sperm retrieval, fertilization, clinical pregnancy and live birth among normal testicular histology, maturation arrest, sertoli only syndrome and peritubular hyalinization and tubular atrophy groups. However, in hypospermatogenesis group sperm retrieval (SRR) and pregnancy rates (PR) were higher in younger patients.
What is known already: Although testicular histology is the most significant variable regarding the success of TESE and ICSI outcome, this is the first study evaluating the impact of clinical and endocrinologic factors on sperm retrieval, fertilization, clinical pregnancy and live birth rates among azoospermic patients in most prevalent testicular histopathologic groups.
Study design, size, duration: A retrospective analysis of 271 patients with non-obstructive azoospermia who underwent TESE procedure for ICSI between 2003 and 2013.
Participants/materials, setting, methods: We searched our database for patients who were diagnosed with azoospermia and treated with TESE. Main outcome measures were the impact of male age, FSH, LH and testosterone levels, sperm parameters, duration of infertility on sperm recovery and pregnancy rates after TESE and ICSI cycles.
Main results and the role of chance: In baseline data, there was no significant difference between groups for male and female age, infertility duration, and total and free testosterone concentrations. FSH concentration was statistically higher in peritubular hyalinization and tubular atrophy group ($p < 0.0001$). Patients with motile sperm retrieved and conceived after TESE were younger in hypo-spermatogenesis group. There was no significant clinical and hormonal variable to predict sperm retrieval, fertilization, clinical pregnancy and live birth among normal testicular histology, maturation arrest, and sertoli only syndrome groups. The highest SRR and PR was in normal spermatogenesis (91,7 and 47,9%, respectively). The lowest SRR and PR was in maturation arrest (21,1%), and peritubular hyalinization + tubular atrophy groups (7,1%), respectively.
Limitations, reason for caution: Retrospective design was the major limitation of this study.
Wider implications of the findings: Younger azoospermic patients with hypospermatogenesis have higher chance for sperm retrieval and pregnancy after TESE. Only patients with testicular atrophy had higher FSH levels as compared to other histopathologic groups. This study also proposed that all azoospermic patients regardless of testicular histopathological diagnosis had some chance for sperm retrieval and pregnancy by TESE and ICSI.

Study funding/competing interest(s): Funding by University(ies) – Gazi University.
Trial registration number: NA.
Keywords: TESE, azoospermia, IVF, sperm retrieval, pregnancy rates

P-089 Sperm DNA fragmentation testing as a general marker for pregnancy outcome in IVF patients

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Study question: Can sperm DNA fragmentation testing be used as a useful tool to predict ART outcome independent of female factors.
Summary answer: Sperm DNA fragmentation analysis was retrospectively shown to be an important independent variable in ART and thereby proves its inherent value as a useful laboratory test.
What is known already: Reactive oxygen species generated within sperm from IVF can have singularly destructive affects on susceptible sperm DNA and thus have knock on effects on fertilisation, embryo formation and pregnancy rate. Many techniques are available to determine this susceptibility but to-date none have shown a clear correlation between the technique used, the use of IVF or ICSI and the CPR.
Study design, size, duration: From January to August 2014 178 SDA tests were performed and centile charts created based on the distribution of the observed DNA fragmentation pattern. These outcomes were then retrospectively matched with the ART procedure employed and the clinical outcome.
Participants/materials, setting, methods: An in-house sperm DNA fragmentation test (SDA) was developed based on the technique described by Evenson in 2002. Following validation versus commercially available SCSA the test was offered commercially within Sims IVF clinic.
Main results and the role of chance: 231 sperm samples were analysed using this technique and centile charts of the distribution were calculated. At the time of analysis 137 cycles were performed in 104 of these couples, and the observed fragmentation rates were correlated with outcomes. Mean DFI was 21.4,with a standard deviation of 11.3. A DFI less than 21.4% was associated with a CPR with ICSI of 31.0% and IVF of 33.3% ($p = 0.84$). In the group with borderline DNA fragmentation (21.4–32.7%) CPR was higher with ICSI than IVF, 31.3 vs. 16.7% However, this difference was not statistically significant ($p = 0.44$). No patients with a DFI above 31.4% had IVF used as the method of fertilisation.
Limitations, reason for caution: There are a limited number of observations ($n = 231$), giving insufficient statistical power to draw conclusions in the borderline DFI group. Although not described, clinical decisions were made based on many factors and so may have influenced the ongoing CPR independent of SDA values or assessment.
Wider implications of the findings: Sperm DNA fragmentation analysis is an unbiased laboratory technique with merit as part of a male evaluation in preparation for ART. Further research is needed to assess if couples with DFI between 20 and 30% would benefit from ICSI rather than IVF. A power calculation demonstrates that 294 cycles would be needed to identify if the difference in pregnancy rates in this group of patients is statistically significant. A prospective RCT to assess this further would be beneficial to clarify the answer to this question.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Sims IVF Clinic.
Trial registration number: NA.
Keywords: sperm, fragmentation, pregnancy, outcome

P-090 Estrogenic regulation of bicarbonate transporters from SLC4 family in rat Sertoli cells

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Study question: Do high levels of 17β-estradiol (E2) change the expression and functionality of solute carrier 4 (SLC4) bicarbonate transporters in rat Sertoli cells (SCs)?
Summary answer: In SCs treated with high levels of E2 we observed an increase in the expression levels of anion exchanger 2 (AE2) and of the electroneutral Na^+/HCO_3 – co-transporter (NBCn1), as well as altered transcellular transport, as perceived by the perturbation on the ATP-induced short-circuit transcellular current.
What is known already: Bicarbonate is essential not only for ion homeostasis but also for the maintenance of pH along the male reproductive tract. SCs play a major role in control of seminiferous tubules pH, partly due to the action of some of bicarbonate transporters. Previous studies support an association of high E2 levels with modulation of specific ion transporters expression involved in H^+ homeostasis in the efferent ductules.
Study design, size, duration: Primary cultures of SCs obtained from male Wistar rats were exposed to high concentrations of E2 (100 nM) during 24 h (at 33°C; 5% CO_2).
Participants/materials, setting, methods: Primary cultures of SCs were obtained from 22 days old male Wistar rats. SCs were cultured using a bicameral chamber system. mRNA expression was determined by conventional and quantitative RT-PCR. Protein expression was assessed by the slot-blot technique. Transcellular transport on cultured SCs was evaluated by voltage-clamp technique.
Main results and the role of chance: AE2, NBCn1, electroneutral Na^+/HCO_3^- co-transporters (NBCe1) and Na^+-driven Cl^-/HCO_3^- exchanger (NDCBE) were identified in SCs, being AE2 and NBCn1 the most abundant. E2-treated cells presented an increase in AE2 and NBCn1 protein levels, as well as altered transcellular transport. E2-treated SCs presented a perturbation of ATP-induced short-circuit current. This alteration was concurrent with augmented AE2 and NBCn1 levels. Overall, we report a relation between increased E2 levels and the expression/function of AE2 and NBCn1 in rat SCs, providing new evidence on the mechanisms by which E2 can regulate SCs physiology and consequently spermatogenesis, with direct influence on male reproductive potential.
Limitations, reason for caution: Although our study demonstrate significant effects of E2 on SCs physiology, an *in vitro* study (even when using primary cultures) does not exactly mimics the *in vivo* conditions, where cells are exposed to other biological influences.
Wider implications of the findings: E2 induces alterations on the expression levels of specific bicarbonate transporters and is able to modulate their functioning in rat SCs. In face to our results, it is imperative to further disclose the molecular mechanisms involved on bicarbonate transport and regulation in SCs, to identify and counteract possible alterations associated with pathological conditions associated with altered E2 levels that compromise the male reproductive potential.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the "Fundação para a Ciência e a Tecnologia" – FCT (PTDC/QUIBIQ/121446/2010) co-funded by Fundo Europeu de Desenvolvimento Regional – FEDER via Programa Operacional Temático Factores de Competitividade – COMPETE/QREN. UMIB is funded by FCT-Foundation for Science and Technology, under the project Pest-OE/SAU/UI0215/2014. M. G. Alves (SFRH/BPD/80451/2011) was funded by FCT. P. F. Oliveira was funded by FCT through FSE and POPH funds (Programa Ciência 2008).The authors declare no conflict of interest. MG Alves and RL Bernardino contributed equally for this work.
Trial registration number: NA.
Keywords: bicarbonate transporters, male fertility, sertoli cells, SLC4 family, estrogens

P-091 Is there an impact of ionizing irradiation (Chernobyl accident) on the relationship between semen parameters and demographic, anthropometric and infectious factors?

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Study question: Are there any modifications in sperm count regarding demographic data, life-style and background pathology correlated to men age at Chernobyl event.
Summary answer: The reproductive health of men born after April 1986 is modified only by an increased BMI and mumps history while in men born before Chernobyl event the toxic environment and age have the highest impact on sperm count.
What is known already: Factors that determine the performance of sperm are supposed to be under intense selection because of their close correlation with fertilization success. A number of different factors have been suggested to account for fertilization probability including sperm concentration, sperm motility morphology. Sperm morphology and performance can be affected by radiation of the Chernobyl event. Some studies in humans have shown changes in ultra-structure of the sperm head when exposed to high levels of radiation.
Study design, size, duration: Cross-sectional study of 1442 men who performed a sperm evaluation for infertility between January 2014 and December 2014. The patients were divided in 2 groups according to the date of birth (before and after Chernobyl event – April 1986).
Participants/materials, setting, methods: 1442 men (mean age 35.2 years, range 18 – 82 years was recruited. A semen analysis count was performed by the same embryologist. Data regarding personal medical history, demographic data and life style (including smoking and alcohol frequency intake) were collected. Statistical analysis was performed using SPSS v.19. Linear regression analyzis and ANOVA test were applied for comparisons between groups.
Main results and the role of chance: In patients born after 1986 higher BMI was associated with a decreased percentage of sperm motility ($p = 0.03$) and the medical history of mumps was associated with a lower pH ($p = 0.012$).

For the patients born before April 1986 the results were completely different. In this group the age modifies semen volume ($p = 0.014$), pH ($p < 0.001$) and percentage of motile sperm cells ($p = 0.035$). Also for this second group the exposure to toxic environment affects the sperm concentration ($p = 0.002$) but the mumps medical history has no impact on sperm parameters. For both groups the alcohol consumption and smoking doesn't influence the sperm parameters.
Limitations, reason for caution: Although this is a prospective observational study and the statistical biases were excluded there are still potential errors in individual's questionnaire filling. Furthermore we cannot exclude that our findings are due to unmeasured factors including diet, exercise and stress.
Wider implications of the findings: Besides being a regional study of reproductive men health it has an international implication in how Chernobyl event affected male sperm parameters. Our study suggested that smoking and alcohol intake are not the most important factors in reproductive men health but BMI, age and toxic environment. In the group of men born before Chernobyl event the BMI is the most important (the age is not related with the sperm analysis) while in the second group the toxic agents affect the sperm count.
Study funding/competing interest(s): Funding by hospital/clinic(s) – MedLife Memorian Hospital, Bucharest R.
Trial registration number: NA.
Keywords: ionizing irradiation, mumps, semen analysis, chernobyl event

P-092 Reduced sperm quality is reflected in clinical outcome in advanced maternal age – independently of blastocyst quality – rendering sperm selection increasingly important

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Study question: The aim of the study was to evaluate whether blastocysts derived from sperm with reduced quality have a reduced chance to implant. This question was tested for young IVF-patients as well as for patients with advanced maternal age (AMA).
Summary answer: Our results demonstrate that blastocysts originating from ejaculate with diminished sperm quality (quantity, motility and morphology and absence of intracytoplasmic morphologically selected sperm injection IMSI class I) have a reduced chance to result in a live birth in the AMA group as compared to blastocysts from normozoospermic men.
What is known already: It is well recognized that semen of sub-fertile men show higher rates of numerical and structural chromosomal abnormalities. Therefore sperm selection is important and will result in a higher blastocyst rate, especially in patients with severely reduced sperm quality. Maternal age plays an important role in oocyte competence and the efficacy of repair mechanisms, being one important reason why implantation rates decrease with AMA.
Study design, size, duration: We conducted a retrospective study on 247 IVF/IMSI cycles between 2012 and 2013. All patients received a fresh single embryo transfer (SET). Clinical outcome was evaluated in respect to sperm quality, blastocyst morphology and maternal age. Final outcomes were pregnancy rate (PR), birth rate (BR) and abortion rate.
Participants/materials, setting, methods: For all cycles fresh semen samples were collected on the day of ovum pick-up, sperm quality was evaluated and the samples were processed by discontinuous density gradient centrifugation before IMSI. Embryos were cultured in a single step medium. On day 5 blastocyst morphology was evaluated and SET was performed.
Main results and the role of chance: No differences in clinical outcome according to sperm quality were observed in 165 cycles of women ≤38 years. 51 patients presented with normozoospermic partners (PR:54.9%,oPR:47.1%,BR:45.1%), 90 with reduced sperm quality but IMSI class I sperm (PR:53.3%,oPR:48.9%,BR:45.6%) and 24 had reduced sperm quality without class I sperm (PR: 66.7%,oPR:58.3%,BR:54.2%). The rate of top-blastocyst transfers and mean female age was similar in all groups.

In contrary, sperm quality seems to play an important role in patients with AMA (>38 years, 82 cycles). 29 patients presented with normozoospermia (PR:44.8%,oPR:37.9%,BR:31.0%), 37 with reduced sperm quality but IMSI class I sperm (PR:35.1%,oPR:29.7%,BR:21.6%) and 16 had reduced sperm quality without class I sperm (PR:18.8%,oPR:12.5%,BR:12.5%). Again, rate of top-blastocyst transfers and mean female age was similar.
Limitations, reason for caution: These preliminary data have to be confirmed in a larger setting including more data on the health of children born.
Wider implications of the findings: Our results show that blastocysts originating from poor sperm quality have a reduced chance to result in a live birth in AMA-couples. This effect is not present in younger patients, underlining that the oocyte competency and repair mechanisms diminish over time rendering sperm selection more crucial. Our findings indicate that IMSI is most important in poor sperm quality in combination with AMA.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study was not externally funded.
Trial registration number: NA.
Keywords: IMSI, paternal effect, male infertility, pregnancy rate, birth rate

P-093 Comparison of ICSI outcomes between ejaculated versus extracted testicular spermatozoa in extreme oligozoospermic men and non-obstructive azoospermia

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Study question: To assess whether the origin of sperm (testicular or ejaculate) has any influence on ICSI outcomes (fertilization rates, embryo quality, implantation rates, pregnancy rates) in case of extreme alterations of spermatogenesis.
Summary answer: There is no difference in ICSI outcomes between cycles performed with ejaculated spermatozoa in oligozoospermic men and those performed with testicular spermatozoa in non-obstructive azoospermia. Testicular sperm extraction (TESE) is an invasive procedure, so, it should be recommended to use ejaculated spermatozoa as often as possible.
What is known already: Some patients have very low sperm count on direct examination (extreme oligozoospermia) or after centrifugation (cryptozoospermia). The therapeutic management of these patients is a real challenge for physicians and biologists. Some authors proposed the use of testicular sperm in case of very low sperm count to improve ICSI outcomes. However, data in the literature are controversial.
Study design, size, duration: We compared, in a retrospective study, 75 patients with extreme oligozoospermia who underwent 161 ICSI cycles with ejaculated spermatozoa (group 1) and 74 patients with non-obstructive azoospermia (NOA) who underwent 150 ICSI cycles with extracted testicular spermatozoa (group 2), between January 2007 and June 2013 at the Lille University Hospital.
Participants/materials, setting, methods: Physical, hormonal, and ultrasound assessments were performed. Semen samples were analyzed, followed by centrifugation. We chose the threshold of 10,000 spermatozoa per ejaculate to define "extreme" oligozoospermia. The testicular disorder of spermatogenesis in both group was defined by a testicular volume <16 mL and/or FSH levels >10 UI/L.
Main results and the role of chance: Cryptorchidism was significantly more common in the NOA group (60.8 versus 22.6%, $p = 0.001$). FSH levels were significantly higher (18.9 IU/L versus 15.3 IU/L, $p = 0.001$) and Inhibin B levels were more often undetectable (31.1 versus 10.7%, $p = 0.0004$) in NOA group, reflecting a deeper alteration of spermatogenesis in case of NOA. There were no significant differences in fertilization rates (48.9 and 43.3%, $p = 0.43$), implantation rates (17.4 and 15.9%, $p = 0.77$) and percentage of top quality embryo (22.4 and 20.4%, $p = 0.73$) between the two groups. The clinical pregnancy rates per embryo transferred were comparable in both groups (28.3 and 27.4%, $p = 0.89$).
Limitations, reason for caution: There is no threshold to define "extreme" oligozoospermia. We chose the threshold of 10,000 spermatozoa per ejaculate because below this threshold ICSI seems to be technically more difficult and may impair fertilization rates. Extreme oligozoospermia and cryptozoospermia were gathered into the same group because the therapeutic management is strictly similar.
Wider implications of the findings: This study shows similarly ICSI outcomes in extreme oligozoospermia (ejaculated sperm) and in NOA (testicular sperm). In case of extreme oligozoospermia, we should strive to use ejaculated sperm because: (1) TESE is an invasive technique, (2) it is in the epididymis that spermatozoa completes its maturation and undergoes epigenetic changes, (3) testicular sperm does not seem to improve ICSI outcomes. Moreover, few studies are available about future of children born after ICSI with testicular sperm.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Lille University Hospital.
Trial registration number: NA.
Keywords: extreme oligozoospermia, non-obstructive azoospermia, ICSI outcomes, testicular sperm extraction, ejaculated sperm

P-094 Sperm DNA oxidation: a new marker of fertility?

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Study question: Can the measure of human sperm DNA oxidation be standardized as a biomarker of sperm quality and men fertility in clinical practice?
Summary answer: Our study demonstrate accuracy and reliability of human sperm 7,8-dihydro-8-oxo-2 '-deoxyguanine (8-OHdG) immuno-detection to measure human sperm DNA oxidation by flow cytometry (FCM) or fluorescence microscopy (FM) in clinical practice, in relationship with semen parameters.
What is known already: Oxidative stress is involved in many disorders including male infertility, notably by inducing sperm DNA decondensation and fragmentation. Sperm nuclear integrity is essential to conduct paternal genome transmission to offspring and normal embryonic development. We previously validated an immuno-detection protocol to measure sperm 8-OHdG biomarker using microscopy in a murine model of oxidation (snGPx4 and GPx5 double mutant). To our knowledge, no standardized protocol exists to assess human sperm nuclear oxidation in clinical practice.
Study design, size, duration: In this prospective study, we compared immuno-detection methods of human sperm 8-OHdG to select standardized protocols to measure human sperm DNA oxidation in clinical practice. During 12 months, using the validated protocols, sperm DNA oxidation of 55 infertile patients (36 ± 6 years) were measured in relation with their semen parameters.
Participants/materials, setting, methods: Immuno-detection protocols were developed and compared to measure human sperm DNA oxidation using visible or fluorescence microscopy (anti-8-OHdG primary antibody and peroxidase-conjugated or Alexa488-conjugated secondary antibody), or FCM (anti-8-OHdG + Alexa488-conjugated antibodies or commercial OxyDNA assay® kit). FCM measures were confronted with semen parameters of infertile patients.
Main results and the role of chance: Oxidation values obtained after peroxidase-conjugated *versus* Alexa488-conjugated antibodies using microscopy detection were moderately concordant ($\kappa = 0.65$) but significantly correlated ($r = 0.60$; $p < 0.05$). In addition, oxidized sperm proportion detected with anti-8-OHdG and Alexa-conjugated antibodies were underestimated (-11%, $n = 5$) with FM in comparison with FCM. Finally, the proportions of 8-OHdG positive sperm revealed by FCM were significantly different for 8-OHdG/Alexa protocol (60 ± 3%) and OxyDNA kit (85 ± 1.7%) $p < 0.001$. The values measured with the two methods were positively correlated ($r = 0.43$; $p < 0.001$) but not concordant (bias of 25%). For this last comparison, sperm parameters of 55 infertile patients, presenting astheno- necro- leuco- or teratozoospermia, were confronted to oxidation measures. We observed significant correlation between sperm DNA oxidation and spermatozoa quality, notably vitality and mobility.
Limitations, reason for caution: All the protocols lack of a true negative control of human sperm DNA oxidation. Discrimination of the different patterns obtained after peroxidase-conjugated detection using FM was extremely difficult and presented an important inter-observer variability, which highlights the need for fluorescence detection and FCM.
Wider implications of the findings: Our study shows the accuracy and reliability of measuring human sperm DNA oxidation level by FCM and FM. The determination of an oxidation threshold would improve male infertility diagnosis, ART prognosis, and adapt the antioxidants taking for patients having abnormal DNA oxidation levels. Furthermore, our results open on studying prospect of the impact of sperm DNA oxidation on embryonic development and the pregnancy chances obtained by ART.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Clermont Ferrand CHU Estaing Hospital, Laboratory of Developmental Biology and Reproduction: AMP-CECOS, F-63003 Clermont-Ferrand, France; UMR CNRS 6293; 2 UMR-CNRS 6293-INSERM U1103, GReD Clermont University, F-63000 Clermont-Ferrand, France.
Trial registration number: NA.
Keywords: human spermatozoa, DNA oxidation

P-095 Trequinsin HCl: A novel specific human sperm motility inducer identified by high-throughput screening using intracellular Ca^{2+} signalling as a surrogate physiological response

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Study question: Does Trequinsin HCl, an ultrapotent phosphodiesterase type 3 (PDE 3) specific inhibitor, has any effect on human sperm motility and acrosome reaction?
Summary answer: Trequinsin HCl was found to be an effective drug in stimulating human sperm total and progressive motility at 10 µM without stimulating premature acrosome reaction (AR).
What is known already: Both Ca^{2+} and cAMP play a pivotal role in controlling human sperm motility. Thus, several non-specific PDE inhibitors (such as methylxanthines) have been used historically as motility inducers. However, their use is compromised by induction of premature acrosome reaction.
Study design, size, duration: This was a prospective study investigating the effect of Trequinsin HCl on human sperm motility and acrosome reaction. The effect of 10 µM Trequinsin HCl on motility was assessed by CASA and Kremer penetration over 2 h. The acrosome reaction was evaluated using FACS.
Participants/materials, setting, methods: All experiments were performed at University of Dundee, School of Medicine. Sperm from 4 healthy research donors and one patient (attending Assisted Conception Unit, Ninewells Hospital, Dundee) were treated with Trequinsin and motility responses were evaluated over 2 h. Acrosome reaction was evaluated by FACS by incubating sperm cells with PSA-FITC dye for 1 h, co-incubated with Trequinsin or controls.
Main results and the role of chance: 10 µM Trequinsin significantly increased both 40 and 80% fraction donor sperm total and progressive motility under both capacitating and non-capacitating conditions ($n = 4$, $p < 0.05$). There was a significant increase in sperm number following Kremer penetration assay ($p < 0.05$). Sperm from an ICSI cycle with total failed fertilisation case were also treated with 10 µM Trequinsin HCl and showed significant increase in both total and progressively motile sperm population under both capacitating and non-capacitating conditions ($n = 1$, $p < 0.05$). Acrosome reaction, on the other hand, showed no significant increase.
Limitations, reason for caution: This is an *in vitro* study using healthy donor sperm to investigate the effect of Trequinsin HCl on human sperm motility and acrosome reaction. Although the 40% fraction sperm is known to have similar motility parameters with asthenozoospermic specimens it is important to test Trequinsin HCl on patient samples.
Wider implications of the findings: It is clear that Trequinsin HCl is an effective and specific stimulator of human sperm motility with preliminary data also supporting its effectiveness in patient sperm, and may represent a novel therapeutic approach to male subfertility.
Study funding/competing interest(s): Funding by national/international organization(s) – Study funded by TENOVUS Scotland (Dr Sarah Martins da Silva Principal Investigator). The investigators have no competing interests.
Trial registration number: Ethical approval 08/S1402/6.
Keywords: sperm motility, acrosome reaction, PDE inhibitor, specific

P-096 Impact of early apoptosis in sperm cells: our results in 598 patients

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Study question: Apoptosis is a necessary process for natural cell regeneration, but how is it manifested in sperm cells depending on the basic semen parameters?, how useful is to know the level of cell apoptosis?
Summary answer: Oligozoospermic patients have a higher incidence of apotosis in live cells. The use of these cells in fertility treatments could jeopardize the success of the technique. It is important to identify the patients with elevated apoptosis to make a correct sperm selection, using magnetic activated cell sorting (MACs).
What is known already: Although there are numerous cytometry tests available to study functionality of the sperm cells, this technology is not routinely used for the diagnosis of male factor. Much work remains to set up new techniques and determine which one has more prognostic value.
Study design, size, duration: This is a retrospective observational study with a total of 598 advanced semen analysis by flow cytometry from patients undergoing infertility treatment since 2013.
Participants/materials, setting, methods: After a basic semen analysis (concentration, motility and morphology), assessment of % apoptosis was performed by flow cytometry (MACSQuant Analyzer, Milteniy Biotec), with Anexin V-FITC and propidium iodide. Results were grouped according to the etiology of male factor: normozoospermic (32.6%), oligozoospermic (12.0%), asthenozoospermic (22.3%), teratozoospermic (26.3%) and oligoasthenoteratozoospermic (OAT) (6.8%).
Main results and the role of chance: The major group of patients with increased % of apoptosis (>15%) presented also low sperm concentration (25.6% of oligozoospermic patients) followed of 18.6% of OAT patients. Only 9.7% of normozoospermic patients showed apoptosis (>15%).
Limitations, reason for caution: None.
Wider implications of the findings: The incorporation of new diagnostic tests in the Andrology Lab should improve treatment outcomes, applying techniques of sperm selection according to the etiology of each patient. Oligozoospermic patients have a major probability of early apoptosis, in this cases ii would be recommendable a selection by MACS prior to ICSI.
Study funding/competing interest(s): Funding by national/international organization(s) – CIRH Foundation, Barcelona, Spain
Trial registration number: NA.
Keywords: apoptosis, flow cytometry, advanced semen analysis

P-097 Analysis of meiosis in testicular biopsy: relationship to sperm DNA fragmentation (SDF), ploidy status and apoptosis measured by flow cytometry

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Study question: It is known that meiotic chromosomal anomalies can cause alterations to one or more of the basic seminal parameters including sperm count, motility and morphology. However, is there any relationship between meiotic abnormalities and advanced seminal parameters measured by flow cytometry such as DNA fragmentation, ploidy status and apoptosis?
Summary answer: Patients presenting alterations on the results of an advanced semen testing (>30% SDF, >3% diploidies and >40% apoptosis) are more likely to present also meiotic chromosomal anomalies.
What is known already: Basic semen analysis may not provide all information to completely evaluate male fertility status. Consequently, several investigators have considered optimizing conventional routine methods to improve male infertility diagnoses. These advances have resulted in numerous techniques for evaluating sperm chromatin quality and DNA fragmentation. Both sperm chromosomal alterations and sperm DNA integrity are important parameters of sperm quality in the prognosis of infertility and in the outcome of assisted reproductive.
Study design, size, duration: In this cohort study, the incidence of meiotic abnormalities and their relationship with different advanced seminal parameters was assessed prospectively in 29 male patients. All had a history of implantation failure after two cycles of IVF/ICSI and ≥6 embryos transferred. The study was carried out between January and December 2014.
Participants/materials, setting, methods: All male underwent both testicular biopsy (TB) and advanced seminal analysis. Each sample was analysed by flow cytometry for %SDF using Sperm Chromatine Structure Assay (SCSA), diploidy test based on propidium iodide and apoptosis markers. Altered tests were considered when %SDF was >30%, apoptosis was >30%, and diploidies >3.3%.
Main results and the role of chance: Meiotic abnormalities were found in 15 cases (51.7%). When comparing abnormal meiotic group with normal group, no differences were found on the %SDF average. However, none (0/14) of the normal meiosis, in comparison to 3/15 (20%) of the altered meiosis had a %SDF >30%. Average percentages of diploidy were 1.5% in normal TB and 2.2% in altered TB. 1/14 (7.1%) of patients with normal meiotic results and 4/15 (26.6%) of patients with meiotic abnormalities showed also abnormal values of diploidy. Regarding apoptosis percentages, no differences were found between both groups. However, half (5/10) of patients with >30% apoptotic sperm and 100% (3/3) of patients with >40% apoptotic sperm showed also meiotic abnormalities.
Limitations, reason for caution: The present findings should be interpreted taking into account some limitations of the study, including the small study population and the lack of stiffness of the inclusion criteria. Moreover, further

data regarding clinical outcome must be included in order to confirm the predictive value of the analysed parameters.
Wider implications of the findings: Our present findings suggest a positive relationship between the results obtained from the meiosis study in testicular biopsy and the data from the advanced seminal analysis. In agreement with the published literature it seems that meiotic chromosomal anomalies, besides causing alterations in basic seminal parameters, can also cause alterations to one or more of the advanced seminal parameters.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fundación CIRH.
Trial registration number: NA.
Keywords: TESE, meiosis, sperm DNA fragmentation, ploidy, sperm apoptosis

P-098 Correlation between two parameters of sperm DNA integrity, DNA fragmentation index and big halo pattern, with respect to sperm decondensation index and semen characteristics

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Study question: Do the DNA fragmentation index (DFI) and halo pattern correlate with sperm decondensation index (SDI) and WHO sperm parameters?
Summary answer: Both parameters of sperm DNA integrity evaluated by the Halosperm test correlate with SDI and WHO sperm parameters. However, the DFI has a stronger prediction than the big halo pattern on the proportion of spermatozoa with DNA condensation.
What is known already: The halosperm test measures the susceptibility of sperm DNA to acid denaturation. Spermatozoa with fragmented DNA fail to produce halos of dispersed DNA, which are characteristic for intact DNA. The DFI estimates the proportion of spermatozoa with fragmented DNA. Among cells with intact DNA, the resulting halo shows different patterns of dispersion. Several studies have demonstrated that men with abnormal semen parameters are more likely to have a higher percentage of sperm nuclear DNA damage.
Study design, size, duration: Until December 2014, a prospective study included 190 males with both normal and abnormal semen parameters as a part of semen analysis for fertility evaluation. The sperm samples were analysed for WHO semen parameters, sperm DNA integrity by Halosperm test and DNA decondensation by aniline blue assay.
Participants/materials, setting, methods: The patients included in the study were 24 normospermic, 140 moderate OAT (sperm concentration $\geq 5 \times 10^6$/ml), and 26 severe OAT (sperm concentration was $< 5 \times 10^6$/ml). A big halo was defined as a dispersion greater or equal to the length of the minor diameter of the core.
Main results and the role of chance: For DFI, negative correlations were found with progressive motility (r = -0.54, p = 7.13E-16), total motility (r = -0.59, p = 2.60E-19) morphology (r = -0.44, p = 4.10E-10) and vitality (r = -0.51, p = 2.00E-13); while positive correlation was found with SDI (r = 0.44, p = 3.61E-10). For the big halo pattern, positive correlations were found with progressive motility (r = 0.34, p = 1.64E-06), total motility (r = 0.37, p = 2.03E-07) morphology (r = 0.38, p = 7.60E-08) and vitality (r = 0.40, p = 7.60E-08); negative correlation was found with SDI (r = -0.34, p = 1.90E-06). The degree of correlation was stronger for DFI than for the big halo pattern.
Limitations, reason for caution: The Halosperm test permits to discriminate between sperm with fragmented DNA and sperm with intact DNA. Unfortunately, the invasivity of the Halosperm tests does not permit its use as a selection technique at the time of ICSI.
Wider implications of the findings: Testing the semen conventional parameters provides limited prediction of male fertility potential and is not always able to explain the cause of infertility. Here, DFI has a stronger correlation than the big halo pattern with respect to the sperm chromatin condensation status and semen parameters. Given the reports documenting the negative effect of DFI and embryo viability, its evaluation on sperm cells could contribute relevant information to the prediction of the male reproductive potential.
Study funding/competing interest(s): Funding by hospital/clinic(s) – S.I.S.Me.R.
Trial registration number: NA.
Keywords: semen analysis, halosperm test, DNA fragmentation index, big halo, sperm decondensation index

P-099 State of chromatin compaction and its impact on sperm DNA damage under oxidative stress conditions

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Study question: Does chromatin compaction have an impact over DNA fragmentation under oxidative stress conditions?
Summary answer: Our results demonstrate that the induction of oxidative stress by hydrogen peroxide incubation significantly increased levels of sperm DNA fragmentation in sperms that have impaired chromatin compaction. The presence of sperm plasma has a protective function at initial incubation periods.
What is known already: It has been described that an altered chromatin compaction could be associated to DNA fragmentation in infertile patients.
Big vacuoles (over 50% of nuclear surface) are associated to DNA fragmentation in infertile patients.
Study design, size, duration: This is a case control study. Thirty samples (15 each group) were recruited for the present study. Six month were used to perform the study.
Participants/materials, setting, methods: Chromatin compaction (CC) was assessed by Acridine Orange and MSOME. Two groups: normal CC and altered CC. Incubated with hydrogen peroxide (15 min) (with (A) and without (B) seminal plasma) and incubated without hydrogen peroxide (with (C)and without (D)seminal plasma). An aliquot was obtained to assess DNA damage and lipid peroxidation at 0–2–4–22 h.
Main results and the role of chance: Seminal parameters of the group with alterations in the CC: volume 2.6 ± 1.1, concentration 63.2 ± 24.2, progressive motility 47.5 ± 14.3, vitality 76.8 ± 15.1, morphology 4.6 ± 1.5, DNA fragmentation 17.2 ± 5.1, lipid peroxidation 11.3 ± 5.2. Seminal parameters in the group without alterations in the CC were: volume 2.8 ± 0.9, concentration 20.2 ± 72, progressive motility 45.1 ± 10.8, vitality 79.2 ± 8.8, morphology 5.8 ± 2.5, DNA fragmentation 13.1 ± 2.9, lipid peroxidation 9.5 ± 2.4. After incubation with peroxide, lipid peroxidation levels progressively increased over time, being statistically significant after 4 h for both groups. Levels of DNA fragmentation increased significantly from 22 h in the group with normal CC and from 4 h in the group with alterations in the CC. In both groups after 22 h, samples A vs. C showed greater resistance (statistically significant) to peroxidation (31 vs. 42%) and DNA fragmentation (44 vs. 65%).
Limitations, reason for caution: Evaluation of the parameters might have been measured at 8 and 12 h in order to have a more precise increase over time.
Wider implications of the findings: We consider our findings significant since in infertile men it is of major importance to know the spermatic oxidative status in order to give genuine relevance to the chromatin state; since in previous studies we have shown that men with impaired CC and oxidative damage, DNA damage occurs in both sperm with chromatin abnormalities and those without it. However, in patients without CC alterations and without oxidative damage, sperm with alterations in chromatin specifically show greater DNA damage. Confirm the protective effect of seminal plasma against oxidative stress.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CEGyR Fundation.
Trial registration number: No trial registration number-experimental design.
Keywords: sperm, DNA damage, oxidative stress, chromatin

P-100 Evaluation of the efficiency of two different cryoprotectants and two different protocols to preserve human spermatozoa from cryoinjury

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Study question: To evaluate the efficiency of two different cryopreservation protocols and two common cryoprotectants (CPs), TEST Yolk Buffer (TYB) and Sperm Freeze (SF), to preserve sperm quality in terms of cryosurvival and post-thaw motility.
Summary answer: TYB resulted in the best cryoprotectant and CP addiction directly to seminal plasma without sperm washing appeared to be the most efficient freezing protocol.
What is known already: It is universally recognized that cryopreservation impairs sperm quality. In order to improve post-thawing sperm survival and motility, media of different composition have been proposed. Basically, all semen

CPs contain glycerol as main permeating cryoprotectants, in other cases they also contain other macromolecules, such as egg yolk. Sperm washing is often performed before CP addiction. However, no clear evidences are available to understand which is the most efficient protocol and CP for sperm cryopreservation.
Study design, size, duration: This prospective cohort study enrolled a total of 188 sperm samples undergoing sperm evaluation from September to December 2014. Samples were cryopreserved using two different CPs and two different protocols. Sperm vitality and progressive motility were the outcome measures assessed after thawing by a blinded observer according to WHO 2010.
Participants/materials, setting, methods: A total of 92 were split into 2 aliquots and cryopreserved by TYB and SF after washing and resuspension. Then, other 92 sperm samples, were split into 2 aliquots and cryopreserved using the same CPs adding them directly to the semen. Sperm vitality and motility were compared between the aliquots.
Main results and the role of chance: TYB provided better post-thaw vitality respect to SF whether for the washed aliquots, respectively 21.20 vs. 15.71% ($P = 0.00001$), or for the aliquots cryopreserved without washing, respectively 27.21 vs. 21.71% ($P = 0.022$). Progressive motility was significantly different in favour of TYB only when aliquots were washed and resuspended, respectively 18.41 vs. 13.99% ($P = 0.001$) and no when CPs were added directly to the sperm sample (16.63% TYB vs. 13.36 SF, $P = 0.14$). Then, the freezing protocol was investigated. Sperm vitality was significantly higher for not washed samples compared to washed ones, respectively 21.20 vs. 27.21% TYB ($P = 0.06$) and 15.71 vs. 21.71% SF ($P = 0.0003$). No significant difference was detected in post-thaw progressive motility comparing washed and not washed samples, respectively 18.41 vs. 16.63% TYB and 13.99 vs. 13.38% SF.
Limitations, reason for caution: This was a prospective cohort study of a limited sample size. Logistic regression analysis was used to control for possible confounding factors (i.e., sperm quality), however, a bigger sample size should be examined in order to confirm these preliminary data.
Wider implications of the findings: This study provides advice concerning the best strategy to cryopreserve sperm samples. Our data suggest that TYB provides better results especially when samples are washed before freezing. Probably, seminal plasma exerts a natural protective role and, when samples are washed before freezing, macromolecules such as egg yolk in addition to glycerol as main CP, can be crucial to protect sperm from cryodamage, replacing the role of seminal plasma.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ferticlinic, Villa Margherita, Rome, Italy.
Trial registration number: NA.
Keywords: sperm cryopreservation, test yolk buffer, sperm freeze, cryopreservation protocol, sperm washing

P-101 Improved clinical outcomes of IVF-ICSI cycles with preimplantation genetic screening by CGH in couples affected of severe male factor and synaptic chromosome anomalies

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Study question: Is Preimplantation Genetic Screening by CGH (CGH-PGS) an alternative management for IVF-ICSI cycle in male factor with synaptic chromosome anomalies?
Summary answer: Clinical outcomes improved by using CGH-PGS, despite off risk of no embryo transfer.
What is known already: The incidence of meiotic chromosome anomalies limited to the germ cell line in patients with severe low sperm counts ($<5 \times 10^6$ sperm/ml) increases with decreased sperm count. Furthermore, oligozoospermic patients have a significant increase in de-novo sex chromosome and autosomal aneuploidies in a series of children born after IVF-ICSI.
Study design, size, duration: In a private IVF clinic, from January 2010 until March 2014, one hundred and nine couples with severe male factor and synaptic anomalies (abnormalities in chromosome pairing) underwent an IVF-ICSI cycle. CGH-PGS was offered to these couples and decided whether to undergo PGS or not. Clinical outcomes are presented.
Participants/materials, setting, methods: Synaptic anomalies were diagnosed in 109 males by FISH in severe oligozoospermia ($<5 \times 10^6$ sperm/ml) or by testicular biopsy in azoospermia. 129 IVF-ICSI cycles were analyzed: 60 underwent PGS and 69 did not. We compared the fertilization, implantation, ongoing pregnancy, miscarriage and live birth rates between both groups.
Main results and the role of chance: No statistically significant differences were observed between PGS and non-PGS groups in maternal age (34.6 ± 2.8 years vs. 34.5 ± 5.8 years), number of inseminated oocytes (14.0 ± 3.7 vs. 13.0 ± 5.8), sperm concentration (3.1 ± 3.9 million/ml vs. 2.2 ± 2.7 million/ml) and fertilization rate (67.7% vs. 70.6%). One hundred and thirty three embryos were chromosomally normal (25.6% of the biopsied embryos and 28.3% of the diagnosed embryos). Significant differences were observed in the number of transferred embryos (1.27 ± 0.80 vs. 2.17 ± 0.54) ($p < 0.0001$) and in the number of transfers (48(80%) vs. 69(100%)) ($p < 0.0001$). No differences between groups were observed regarding ongoing pregnancy rate (51.7% vs. 40.8%) and miscarriage rate (12.9% vs. 32.1%). Significant clinical differences were observed in implantation rate (56% vs. 24.7%) ($p < 0.05$) and in live birth rate (45% vs. 27.5%) ($p < 0.05$).
Limitations, reason for caution: This is an observational study, and further studies are needed in order to confirm these preliminary results. The risk transfer of non normal embryos cannot be excluded.
Wider implications of the findings: Selection of chromosomally normal embryos by CGH-PGS can improve clinical outcomes in couples affected by severe male factor and synaptic chromosome anomalies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – "Cátedra de Investigación en Obstetricia y Ginecología" of the Department of Obstetrics, Gynecology and Reproduction, Hospital Universitario Quiron Dexeus.
Trial registration number: NA.
Keywords: severe male factor, synaptic chromosome anomalies, PGS

P-102 Association between adherence to the Mediterranean diet and semen parameters in men of subfertile couples undergoing in vitro fertilization treatment

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Study question: Is adherence to the Mediterranean Diet (MD) associated with semen quality in men undergoing in vitro fertilization treatment (IVF)?
Summary answer: Greater adherence to the MD, as assessed through a Mediterranean Diet score (MD-score), was significantly and positively associated with higher semen concentration, total semen count, and total and progressive motility, after adjustment for potential confounders including age, body mass index (BMI), smoking, physical activity level and state/trait anxiety.
What is known already: A dietary pattern characterized by high intakes of fruits, vegetables, fish and whole grains was recently shown to be associated with better semen quality in men undergoing IVF treatment. In addition, a 'prudent' dietary pattern characterized by high intakes of fish, chicken, fruit, vegetables, legumes and whole grains was found to be associated with higher semen progressive motility in young men. Whether adherence to the MD affects semen parameters remains to be evaluated.
Study design, size, duration: This is an ongoing cross-sectional cohort study assessing dietary and lifestyle habits in men of subfertile couples undergoing IVF/ICSI at the Embryogenesis Unit, Athens, Greece. The study started in September 2014 and was designed to evaluate the influence of habitual dietary intake and lifestyle on fertility and pregnancy outcome.
Participants/materials, setting, methods: Seventy-one male participants filled out a general questionnaire comprising information on lifestyle/demographic factors and a validated food-frequency questionnaire to assess habitual dietary intakes. Adherence to MD was assessed with a MD-score (ranging from 0 to 55; higher scores indicate better adherence to MD) that incorporates the inherent characteristics of this diet.
Main results and the role of chance: Mean (±SD) sample age was 40.1 ± 4.5 y, mean BMI was 26.6 ± 3.5 kg/m^2 and mean MD-score was 29.6 ± 7.4, respectively. A highly significant positive correlation was observed between MD-score and semen concentration ($r = 0.72$), total semen count ($r = 0.69$), total motility ($r = 0.75$) and progressive motility ($r = 0.73$) (all p-values < 0.05). Moreover, subjects in the highest tertile of MD-score had significantly higher values in the above semen parameters, compared to subjects in the lowest

tertile of the score. The MD-score was unrelated to sperm morphology and volume. Multiple regression models controlling for potential confounders (age, waist, BMI, anxiety, physical activity) confirmed that MD-score is a significant predictor (β coefficient ± SE) of semen concentration (2.61 ± 0.23), total semen count (8.75 ± 0.95), total motility (2.26 ± 0.21) and progressive motility (1.51 ± 0.18), (all *p*-values < 0.05).

Limitations, reason for caution: The main limitation of the study stems from its small sample size, while its cross-sectional nature limits our ability to determine causality of adherence to the MD on semen quality parameters.

Wider implications of the findings: This study demonstrates that habitual dietary intake affects semen quality and support the suggestion that counseling men to increase adherence to the Mediterranean Diet may be an easy and safe way to improve measures of semen quality. Additionally, these findings are consistent with previous studies showing that dietary patterns with some of the characteristics of the traditional MD, i.e. rich in fruit, vegetables, legumes and whole grains, are associated with better measures of semen quality.

Study funding/competing interest(s): Funding by hospital/clinic(s) – None.

Trial registration number: NA.

Keywords: Mediterranean diet, semen quality, infertility, IVF

P-103 The phospholipase Phospholipase A2 group 2A (PLA2G2A) membrane protein as a potential infertility marker within human sperm

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Study question: Is there a relationship between the protein presence and abundance of PLA2G2A in human sperm samples (SS) and their competence to achieve a pregnancy with an ICSI treatment once the main male, female and cycle potentially biasing factors are controlled?

Summary answer: The percentage of stained cells and protein levels for PLA2G2A in sperm from infertile males achieving or not pregnancy by means of ICSI are comparable, regardless the oocyte source, after controlling for potential clinical and phenotypical confounders, leading to conclude that PLAG2A2 involvement in sperm function seems limited so far.

What is known already: We previously reported all the sperm mRNAs exclusively present or differentially expressed in SS achieving or failing to achieve pregnancy in different assisted reproduction techniques (IUI, IVF and ICSI). Among all, the one that codifies for PLA2G2A (a membrane-associated protein whose main function is the regulation of the phospholipid metabolism in biomembranes) has been found to be overexpressed in samples unable to obtain pregnancies, then being considered as a potential sperm infertility marker.

Study design, size, duration: Nested cases and controls study analyzing spermatozoa from 48 ejaculates that achieved clinical pregnancy (*P*+) and spermatozoa from 25 semen samples that did not achieve it (*P*-) after undergoing ICSI treatment with own or donated oocytes, prospectively collected, where PLA2G2A was measured by means of flow cytometry.

Participants/materials, setting, methods: From SS prepared for the ICSI treatments, aliquots were fixed and stored for ulterior analysis once the pregnancy results were known. SS were incubated with Anti-PLA2G2A-Ab followed by secondary FITC-conjugated-Ab. PLA2G2A positive cells and mean fluorescence intensity was quantified and groups compared using U-Mann-Whitney's and logistic regression controlling confounding parameters.

Main results and the role of chance: Mean volume, concentration, motility and total motile count (TMC) were 2.8 ml CI 95% (2.6–3.1), 52.4 mill/ml CI 95% (43.6–61.2); A + B forms 42.1% CI 95% (38.6–45.6), TMC 59.1 mill CI 95% (48.8–69.4), respectively. In prepared sperm, 10.3 mill/ml CI 95% (8.8–11.8), A + B 95.6% CI 95% (94.4–96.9) and 4.0 mill CI 95% (3.0–5.0). A mean number of 1.9 CI 95% (1.8–2.0) embryos were transferred, in 49 donation and 23 own oocytes' cycles. Women's mean age 38.1y CI 95%(37.1–39.2). PLA2G2A + ve cells were 4.6 CI 95% (2.6–6.5) in *P*+, and 6.9 CI 95% (1.1–12.8) *P*-, while the mean staining intensity registered was 194.3 CI 95% (134.5–254.2) vs. 140.2(66.3–214.0). The odds ratio (OR) of *P*+/*P*- depending on the PLA2G2A% or stained cells was 0.98 CI 95% (0.93–1.03), B = -0.024, while regarding the staining intensity, OR = 1.00 CI 95% (1.00–1.00), B = 0.002. Once adjusted for potential clinical confounders (day of embryo transfer, own or donated oocytes, age, female etiology, sperm features, embryos transferred…) Adj (OR) PLA2G2A% of staining was 0.96 CI 95% (0.87–1.06), B = -0.042, while regarding the staining intensity, Adj (OR) = 1.00 CI 95% (1.00–1.00), B < 0.001.

Limitations, reason for caution: Other sperm factors not included within this analysis could be biasing the studied relationships, given that sperm function has been demonstrated to be multiparametric. Further confirmation of our results is needed from other studies, confirming or not if our results are extrapolable to other subpopulations or different assisted reproduction protocols.

Wider implications of the findings: PLA2G2A is a sperm membrane associated protein whose mRNA has been described as an infertility marker, making it suitable for the development of a sperm selection tool based on the use magnetic activated cell sorting (MACS) technology to eliminate PLA2G2A positive spermatozoa and improve ART results. Nevertheless, the first step towards it is to demonstrate the link between the protein in sperm and clinical results with ICSI, and this has not been attained yet.

Study funding/competing interest(s): Funding by national/international organization(s) – Department of Industry, Innovation, Trade and Tourism and FEDER funding (EU) (IG-2011/0000681 and IG-2012/0000497).

Trial registration number: NA.

Keywords: PLA2G2A, sperm, male fertility, infertility marker, flow cytometry

P-104 Mitochondrial mass (MM) – the best candidate, among mitochondrial functionality markers in human spermatozoa for prediction of successful embryo implantation

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Study question: Which of the mitochondria-related parameters, measured in human spermatozoa, is the best prognostic marker for determination of embryo quality and implantation potential of resulted embryos.

Summary answer: Among all the measured mitochondrial variables (mitochondrial membrane potential (MMP), mitochondrial mass (MM), superoxide radical (SR) and adenosine triphosphate (ATP)) mitochondrial mass showed the best predictive value in relation to the embryo implantation.

What is known already: Mitochondria are best known to participate in ATP production, generation of reactive oxygen species (ROS), apoptotic pathway, and calcium homoeostasis. However, there are contradictory data published in the literature, connecting sperm mitochondrial functionality, measured by assessment of MMP, MM, SR and ATP production and ART outcome. According many authors sperm mitochondrial functionality seems to be critical for fertilisation, but it's still not clear which parameter is more important for predicting embryo quality and pregnancy rate.

Study design, size, duration: A retrospective study was performed during the period 2013–2014. A native sperm from 100 patients undergoing ICSI procedure were used for analysis of mitochondrial status, oxidative stress and ATP production.

Participants/materials, setting, methods: The integrity of the sperm mitochondrial membrane potential (MMP) is determined by JC-1, mitochondrial mass – using Mito Tracker Green and ROS production were assessed by MitoSOX Red. The described variables were tested by flow cytometry. ATP concentration in sperm was measured by using bioluminescence assay.

Main results and the role of chance: Significant differences were observed between the group One (successfully implanted embryos) and group Two (unsuccessfully implanted embryos) regarding – MM (28.69% vs. 16.64%, respectively) ($p < 0.041$). No significant differences were found between group One (good quality embryos) and group Two (bad quality embryos) regarding - MMP (44.51% vs. 39.33%); MM (32.45% vs. 24.65%) and ATP (0.04% vs. 0.03%). ATP values showed a statistically significant association with the outcome of fertilization process (r = -0.300; $p = 0.013$).

Limitations, reason for caution: Present technique allows evaluation of all mitochondrial parameters (MMP, MM, ATP and ROS production) for a patient only in cases when the sperm count exceeds 4 mln/mL.
Wider implications of the findings: Mitochondrial related parameters of human sperm can be useful for better prediction of embryo quality and pregnancy rate after ART. Mitochondrial variables can be included in basic semen analysis for quality assessment and sperm selection in reproductive biology.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Nadezhda Fertility Clinic, Sofia, Bulgaria.
Trial registration number: NA.
Keywords: Spermatozoa, mitochondrial mass, mitochondrial membrane potential, biochemical pregnancy, adenosine triphosphate

P-105 Age related effects on semen parameters in infertile men – results from a cross sectional study

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Study question: We aimed at assessing possible impact of patient age over semen parameters in men with oligospermia.
Summary answer: Current findings showed a linear increase of sperm concentration with age in primary infertile men with oligospermia. No further correlation between age and either sperm motility or normal morphology was observed.
What is known already: Conflicting data exist in terms of relationship between age and semen quality in fertile men. If on one hand several studies suggest that increased male age is associated with a decline in semen volume, sperm motility, and sperm morphology on the other evidences exist supporting a relationship between medical relevant comorbidities and semen parameters.
Study design, size, duration: Cross sectional study. Complete demographic, clinical and laboratory data from 618 infertile men with pathological sperm concentration presenting for primary couple's infertility were analyzed.
Participants/materials, setting, methods: Comorbidities were scored with the Charlson Comorbidity Index (CCI). Testicular volume was assessed with a Prader orchidometer. Semen analysis values were assessed based on the 2010 WHO reference criteria. Descriptive statistics and linear regression models tested the impact of age over sperm parameters.
Main results and the role of chance: Mean (median) patient age, BMI, CCI, sperm concentration, total sperm progressive motility, morphology, left testis volume, FSH, and inhibin B levels were 42.1 (42) years, 25.7 (25.4) kg/m^2, 0.13 (0.0), 5.4 (4.5) million/mL, 11.6% (2%), 17.3% (14%), 15.3 cc (15), 9.5 (6.2) mUI/ml, and 99.3 (91.7) pg/ml, respectively. At univariable linear regression analysis sperm concentration increased with age (Beta: 0.08; $p = 0.04$), left testis volume (Beta: 0.27; $p < 0.001$), and inhibin B (Beta: 0.27; $p < 0.001$); conversely, sperm concentration decreased along with FSH (Beta: -0.18; $p < 0.001$). The linear association between age and sperm concentration was confirmed at multivariable regression analysis (Beta: 0.17; $p = 0.01$) even after accounting for CCI, left testis volume, FSH, and inhibin B values. No significant correlations were observed between age and other sperm parameters.
Limitations, reason for caution: Main limitation is the cross sectional design of our study.
Wider implications of the findings: This is the first study focused on the impact of age over semen parameters in men with oligospermia. Existing data suggest how semen quality may be a fundamental biomarker of overall male health, and an independent predictor of mortality.
Study funding/competing interest(s): Funding by hospital/clinic(s) – ospedale San raffaele
Trial registration number: NA.
Keywords: semen, parameters, infertile, men, age

P-106 Hydrogen molecule treatment improves the sperm motility of the oligoasthenozoospermia patients

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Study question: Does H_2 treatment improve the sperm motility and fertilization ability of oligoasthenozoospermia patients?
Summary answer: H_2 treatment seemed to be the powerful therapeutic alternative to the oligoasthenozoospermia patients.
What is known already: Oxidative stresses are thought to have detrimental effects on sperm motility of the oligoasthenozoospermia patients. However the precise mechanisms are not known, oxidative damages to the axoneme of the sperm and the depletion of intracellular ATP appear to be involved. Recently some researchers reported that H_2 molecule selectively reduces toxic reactive oxygen species, such as hydroxyl radicals, and that the effectiveness in the treatment of oxidative stress related diseases.
Study design, size, duration: Sperm suspension from 21 oligoasthenozoospermia patients divided into 4 groups: control was centrifuged by medium (equivalented N_2-mixed gas 5%CO_2, 20%O_2, 75%N_2). H2 treated group were divided into 3 groups by H_2 concentration, that is, 50, 75, 100%. In addition, fertilization ability of H2 treated sperm was investigated using mouse oocytes.
Participants/materials, setting, methods: Sperm suspensions were frozen on the collected day. H2 treated group were centrifuged with the H2 contained medium. The sperm of the H_2 treated 75% group and control group were injected to mouse oocytes by piezo micro-manipulator. Forward motility was measured with Makler chamber and Sperm Count Analyser.
Main results and the role of chance: In terms of sperm forward motility, 75% of H_2 concentration showed the best results compared with those of 50% and 100% ($P < 0.05$). We further found that the normal fertilization rate of the mouse oocytes was significantly increased by H_2-treatment ($P < 0.05$, versus the untreated sperm suspension.).
Limitations, reason for caution: This is a pilot study on a relatively small sample size with limited conditions. The confirmation using larger samples under various conditions may be required. Furthermore, we need to check the safety of H_2 treated sperm use in ART.
Wider implications of the findings: The findings of this study indicate that H_2 treatment improves the sperm motility of the oligoathenozoospermia patients. Possibly, men with severe sperm dysfunction could select IVF instead of ICSI by using H_2 treatment. It may also useful in the selection of the good sperm for ICSI or ROSI using the testicular sperm or round spermatid.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Yamashita shonan yume clinic
Trial registration number: NA.
Keywords: sperm motility, hydrogen molecule, oligoasthenoteratozoospermia

P-107 Origin and morphometric parameters of nuclear vacuoles in human sperm

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Study question: The aim of this study was the analysis of presence, origin and morphometric parameters in human sperm nuclear vacuoles from

normozoospermic subjects assessed by Transmission Electron Microscopy (TEM).

Summary answer: Our findings determined that nuclear vacuoles are an usual structure in the sperm heads from normozoospermic subjects, mainly located in the anterior part of sperm heads. These vacuoles are originated from invaginations of the nuclear envelope

What is known already: The presence of sperm vacuoles seemed relatively common in sperm heads from both fertile and infertile men. However, the presence of larger vacuoles in size (which occupy between 13% and 50% of the sperm head's surface area) is relatively contradictory. Moreover, the molecular mechanism responsible of the nuclear vacuoles formation is unknown.

Study design, size, duration: Prospective, randomized study, 15 normozoospermic samples were processed for sperm head analysis by TEM, from March to December 2014. A total of 227 sections were analyzed, with an average between 15 and 20 sections per sample, magnification factor 2000x. Images were analyzed by computer image analysis software.

Participants/materials, setting, methods: Sperm samples collected after informed consent of 15 participants. After sperm analysis according to WHO-2010, fresh samples were fixed, included in EPON, and ultrathin sections were obtained and analyzed by TEM in order to better define the ultra structure of human sperm cephalic vacuoles.

Main results and the role of chance: It was observed that 43.91 % of sperm showed nuclear vacuoles. The mean values for area of vacuoles were 0.27 μm^2 versus total area of nucleus (2.62 μm^2). These vacuoles correspond to 9.136% of the total area of the nucleus. There is a positive correlation between vacuole area and total nuclear area ($r = 0.49$; $p < 0.0001$). Furthermore, TEM analyses confirmed that a high percentage of vacuoles (81.84 %) is located in the anterior part of sperm heads. Finally, regarding the origin of vacuoles we observed that these vacuoles are nuclear envelope invaginations.

Limitations, reason for caution: This work was performed using TEM, thus vacuoles cannot be entirely analyzed, only sections.

Wider implications of the findings: Our results describe, at ultra structural level, morphometric parameters related to sperm head vacuoles and provide new information about origin of these structures, which do not support the acrosomal or plasma membrane residues implication. These findings suggest the possibility of creation new diagnostic tests to differentiate structural from functional vacuoles.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by private and public hospital/clinic.

Trial registration number: A trial registration number is only required for clinical trials.

Keywords: TEM, vacuole

P-108 How efficient is ICSI for treating infertile men with complete globozoospermia?

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Study question: What is the real efficiency of ICSI procedure with or without calcium ionophore (Ca-I) for treating infertile men with complete globozoospermia?

Summary answer: Infertile patients with globozoospermia had poor results after conventional ICSI in our study. Furthermore, these patients reached a plateau in their cumulative clinical pregnancy rates after the third ICSI cycle. Although Ca-I significantly increased the fertilization and clinical pregnancy rates, it did not increase time to pregnancy.

What is known already: Men with globozoospermia have an increased rate of fertilization failure following ICSI, possibly due to a hindered oocyte activation capacity associated with the absence of an acrosome. Oocyte activation measures, namely Ca-I, may increase these fertilization rates. However, since the evidence of this intervention is limited, we are unable to determine until when it is no longer reasonable to offer treatment to these patients using their own sperm.

Study design, size, duration: We performed a retrospective single-centre cohort study of all consecutive ICSI cycles (91 cycles performed in 34 couples) using sperm from infertile men with a confirmed diagnosis of complete globozoospermia between 1992 and 2014.

Participants/materials, setting, methods: Cycles were classified into three groups according to the percentage of oocytes in which ICSI was performed using Ca-I: in all (100% Ca-I, $n = 41$), half (50% Ca-I, $n = 10$) or none (0% Ca-I, $n = 38$). Baseline cycle characteristics and clinical pregnancy rates were compared amongst the groups using multivariable logistic regression.

Main results and the role of chance: The average female age, number of oocytes retrieved, embryo developmental stage and number of embryos transferred did not vary significantly amongst the groups. Fertilization rates were significantly lower in the 0% Ca-I group (27.0%) when compared to the 100% Ca-I (46.9%, $p = 0.004$), but not versus the 50% Ca-I (41.1%, $p = 0.190$). Most importantly, clinical pregnancy rates were significantly lower in the 0% Ca-I group (7.6%) when compared to the 100% Ca-I (30.9%, $p = 0.021$), but not versus the 50% Ca-I (10.0%, $p = 0.869$), even after adjusting for female age, number of oocytes retrieved and year of treatment. No globozoospermic patient in our sample was pregnant after the third ICSI cycle, regardless of their use of Ca-I (HR = 2.45, CI 95% 0.69–8.69).

Limitations, reason for caution: As this was a retrospective study with a small sample size, we were unable to account for all foreseeable heterogeneity or confounding amongst our study groups, namely the presence of the DPY19L2. Nonetheless, previous studies have shown that this mutation has a limited effect on pregnancy after ICSI.

Wider implications of the findings: Oocyte activation using Ca-I improves fertilization and clinical pregnancy rates during ICSI in infertile men with globozoospermia. However, this treatment does not seem to completely resolve the poor prognosis of these patients. Despite the use of Ca-I, it may be reasonable to advise patients to consider the use of donor sperm after three failed ICSI cycles.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Universitair Ziekenhuis Brussel.

Trial registration number: NA.

Keywords: globozoospermia, calcium ionophore, ICSI

P-109 Comparison of two different globozoospermia groups according to prevalence of round headed forms

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Study question: Comparison of two different globozoospermia groups according to prevalence of round headed forms

Summary answer: Especially for teratozoospermia cases with the dominance of round headed spermatozoa, our results emphasize the necessity and the importance of detailed sperm morphology analysis before ICSI.

What is known already: Globozoospermia is one of the most severe form of sperm morphological defects which usually results in low fertilization.

Study design, size, duration: The aim of our study was to analyze and compare the ART outcomes of cases which are grouped according to the prevalence of round-headed spermatozoa in the initial semen samples.

Participants/materials, setting, methods: According to the round-headed sperm concentration in the semen, patients were classified into two groups: Group I consisted of 10 couples with 14 cycles with sperm sample containing >70% globozoospermic forms (between 70 and 100%). Group II included 21 patients with 27 cycles in which male partner was diagnosed as severe teratozoospermia and the abundance of round-headed forms were 70%. Semen samples of 7 patients in group I and 10 patients from group II were also analyzed with acridine orange staining method in order to assess the degree of nuclear maturity. In both groups the mean values of sperm concentration, motility, round head shape, acrosomal content and other accompanying abnormalities such as macrocephalic and multiple head forms, mid-piece and tail defects were compared.

Main results and the role of chance: No difference in sperm concentration and motility were observed ($p > 0.05$). However, statistically significant differences were found in terms of spermatozoa having reduced acrozomal content, macrocephalic head, multiple head as well as mid-piece defects between these two globozoospermia groups ($p < 0.01$). Low fertilization rate, which is a common finding for globozoospermia cases, was observed for both groups (21.3% and 39.9% for group I and group II, respectively). Also, no significant difference was found between these two groups with respect to embryo

developmental ability on each day starting from prezygote stage until embryo transfer, pregnancy rates and implantation rates ($p > 0.05$). Spermatozoa of the patients from both groups were observed as having higher immature nuclear structures according to acridine orange test results.
Limitations, reason for caution: No difference in sperm concentration and motility were observed ($p > 0.05$).
Wider implications of the findings: Although the degree of dominance may differ in different semen samples, once it is the dominant form, presence of round-headed spermatozoa can equally and negatively affect the ART outcome in both groups. Once fertilized, resulting embryos may develop normally and generate successful pregnancies. However, such patients should be informed about lower fertilization, embryo development and pregnancy rates.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Göztepe Medical Park IVF Center, Istanbul Medeniyet Üniversitesi.
Trial registration number: 3
Keywords: Globozoospermi, Severe Male Infertility, Tip II Round, ICSI

P-110 Metabolic fingerprints in testicular biopsies from type 1 diabetic patients

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Study question: Can type-1 diabetes (T1D) alter the metabolic profile of testicular tissue?
Summary answer: T1D depressed lactate and creatine content in testicular tissue and altered glycolysis-related enzymes and transporters.
What is known already: Diabetes mellitus (DM) is a metabolic disease that has grown to pandemic proportions and the number of diabetic patients is expected to grow further in the next decades. Recent reports have highlighted that T1D alters male reproductive function, but the mechanisms remain unknown.
Study design, size, duration: Testicular biopsies obtained from patients under treatment for recovery of male gametes were used after informed written consent, and after treatment. Ten men were selected: 5 with conserved spermatogenesis and 5 T1D patients, diagnosed for more than 10 years and following an insulin therapy to control blood glucose levels.
Participants/materials, setting, methods: Testicular tissue from normal and T1D men was analyzed by High-Resolution Magic-Angle Spinning (HR-MAS) Nuclear Magnetic Resonance (NMR) spectroscopy. mRNA and protein expression of glucose transporters and glycolysis-related enzymes were also evaluated.
Main results and the role of chance: Our results show that testicles from diabetic men presented decreased levels of lactate, alanine, citrate and creatine. The mRNA levels of glucose transporter 1 (GLUT1) and phosphofructokinase (PFK) were decreased in testicles from diabetic men, but only GLUT3 presented decreased mRNA and protein levels. Lactate dehydrogenase (LDH) and glutamate pyruvate transaminase (GPT) protein levels were also found to be decreased in testicles from diabetic men.
Limitations, reason for caution: Diabetes mellitus (DM) is often associated with several "silent" comorbidities difficult to address. Moreover, the biological material available was scarce. Nevertheless, to avoid major deviations, the same piece of tissue was used for all the analysis performed in this study providing robust metabolic cues in testicular tissue from diabetic men.
Wider implications of the findings: Since lactate and creatine are essential for germ cells development and support, the mechanisms discussed herein open new insight on the molecular mechanism by which DM promotes subfertility/infertility in human males.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the 'Fundação para a Ciência e a Tecnologia' – FCT (PTDC/QUI-BIQ/121446/2010 and PEst-C/SAU/UI0709/2014) co-funded by Fundo Europeu de Desenvolvimento Regional – FEDER via Programa Operacional Factores de Competitividade – COMPETE/QREN. M.G. Alves and P.F. Oliveira were funded by FCT through SFRH/BPD/80451/2011 and FSE and POPH funds (Programa Ciência 2008), respectively.
Trial registration number: NA.
Keywords: Testicular Metabolism, Male Infertility, Diabetes Mellitus, Lactate

P-111 Three-dimensional sperm surface reconstruction analysis yields more consistent and accurate results than conventional bi-dimensional morphological analysis

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Study question: We are concerned that standard sperm morphological analysis may be limited by asymmetrical abnormalities, orientation, or unpredictable sperm head positioning; we question whether a three- dimensional (3-D) sperm surface reconstruction model of a specific single sperm offers superior information.
Summary answer: Because of our unique 3-D imaging modality, spermatozoa are kept in a fluid environment eliminating positional artifacts and allowing analysis of the entire sperm head; as such, 3-D sperm surface reconstruction generates a high-fidelity profile that is more detailed and reliable than standard morphological techniques.
What is known already: Abnormal sperm morphology has been correlated with unpredictable oocyte fertilization rates with standard insemination and is a frequent indication for intracytoplasmic sperm injection (ICSI) in subfertile males. Traditional/standard bi-dimensional microscopy gives information about a single plane of the cell, potentially ignoring unique facets/surfaces and highlighting optical artifacts. These limitations may decrease the accuracy of the male infertility assessment.
Study design, size, duration: In this prospective, randomized, double-blind study, a single aliquot of semen was analyzed; nine spermatozoa were imaged and processed; 93 images were obtained and 9 resultant sperm surface reconstruction models were generated as videos. A team of 7 andrologists independently participated in the study.
Participants/materials, setting, methods: Still images were obtained at 600x; sperm surface reconstruction results were displayed as video depictions. Images and videos were randomly displayed; sperm heads were assessed for the presence and severity of morphological irregularities. A two-sided paired student's t-test was performed with statistical significance evaluated at the 0.05 alpha level.
Main results and the role of chance: The semen sample used in the study had a concentration of 84 x 10^6/ml, motility of 49%, and 4% normal morphology according to 2010 WHO criteria. Nine sperm were selected, ten-second videos were obtained, 93 images were captured, and only spermatozoa that demonstrated at least an anterior, posterior, and two lateral images were included.

Participants' ability to see a 3-D profile of the sperm surface, which was generated from the bi-demensional images, caused more sperm to be scored as normal cells. When all scores across the 9 sperm were averaged (1 = major head defect, 2 = minor head defect, 3 = normal), the mean ± SD image-based score was 1.96 ± 0.09 vs. reconstruction video-based 2.44 ± 0.13 (P = 0.008).
Limitations, reason for caution: This sophisticated computerized method of assessing sperm morphology offers a detailed profile of the spermatozoa, a new mode of evaluation. Although we consider these results promising, this study was solely performed on a single aliquot of semen and on a limited number of cells.
Wider implications of the findings: These results emphasize the highly subjective nature of bi-dimensional morphological assessment. Our findings suggest that evaluating an individual spermatozoon in a single plane may increase false-negative interpretations. This 3-D sperm morphological analysis is promising in that it may not only allow for a new method of sperm morphological analysis during the infertility evaluation, but it may assist in the selection of live male gametes prior to ICSI.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: NA.
Keywords: morphology, sperm, 3D three-dimensional, ICSI, bioinformatic(s)

P-112 Clinical outcomes of artificial oocyte activation with calcium ionophore in intracytoplasmic sperm injection (ICSI) using testicular spermatozoa on sibling oocytes

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Study question: To analyze whether artificial oocyte activation (AOA) with calcium ionophore after ICSI using testicular spermatozoa improves fertilization, embryonic development and pregnancy outcome in patients with obstructive azoospermia (OA) or non-obstructive azoospermia (NOA).
Summary answer: AOA with calcium ionophore showed favorable effect on fertilization rate in patients with NOA but OA. The sperm source will strongly affect the fertility potential or clinical outcomes. Severe male factor infertility, especially NOA, is an indication for application of AOA.
What is known already: Even using normal-morphology oocytes, fertilization failure was often observed on poor morphology or poor motility from testicular spermatozoa. AOA using several materials (calcium ionophore, ionomycin or strontium) has been tried to prevent that. However, it is not clear whether AOA is effective which type of materials when and how to use them.
Study design, size, duration: This prospective study was performed between October 2013 and December 2014 at a reproductive center. All patients involved gave written consent, and institutional review board approval was granted. This study includes 18 OA and 37 NOA couples.
Participants/materials, setting, methods: AOA was randomly performed on sibling MII oocytes after ICSI with motile testicular spermatozoa from OA or NOA. Two pronuclei (2PN) oocytes, blastocysts development, good-quality blastocysts, biochemical pregnancies, and clinical pregnancies rates were compared between two groups.
Main results and the role of chance: In terms of OA couples, there are no significant difference in 2PN oocytes, blastocysts development, and good-quality blastocysts rates (70.3%, 56.5%, and 45.7% with AOA and 65.6%, 41.8%, and 42.9% without AOA, respectively). For NOA couples, 2PN oocytes with AOA (75.0%) was significantly higher than those without AOA (59.6%). Blastocysts development, and good-quality blastocysts rates for NOA couples were 54.3% and 42.1% with AOA and 56.0% and 41.5% without AOA, respectively (no significant differences). There are no differences in biochemical and clinical pregnancy rates with or without AOA regardless of OA or NOA. In AOA oocytes, blastocyst and good-blastocyst rates per MII were tended to be better than without AOA, although it was no significant differences, even pregnancy rates.
Limitations, reason for caution: The participants are limited to the azoospermia whom had retrieved motile testicular spermatozoa because immotile spermatozoa may have some confound factors. We included <39 years age of the spouses at the time of ICSI cycles and 8 or more MII oocytes in this study.
Wider implications of the findings: This is the first study of AOA in couples using testicular spermatozoa on sibling oocytes. Those data showed oocytes with AOA was better clinical outcomes compared with sibling oocytes without AOA. However, long-term follow-up studies of children born after AOA using a calcium ionophore are not yet available. Thus, ICSI with split AOA might be better in first ICSI cycle using testicular spermatozoa. In addition, we should consider which case is benefit from AOA.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproduction Clinic Osaka.
Trial registration number: NA.
Keywords: TESE, ICSI, calcium ionophore, azoospermia, artificial oocyte activation

P-113 Vitamin e and berberine counteract the adverse effects of ros on sperm and seminal parameters

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Study question: is there a role of vitamin E and berbeine on results of ICSI in male infertility?
Summary answer: Treatment with vitamin E had a beneficial effect on improving sperm DNA integrity together with improvement of quality of embryo. In vitro treatment by Berberine during sperm preparation may be used as a substitute to medical treatment with antioxidants.
What is known already: Out of many causes of male infertility oxidative stress (OS) has been attributed to affect the fertility status and thus, it has been studied extensively in recent years. In a state of OS, DNA damage occurs due to oxidation of bases, strand breaks, cross- linking, deletions, frame shifts and rearrangement of chromosomes Also it is well known that the washing of the sperm during the first step in ICSI by centrifugation is associated with the generation of ROS and physical damage to spermatozoa
Study design, size, duration: Prospective controlled study, university based 54 male randomized into 3 groups (normal group, infertile treated with antioxidant, infertile not treated) 10 semen samples prepared twice. Once by traditional sperm wash media and once by addition of berberine to the wash media.
Participants/materials, setting, methods: In vivo study tracked through determination of pro-oxidants/antioxidants status in seminal plasma by determination of TBARs concentration / SOD, GPx & GSH activity and its harmful effect on DNA mutation when analysed against MP77 by RAPD assay and finally correlates these parameters with semen analysis alteration. In vitro study was assessed by measure the fertilization rate and good quality embryos upon adding Berberin on sperm wash media versus traditional media.
Main results and the role of chance: Antioxidant treated group shows a significantly lower seminal TBAR's concentration (9.74 ± 7.88). SOD activity of normal group (39.85 ± 31.45) and group treated with antioxidant (46.20 ± 27.21) were significantly higher than untreated group. Sperm DNA samples showed a RAPD profile with less fragmented bands with Primer MP77 in treated group than the untreated group. the group with Vitamin E showed the highest good quality embryos rate (78.06 ± 22.29). For *in vitro* study, sperm wash with berberine supplementation show higher good quality embryos (P 0.034).
Limitations, reason for caution: wide scale of sperm parameters in the infertile group.
Wider implications of the findings: in vitro supplementation of the sperm wash media with antioxidant (berberine) may be a substitute to long term in vivo antioxidant supplementation.
Study funding/competing interest(s): Funding by University(ies) – Alexandria university.
Trial registration number: NA.
Keywords: Berberin, SOD, GPx, TBAR, GSH

P-114 Validation of SCSA, an alternative to assess sperm DNA fragmentation (SDF) by flow citometry (FC)

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Study question: To evaluate SDF in normozoospermic sperm samples comparing the two classical methods, SCD and TUNEL, with SCSA, a new technique implemented in our lab: Are they three measuring the same? Which would optimize better the laboratory workflow?
Summary answer: SDF values did not show any statistically significant differences. This means that the three techniques, SCSA, SCD and TUNEL, are partially redundant and mostly measuring the same damage. This approach with few runs of cytometry could improve the work flow in the lab assuring most relevant findings.
What is known already: SDF has become a biomarker for male infertility because it has been shown that it could cause defects in embryo development, risk of early pregnancy loss, or problems with foetal development. Several techniques have been developed for the measuring of SDF where studies have compared the specificity and sensitivity of these techniques (Ribas-Maynou, 2013). Their similarity, complementarity and compatibility are being discussed because each technique involves different DNA targets to measure SDF.
Study design, size, duration: A total of 30 normozoospermic human sperm samples were collected since September to November 2014 at a private clinic after 2 days of sexual abstinence. All samples were analyzed in fresh and the SDF was measured by using 3 different methods: SCD test, TUNEL assay and SCSA Test.
Participants/materials, setting, methods: All samples were centrifuged 8 minutes at 600 g in washing media. The pellet was resuspended in washing media. TUNEL assay was performed using the In Situ Cell Death Detection Kit by fluorescence microscope, SCSA methodology by flow citometry and SCD test by using Halosperm® kit.
Main results and the role of chance: For each assay, we calculated the percentage of fragmented DNA spermatozoa from the total sample. Comparisons of SDF between different groups were assessed using the ANOVA test for one factor with repeated measures. All statistical tests were performed taking into account the 95% of the confidence interval. We excluded systemical and technical deviations using appropriate positive and negative controls. The mean and SD of SDF obtained for each technique was: SCD test: 9.44 ± 4.78; TUNEL assay: 10.45 ± 5.45; SCSA: 10.62 ± 5.32. No statistical differences were found between TUNEL assay, SCSA and SCD test ($p = 0.093$). SCD test and TUNEL assay were time-consuming compared to SCSA, which gave the most accurate and precise results.
Limitations, reason for caution: Many factors should be considered to select the most appropriate technique: costs, timing, sensitivity and specificity of each technique. Higher number of samples would be necessary in order to improve the statistical power. Moreover, pathologic samples are missed in this study but further studies are ongoing with these samples.
Wider implications of the findings: To determine which SDF technique is most suitable, the use of FC offers consistent and reliable data in a short time, but it is an expensive tool. Alternatively TUNEL and SCD offer similar and reproductible results but many disadvantages. Thus SCSA is the most suitable technique. Moreover, the potential of FC seems not exhausted and more variables like ROS, apoptosis, sperm aneuploidy, mitochondrial status… are being evaluated and will contribute to a new enhanced spermiogram.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University associated private clinic and its fundation
Trial registration number: A trial registration number was not required due to the retrospective study design.
Keywords: SCSA, SCD test, TUNEL assay, Sperm DNA fragmentation, Flow citometry

P-115 Environmental and lifestyle factors associated with sperm DNA methylation

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Study question: Does lifestyle and environmental exposures influence sperm DNA methylation?
Summary answer: Exposure to environmental toxicants and life-style factors associates with changes in methylation at key imprinted genes in human sperm.
What is known already: Aberrant DNA methylation of imprinted genes and of genes important for spermatogenesis has been linked to male infertility. The epigenome is susceptible to modification by the internal and external environment, with risk factors including exposure to environmental toxicants, physical activity levels, alcohol consumption and diet. Therefore, these factors could contribute to male infertility through their influence on DNA methylation.
Study design, size, duration: Semen samples were obtained from consenting male patients recruited from a fertility clinic over 10 months. Lifestyle, occupational and environment data were acquired through a questionnaire completed by participants at the time of their treatment.
Participants/materials, setting, methods: Forty five neat samples were analysed for DNA methylation by bisulphite pyrosequencing. DNA methylation was analysed in prepared sperm from the same samples as a control. CpG methylation at imprinted genes for *H19*, *PLAGL1*, *PEG10* (among others) and genes for *MTHFR* and *CD247* were analysed using multiple regression analysis.
Main results and the role of chance: Here we report a number of key environmental factors linked to altered methylation in neat semen at specific imprinted genes. Additionally, increased weekly alcohol consumption and exposure to chemicals including solvents, pesticides and detergents (self-reported), are linked to an increase in *PEG10* methylation. However, there was no significant association between these factors and *H19*, *PLAGL1* or *MTHFR* methylation when controlling for age, BMI and smoking status. DNA methylation is lower in neat semen than in prepared sperm for all genes investigated. *CD247* had 0.6% less methylation in neat semen than in prepared sperm with individual cases having up to 2.2% lower methylation, which could be indicative of leukocyte contamination.
Limitations, reason for caution: We only focus on a limited number of CpG sites for analysis in each gene and so may miss more informative methylated sites. Lifestyle data was self-reported via questionnaire and may underestimate true lifestyle information.
Wider implications of the findings: Changing lifestyle habits could, to an extent, be part of a solution to improve male fertility as it influences the epigenetic regulation of gene expression. This could ultimately improve offspring health as inheritance of epigenetic dysregulation can have transgenerational implications. In addition, the possibility for environmental toxicants to aberrantly affect DNA methylation is an important risk to consider for fertility health in urban living spaces and industrial work places.
Study funding/competing interest(s): Funding by University(ies) – Manchester Metropolitan University accelerator fund to Michael Carroll.
Trial registration number: Approved by Central Manchester Ethics Committee and the Human Fertilisation and Embryology Authority (HFEA licence R0026).
Keywords: Sperm, Lifestyle, Methylation

P-116 Relevant lipids conditioning reproductive results found in sperm after assessment of the lipidomic profile in spermatozoa of infertile males undergoing assisted reproduction treatments with ICSI

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Study question: Are there any differences between sperm samples that failed (non-pregnant, NP) vs. those achieving pregnancy (*P*) regarding the sperm lipidomic profile exhibited after intracytoplasmic sperm injection (ICSI) cycles?
Summary answer: This work reveals statistically significant differences in the lipidomic profile between samples from group NP vs. P after ICSI, finding higher acylglycerophosphocholines levels, and an increase of ceramides and sphingomyelins in dysfunctional spermatozoa, thus being potential sperm fertility biomarkers deserving further attention and research, to improve assisted reproduction treatments' efficacy.
What is known already: Sperm membrane lipids are known to be involved in sperm key functions as capacitation, interaction between spermatozoa and oocyte and in sperm survival after freezing/thawing processes. Furthermore, their particular lipidic composition, with very high level of phospholipids, sterols, glycolipids, saturated and polyunsaturated fatty acids affects oxidative attack

sensitivity. Despite studying these lipids is of interest in Andrology, the available information until now is scarce given that metabolomics is a very recently applied analysis method.
Study design, size, duration: Prospective, analytical, nested cases and controls study evaluating the lipidomic profile of spermatozoa, from patients' ejaculates (Group NP, n = 16; vs. Group P, n = 22) after ICSI. Aliquots from the same samples employed in ICSI procedures were collected, frozen until the reproductive results were known, and then assigned to their corresponding group.
Participants/materials, setting, methods: Sperm samples from infertile patients undergoing ICSI were analysed using Ultra Performance Liquid Chromatography coupled to Mass Spectrometry with methanol platform for fatty acyls, bile acids and lysoglycerophospholipids and methanol/chloroform for glycerolipids, cholesteryl esters, sphingolipids and glycerophospholipids. Lipidic levels were compared using Wilcoxon-signed-rank test and Multivariate analyses after logarithmic transformation.
Main results and the role of chance: We detected 151 different lipids in the sperm samples, 10 of them significantly overexpressed in sperm samples from NP group with 1.10 to 1.30 fold change and a p value < 0.05. Among them, lipids found to be different, showing formula, mean and SD concentrations, between groups NP and P respectively, included Ceramides Cer(d18:1/22:0)6.04 ± 5.44vs.2.83 ± 3.38, Cer(d18:1/22:0)0.39 ± 0.37vs.0.17 ± 0.16 and Cer(d18:1/24:0)2.02 ± 1.99vs.0.89 ± 0.96, Sphingomyelins SM(38:1)1.74 ± 1.63vs.0.77 ± 0.99, SM(d18:1/22:0)0.95 ± 0.90vs.0.39 ± 0.51, SM(42:1)1.45 ± 1.34vs.0.59 ± 0.76, and SM(d18:1/25:0)0.28 ± 0.23vs.0.12 ± 0.13. Also 1 or 2-Monoacylglycerophosphocholines PC(0:0/20:0)0.07 ± 0.06vs.0.03 ± 0.04, 1-ether, 2-acylglycerophosphocholines PC(O-22:0/20:4)0.31 ± 0.26vs.0.13 ± 0.16 and 1-ether, 2-acylglycerophosphoethanolamines PE(16:1e/18:2)1.15 ± 1.08 vs.0.53 ± 0.54 showed different levels between groups. To be highlighted higher levels of monoacylglycerophosphocholine, and increased ceramides and sphingomyelins, mostly consisting of a sphingosine moiety of 18 carbon number (sphing-4-enine), amide-linked to a long-chain fatty acyl group. Multivariate analyses are not suitable for differentiating between the two groups of samples.
Limitations, reason for caution: Other sperm factors not included within this analysis could be biasing the studied relationship, given that sperm function has been demonstrated to be multifactorial. Further confirmation of our results in needed from other studies, confirming or not if our results are extrapolable to other subpopulations or different assisted reproduction protocols.
Wider implications of the findings: The description of lipids linked to optimal sperm function, open new possibilities regarding the development of male fertility diagnostic tools, culture media formulations to improve sperm quality and enhance reproductive results, or, given that lipids compose the major part of the plasma membrane, to design new sperm selection tools based on these molecular traits, using magnetic activated cell sorting of spermatozoa exhibiting specific lipids associated with the best reproductive results.
Study funding/competing interest(s): Funding by national/international organization(s) – Department of Industry, Innovation, Trade and Tourism and FEDER funding (EU).
Trial registration number: NA.
Keywords: lipid, metabolomics, male infertility, male fertility marker, ICSI

P-117 Non familial sporadic heritable retinoblastoma and it's correlation with sperm DNA damage

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Study question: Loss of sperm DNA integrity: Is it the cause of Non-familial Retinoblastoma?
Summary answer: Sperm DNA damage may be the cause of childhood Retinoblastoma and paternal factors may play a critical role in embryonic development.
What is known already: Sperm is highly vulnerable to oxidative damage to both nuclear as well as mitochondrial DNA and due to minimal cytosolic antioxidants. Paternal smoking may be the cause of childhood morbidity and mortality and Retinoblastoma is the most common childhood tumour but aetiology is not known.
Study design, size, duration: A case control study design with 26 cases and 30 controls. Study duration was 8 months.
Participants/materials, setting, methods: Somatic mutations in Rb1 gene were excluded by PCR. Semen samples were collected and analysed for semen parameters. Biological markers for sperm DNA damage such as DFI by SCSA and ROS levels by Chemiluminiscence assay were measured. Multivariate logistic regression was used to compute the OR for Retinoblastoma.
Main results and the role of chance: Seminal mean ROS levels were significantly higher [56.7 ± 46.3 vs 21.6 ± 7.9 RLU/sec/million sperm; $p < 0.0001$] in cases as compared to controls. There was significant increase in mean DFI levels [30.3 ± 5.9 vs 23.2 ± 9.4%;p = 0.0017] in cases as compared to controls. The adjusted OR for nicotine consumers was [4.2(1.1, 16.3); p = 0.037; 95%CI] with non-alcohol users and ROS ≤ 27 RLU/sec/million sperm (cut-off value) taken as reference whereas, the OR for alcohol users was [2.5(0.7, 8.5); p = 0.139; 95%CI] with nicotine non-consumers and ROS ≤ 27 RLU/sec/million sperm as reference. With non-alcohol users and DFI ≤ 28% as reference, the adjusted OR for nicotine consumers was [4.4(1.8, 16.7); p = 0.028; 95%CI] whereas the OR for alcohol users was [2.7(0.8, 8.6);p = 0.098;95%CI] with nicotine non-consumers and DFI ≤ 28% (cut-off value) taken as reference.
Limitations, reason for caution: Low sample size.
Wider implications of the findings: Nicotine consumption either in the form of smoking or oral tobacco and increased alcohol intake adversely affect DNA quality and thus lifestyle interventions like diet rich in fruits & vegetables, quitting smoking & alcohol intake and yoga & meditation may improve the DNA health and be therapeutic.
Study funding/competing interest(s): Funding by national/international organization(s) – Indian Council of Medical Research, New Delhi, India.
Trial registration number: IESC/T-80/28.02.2014.
Keywords: Retinoblastoma, ROS, DFI, Sperm DNA damage

P-118 Effect of progesterone on motility, kinetic parameters and acrosome reaction on native, vitrified and conventionally cryopreserved sperm

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Study question: Does progesterone have a positive effect on motility, kinetic parameters and acrosome reaction (AR) of human spermatozoa after preparation for ART treatment, cryopreservation or vitrification?
Summary answer: No significant improvement of motility, kinetic parameters (curvilinear velocity, average path velocity, amplitude of lateral head displacement) and AR could be observed after the progesterone treatment of cryopreserved or vitrified spermatozoa. Higher concentration of progesterone increases AR of native spermatozoa.
What is known already: Subfertile men show a reduced responsiveness to progesterone. Progesterone-induced rapid effects and fertilization in IVF are related in infertile men. Capacitation, hyperactivation, chemotaxis and acrosome reaction in mammalian spermatozoa are regulated by progesterone in a dose-dependent manner. Low concentrations seem to affect motility, while high concentrations increase hyperactivation. The effect of progesterone on acrosome reaction and the involved metabolic pathways are still controversial.
Study design, size, duration: This is an ongoing prospective and observer blinded study. We included all semen samples from men presenting in our clinic for diagnostic analyses or infertility treatment (n = 10) with a concentration ≥ 20 Mio/ml after processing by density gradient. The mean age of the cohort was 38.8 years.
Participants/materials, setting, methods: The samples were divided and cryopreserved either using cryoprotectant-free vitrification or conventional slow-freezing. Post-thawing spermatozoa capacitated for 2 h in GM501 Sperm Active at 37°C and treated with progesterone (0 nM, 25 nM and 50 nM) for 30 min. Motility (WHO), kinetic parameters (CASA), capacitation and AR (chlortetracycline staining) were compared.
Main results and the role of chance: No significant effect on motility, kinetic parameters and AR in conventional cryopreserved samples for all concentrations of progesterone could be observed. Furthermore, no statistic relevant effect of progesterone on motility, kinetic parameters and AR could be found. Only in the control group without cryopreservation a significant increase of

acrosome reaction ($p < 0.05$) was found after treatment with 50 nM versus 0 nM. This effect could not be observed after treatment with 25 nM versus 0 nM.
Limitations, reason for caution: The effect of progesterone on sperm function is dose- and time dependent and therefore, the experimental setup may not represent the actual situation at crucial points of sperm activation in the female reproductive tract and during fertilization, as well as after sperm reactivation and especially after cryopreservation.
Wider implications of the findings: Progesterone is a physiologically relevant molecule in the regulation of sperm function. So far, exact molecular pathways have not been completely elucidated. Spermatozoa already react to very low concentrations of progesterone. As premature hyperactivation and AR can be detrimental to sperm function, it is important to find out the threshold progesterone concentration. Therefore, detailed knowledge of the requirements of full functional capability may be helpful in further optimizing therapeutic strategies in ART and cryopreservation protocols.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Team Kinderwunsch Hannover.
Trial registration number: NA.
Keywords: progesterone, acrosome reaction, sperm function, cryopreservation, kinetic parameter

P-119 Associations between DNA double strand breaks and N7-methyldeoxyguanosine in human sperm and IVF/ICSI outcomes

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Study question: Human sperm DNA contains damage arising from exposure to genotoxic agents. To further characterize the significance of such damage in human reproduction, the associations between DNA double strand breaks (DSBs) and N7-methyldeoxyguanosine (N7-MedG), a marker of exposure to alkylating agents, and fertility outcomes, were investigated in a clinical assisted reproduction programme.
Summary answer: DSBs and N7-MedG can negatively affect pregnancy and live birth rate.
What is known already: DNA fragmentation (single and double strand breaks) might negatively affect embryo quality and pregnancy rate.
Study design, size, duration: 98 male partners of couples attending for infertility treatment (in *vitro* fertilization cycles $n = 46$, intracytoplasmic sperm injection cycles $n = 52$) were recruited over 10 months.
Participants/materials, setting, methods: In sperm prepared for treatment use, DSBs were measured by a neutral Comet assay and the median of % tail DNA (% Median) and proportions of sperm with Comet tails containing < 0.005% DNA (few DSBs: FDSB) or > 7.5% DNA (high DSBs: HDSBs) were determined. N7-MedG levels were quantified in extracted sperm DNA from neat sperm by immuno-slot blot assay. The fertilization rate and pregnancy outcomes (implantation rate, clinical pregnancy and live birth) were determined.
Main results and the role of chance: In all couples, regardless of type of treatment, % Median was correlated negatively with embryo implantation ($P = 0.02$). Conversely, % FDSBs was correlated positively with embryo implantation ($P = 0.001$). However, only % FDSBs, but not % Median or % HDSBs, was correlated positively with clinical pregnancy ($P = 0.02$) and live birth ($P = 0.04$). N7-MedG levels were negatively correlated only with live birth with a borderline significance ($P = 0.06$). Taking in consideration the type of treatment, % Median in IVF but not ICSI couples was correlated negatively with only embryo implantation ($P = 0.04$). On the other hand, % FDSBs in ICSI but not IVF couples had significant and positive correlations with embryo implantation ($P = 0.008$) and clinical pregnancy ($P = 0.03$). N7-MedG levels in IVF or ICSI couples were not associated with pregnancy outcomes.
Limitations, reason for caution: The numbers of biochemical pregnancy ($n = 6$) and late miscarriage ($n = 2$) are small and thus it is difficult to draw a conclusion about the impact of DSBs and N7-MedG on miscarriage.
Wider implications of the findings: These results suggest that the impact of DSBs (% Median or % FDSBs) on pregnancy outcomes appears in the early stage of embryo development and thus embryo implantation rather than in early miscarriage (biochemical pregnancy) or late miscarriage. On the other hand, alkylation damage to DNA (N7-MedG) might be a more significant factor in early and late miscarriage.
Study funding/competing interest(s): Funding by University(ies) – Damascus University.
Trial registration number: This study had Local Ethics Committee Approval (Central Manchester REC ERP/91/078) and approval from the UK Human Fertilization and Embryology Authority (HFEA research licence R0026).
Keywords: Infertility, IVF/ICSI, sperm DNA damage

P-120 The Kappa-opioid receptor modulates the human sperm capacitation and acrosome reaction

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Study question: Is the Kappa-opioid receptor involved in the regulation of human sperm capacitation and acrosome reaction?
Summary answer: The selective kappa-opioid receptor agonist, U50488, promotes the human sperm capacitation and inhibits the acrosome reaction modifying the calcium channels.
What is known already: Opioids exert their effects by binding to membrane receptors and nowadays, three types of opioid receptors (μ,δ and κ) have been found in human sperm cells As it is known, μ-opioid receptors and δ-opioid receptors are implicated in sperm motility but κ-opioid receptor doesn't have any effect on it. Therefore, the physiological function of κ-opioid receptor is completely unknown in human sperm cells, as the signalling pathways involved in both processes.
Study design, size, duration: We used 50 human seminal samples obtained from the Cruces Universitary Hospital. The samples were isolated and capacitated by swim up. The isolated sperm cells were used for inhibitory studies of calcium signalling pathways using the following substances: U50488: selective kappa-opioid receptor agonist, U73122: Phospholipase C inhibitor, Mibefradil 1.5 μM: calcium channel inhibitor, Mibefradil 30μM: calcium channel activator and NNC55-0395: CatSper calcium channel inhibitor. The samples were treated with the different substances for an hour, and the control was compared with the treated samples. ($p < 0.05$).
Participants/materials, setting, methods: After the treatments, the acrosome reaction was conducted by Flow cytometry using the anti-CD46 antibody. Immunoblotting studies were carried out to evaluate capacitation using the anti-phosphotyrosin antibody and the results were analyzed by densidometry.
Main results and the role of chance: Immunoblotting studies showed an increase on the tyrosine phosporilation in human sperm cells induced by the U50488 kappa-opioid receptor agonist. The inhibition of CatSper by Mibefradil 1.5 mM and NNC55-0395 also induced tyrosine phosphorylation and this effect was reversed by the U50488 kappa-opioid receptor agonist. The U50488 kappa-opioid receptor agonist inhibited the acrosome reaction in human sperm modifying the calcium channels. U50488 was able to blunt acrosome reaction induced by Mibefradil 30μM, a calcium channel activator. The inhibition of calcium channels and the Phospholipase C by Mibefradil 1.5 μM, NNC55 (CatSper selective inhibitor) and U73122 respectively inhibited acrosome reaction themselves and Kappa-opioid selective agonist had no any sinergic effect.
Limitations, reason for caution: We need further studies to describe the whole signalling pathway.
Wider implications of the findings: The kappa-opioid receptor participates in the regulation of the acquisition of the fertile capacity in human sperm. This supports the idea that the opioid system could be used as a therapeutic target in the finding of male contraceptives.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). This work was supported by grants from the Basque Government (GIC 12/173). IU was supported by a grant from the University of the Basque Country, MG by the Zabalduz grant, IM by a grant from Basque Government and HE was supported by a grant from Gangoiti Barrera Foundation. The authors have no conflicts of interest to declare. The authors have no conflicts of interest to declare.
Trial registration number: CEISH/61/2011.

Keywords: Opioid system, Kappa-opioid receptor, Capacitation, Acrosome reaction, Signalling pathway

P-121 Assessment of sperm quality after magnetic activated sperm cell separation

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Study question: The effectiveness of magnetic activated cell sorting (MACS) in *in vitro fertilization* (IVF) protocols is still controversial. Thus, we examined whether the combination of MACS with other (existing) nontoxic sperm separation techniques increase the chance of finding higher number of competent sperm cells for IVF.
Summary answer: Our results indicated that the spermatozoa obtained from swim up and MACS groups give statistically significant better results compared to other groups, indicating that combined methods may result in better IVF outcomes.
What is known already: Selecting the competent spermatozoa by the current selection techniques, ranging from sperm washing to advanced sperm selection techniques such as the removal of apoptotic spermatozoa or sperm ultra-morphology evaluations, have employed in IVF protocols. MACS was introduced as a novel technique that would remove apoptotic spermatozoa from non-apoptotic ones. However, the potential beneficial effects of MACS in clinical applications are still debatable even in very recently published articles that highlight the importance of further studies.
Study design, size, duration: To examine the sperm quality assessment after combining MACS with swim up (SU) and density gradient (DG) methods, 80 subgroups were created from 10 normozoospermic and 10 oligozoospermic cases. Each sample was further subgrouped as; i) SU, ii) DG, iii) SU + MACS, and iv) DG + MACS.
Participants/materials, setting, methods: Number and morphology of spermatozoa, DNA integrity (with chromomycin-A3), apoptosis (with TUNEL assay), and the distribution and amount of two essential proteins Izumo-1 (fundamental for oocyte fertilization), phospholipase-C-zeta (PLC-zeta, an oocyte activating protein) were evaluated with a confocal microscope and/or flow cytometry. Comparisons were performed with appropriate statistics tests.
Main results and the role of chance: Total and rapid-progressive sperm numbers significantly low in MACS groups. The ratio of spermatozoa having normal morphology was insignificantly higher in SU compared to others. The number of TUNEL positive cells significantly lower in MACS + SU compared to others. No difference was found in among the groups in oligozoospermic cases. DNA integrity was found lower in MACS + SU compared to MACS + DG. The ratio of Izumo-1 sperm in SU group was significantly higher compared to others. Izumo-1 positivity in MACS + SU was similar to that of MACS + DG. Flow cytometry showed that the number of PLC-zeta positive spermatozoa was higher in DG or MACS + DG; however, mean intensity was found higher in SU or MACS + SU, indicating that both SU groups provide isolation of spermatozoa having higher amount of PLC-zeta.
Limitations, reason for caution: Besides ethical concerns, the major limitation of using MACS is the decrease of sperm number, therefore oligozoospermic patients do not seem to be appropriate candidates for MACS applications. It would be ideal to perform IVF cycles following the above cell separation protocols to observe the outcomes of these study groups.
Wider implications of the findings: MACS was presented as one of the pivotal techniques among other advanced sperm selection techniques. Since the combination of MACS with SU gave the higher sperm quality, we suggest using this combination for clinical applications, not the MACS technique alone. To our knowledge, this was the first attempt that MACS was implemented in the oligozoospermic patients, and our findings revealed that MACS does not seem to be appropriate for sperm selection in these cases
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Ankara University BAP. TUBITAK.
Trial registration number: 213S019.
Keywords: sperm selection, MACs, PLC-zeta, Izumo-1

P-122 Effect of the supplementation of myo-inositol in the cryopreservation process of human spermatozoa

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Study question: Can the addiction of myo-inositol influence human sperm quality in terms of motility and DNA fragmentation during the cryopreservation process?
Summary answer: Myo-inositol seems to improve semen parameters after thawing only in a group of patients.
What is known already: Cryopreservation of spermatozoa is associated with an oxidative stress induced by reactive oxygen species that cause a reduction in sperm DNA integrity and motility. Myo-inositol is physiologically released in the testis and it directly increases the membrane potential, an index of sperm motility. Recent studies have suggested a possible role of myo-inositol in the chemotaxis and thermo taxis of spermatozoa.
Study design, size, duration: From July to December 2014 a prospective study included semen samples of 90 men undergoing basic semen analysis or ART. Semen analyses were performed according to WHO (2010) criteria.
Participants/materials, setting, methods: Semen samples were cryopreserved using slow freezing. Each sample was divided into two aliquots: one cryopreserved using freezing medium supplemented with 2 mg/mL of myo-inositol, and one using freezing medium without myo-inositol. Motility and DNA fragmentation (SCD test, Halosperm® kit) were assessed both in fresh and thawed spermatozoa.
Main results and the role of chance: After supplementation of myo-inositol a total of 66/90 patients showed an average reduction of 3.7% of DNA fragmentation after thawing, 9/90 patients showed an average increase of 2% and in 15/90 patients were found no variations. Myo-inositol affected also sperm motility: in 45/90 patients we observed an increased motility, but 28/90 patients had a decreased motility.
Limitations, reason for caution: We did not analyse semen samples with a sperm concentration lower than 5 x 10^6/mL.
Wider implications of the findings: Further studies would be necessary to establish which criteria must be investigated to predict the response to myo-inositol addition in terms of motility and DNA fragmentation. Moreover, the supplementation with myo-inositol could be performed on semen samples of patients with asthenozoospermia, high DNA fragmentation index or on testicular sperm to improve fertilization rate and embryo quality.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by commercial/corporate company(ies) – Azienda Ospedaliera Papa Giovanni XXIII; Lo.Li.pharma s.r.l.
Trial registration number: NA.
Keywords: myo-inositol, sperm cryopreservation, DNA fragmentation

P-123 Angiotensin II AT2 receptor is expressed in human sperm cells and is involved in sperm motility

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Study question: to investigate the presence of angiotensin receptor AT_2 on human spermatozoa and its implication in sperm motility.
Summary answer: We describe the presence of AT_2 receptor in human sperm cells and its necessary for the maintenance of sperm motility
What is known already: Good motility is a central component for normal male fertility, ART outcomes have demonstrated that the fertilization rates, embryo qualities and pregnancy rates are poor when spermatozoa with low motility have been used for fertilization as compared to those with normal motility. It has been described several components of the rennin-angiotensin system in human sperm cells such as AT_1, ACE, neprilisine and aminopeptidase N. However, the presence and role of the AT_2 is completely unknown.
Study design, size, duration: 1) to investigate the presence of AT_2 receptorin human spermatozoa, 2) to determine its correlation with sperm concentration and motility and 3) and with pathogenic samples. Human samples were

obtained from the Clínica IVI Bilbao, from February 2014 until now. Statistics: Spearman´s rank correlation and Mann-Whitney U-test.
Participants/materials, setting, methods: We used 121 human semen samples. All samples were examined for sperm concentration and motility and classified following WHO guidelines. To confirm the presence of AT_2receptors**,** we performed expression assays for AT_2 receptor by western blot and immunofluorescence. The levels of AT_2 receptor were measured by flow cytometry.
Main results and the role of chance: We demonstrated the existence of angiotensin receptor AT_2 in human sperm by western blot. Immunofluorescence studies showed that AT_2 is mainly located at the equatorial segment of the sperm head. AT_2 receptor levels are associated with sperm motility parameters. Particularly, we found a significant positive correlation between AT_2 receptors and AB motility grade spermatozoa (progressive motility) in freshly and capacitated samples ($p < 0.01$ and $p < 0.01$). Moreover a significant negative correlation with D motility grade spermatozoa in freshly and capacitated samples (inmotile) ($p < 0.05$ and $p < 0.01$). Regarding pathological studies, the levels of AT_2 receptor measured by flow cytometry were lower in spermatozoa of asthenozoospermic men as compared to normozoospermic controls ($p < 0.05$).
Limitations, reason for caution: Duration of the study.
Wider implications of the findings: angiotensin receptor AT_2 is present in human semen and may be involved in the control of sperm motility. These findings suggest the angiotensin receptor AT_2 could be used as a therapeutic target in the finding of male infertility. In-depth understanding of the proteins involved in sperm motility can help elucidates the role of these proteins in male infertility as well as establish biomarkers for male infertility
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Department of Physiology, Faculty of Medicine and Dentistry, University of basque Country, Leioa, Spain. IVF Laboratory, Clínica IVI Bilbao, Leio, Spain.
Trial registration number: CEISH/61/2011.
Keywords: rennin-angiotensin system, angiotensin receptor AT_2

P-124 Predictive value of different factors influencing pregnancy rate following intrauterine insemination with homologous semen: results of a prospective observational study of 1041 inseminations

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Study question: This study aimed to examine the extent to which the outcome, i.e. pregnant or not pregnant, of artificial insemination with homologous sperm (AIH) is influenced by certain covariates such as age, smoking/non-smoking, BMI, infertility diagnosis, stimulation method, the insemination procedure itself, sperm quality parameters etc.
Summary answer: Taking all covariates into account, scientific evidence elucidated three predictive factors influencing pregnancy rate: female age, primary or secondary infertility status and the use of natural cycle or ovarian stimulated cycle.
What is known already: Many retrospective studies have examined different prognostic factors influencing pregnancy rates after IUI treatment with homologous semen. Possible effects of covariates such as age, smoking, BMI, ovarian stimulation protocols etc. have been described before. However, in most studies, covariates were studied as independent of each other which may lead to misleading conclusions.
Study design, size, duration: During the period of July 2011 until November 2014, data from 1041 IUI cycles in 454 couples were collected prospectively in a tertiary referral infertility centre. Because outcome results after AIH cannot be analysed independently, the statistical analysis was performed using a Generalized Estimating Equations (GEE) model.
Participants/materials, setting, methods: The examined covariates were female and male age, smoking, BMI, primary/secondary infertility, infertility diagnosis, cycle number, stimulation method, day 0 estradiol/progesterone levels, abstinence period, HCG-insemination interval, the insemination procedure itself, occurrence of blood loss after insemination, sperm quality parameters and inseminating motile count (IMC).
Main results and the role of chance: A clinical pregnancy rate, i.e. presence of fetal heart beat at 6–7 weeks of gestation, of 9.1% per cycle was observed. A univariate statistical analysis of the dataset revealed only one parameter influencing the pregnancy rate per cycle: the ovarian stimulation method. The pregnancy rate per cycle was significantly lower after the use of clomiphene citrate versus natural cycle or gonadotrophin or recombinant-FSH stimulated AIH cycles. A multivariate GEE analysis eventually revealed that the only valuable prognostic covariates included female age, infertility status (i.e. primary or secondary infertility) and ovarian stimulation method. We also observed that a difficult insemination procedure, with or without significant loss of blood, BMI, smoking and the interval between HCG triggering and insemination did not influence the success rate.
Limitations, reason for caution: It is misleading to look at only one covariate at a time since observations are not independent of each other in this series. Furthermore, GEE analysis has a low statistical power to detect statistically significant differences in groups with low success rates, such as observed in an AIH programme.
Wider implications of the findings: According to our results, it seems that female age, infertility status and type of ovarian stimulation are the most important factors influencing the success rate in an AIH programme. Many different prognostic factors described before, such as BMI, smoking, cycle number, easy or difficult insemination procedure etc., did not affect the outcome results in our series. This may be due to the fact that a multivariate GEE statistical analysis was performed.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Funding by national/international organization(s). This study is part of the 'Limburg Clinical Research Program (LCRP) UHasselt-ZOL-Jessa', supported by the foundation Limburg Sterk Merk, province of Limburg, Flemish government, Hasselt University, Ziekenhuis Oost-Limburg and Jessa Hospital.
Trial registration number: 1
Keywords: IUI, pregnancy rate, predictive factors, partner semen, artificial insemination

P-125 Human oocyte calcium analysis (HOCA): a novel test for sperm oocyte-activation potential

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Study question: Does human oocyte calcium analysis (HOCA) contribute to reveal sperm-related activation deficiencies in patients with recurrent fertilisation failures?
Summary answer: Mouse oocyte activation test (MOAT) and mouse oocyte calcium analysis (MOCA) are diagnostic assays that allow classifying sperm depending on its activation capacity. We have shown that the mouse model can only reveal severe sperm deficiencies. The use of HOCA detects more precisely sperm activation defects.
What is known already: Oocyte activation deficiency is the main cause for fertilisation failure after ICSI. The sperm-borne factor PLCζ performs a major role in the oocyte activation process. Its function is highly conserved amongst mammals, allowing successful heterologous fertilisation. Although mouse oocytes are used to evaluate the activation potential in human sperm, we have to take into account that human PLCζ (hPLCζ) shows greater potency than mouse (mPLCζ)**.**
Study design, size, duration: Six patients that showed recurrent fertilization failures after ICSI were selected for the study. Activation potential and the calcium patterns were determined by MOAT and MOCA, respectively. Patients showing moderate to normal MOAT activation rate and MOCA score (amplitude x frequency of oscillations, AxF) were further analysed by HOCA.
Participants/materials, setting, methods: *In vitro* matured GV-MII and MI-MII human oocytes were collected and vitrified. After thawing, oocytes were cultured for 2 h and loaded with 7.5 μM Fura-2AM before ICSI. For HOCA, calcium oscillations were recorded by a radiometric method (λ_{exc}340/380 nm) on an inverted epifluorescence microscope every 30 s for 10 h.
Main results and the role of chance: The following data were considered as normal references: (i) MOAT activation rate >80%; (ii) MOCA: product of

frequency (number of peaks/2 h, F) and amplitude of the peaks (fluorescence difference subtracted from baseline, A) ≥9 and (iii) HOCA: Oscillatory activity (number of oocytes peaking, OA) >75%, F = 0.9–1.6 and A = 0.8–1.7.Patients included in the study showed moderate ($n = 2$) to normal activation rates ($n = 4$) after MOAT compared to a positive control (median = 80.4%(66%-97%)). MOCA revealed aberrant patterns in 3 out of 6 patients (median$_{AxF}$ = 9.2 (3.1–46.3)). Interestingly, HOCA revealed a significant reduced activation capacity in all 6 patients. In 4 out of 6 cases no oscillations were observed. The other 2 patients showed normal OA (63%, 80%)and A(0.72, 0.79), however the F was significantly reduced (0.5 and 0.2).

Limitations, reason for caution: The quality of *in vitro* matured human oocytes is impaired compared to *in vivo* matured oocytes. However, the effect of this *in vitro* maturation on the subsequent calcium pattern has already been described in human.

Wider implications of the findings: The present study compares activation capacity of human sperm using homologous and heterologous ICSI. We showed that the diagnostic tests performed using mouse oocytes cannot be directly extrapolated to the human model. This is probably due to the higher activation potential of hPLCζ. In addition, our findings support that the use of HOCA enables the detection of less severe PLCζ deficiencies and provide novel insights for the role of sperm in modulating calcium dynamics.

Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Ghent University / Ghent University Hospital.

Trial registration number: NA.

Keywords: Fertilisation failure, Oocyte activation, Calcium, Male infertility, PLCz

P-126 Use of clomiphene citrate for male infertility caused by idiopathic secondary hypogonadism

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Study question: Idiopathic secondary hypogonadism is a classic cause of male infertility. In those men we evaluated the use of clomiphene citrate (CC) to increase testosterone secretion and improve spermatogenesis.

Summary answer: Clomiphene citrate is an effective and inexpensive treatment to increase testosterone level in idiopathic secondary hypogonadism. It could also potentially improve spermatogenesis in selected patients. The effect of cc on natural pregnancy rates remains to be evaluated.

What is known already: Patients with secondary hypogonadism have inadequate gonadotropin secretion in presence of normal testes. Clomiphene citrate binds to the hypothalamic estrogen receptors resulting in blocking estrogen negative feed-back. This effect induces an increase in FSH and LH secretion, which can improve testosterone secretion by Leydig cells and potentially improve spermatogenesis. Men who meet the following criteria could benefit from the treatment with cc: idiopathic infertility, sperm concentration > 5 million/ml, low or normal FSH/LH levels.

Study design, size, duration: We performed a retrospective review, between January 2010 and January 2013, of 230 men who were referred to our university hospital fertility unit for infertility assessment.

Participants/materials, setting, methods: Secondary hypogonadism was diagnosed in 30 patients. Fourteen met the inclusion criteria of idiopathic secondary hypogonadism with a sperm count over 5 million/ml. They were prescribed cc for 3 months (25 mg/day). Testosterone level was measured at 1 and 3 months, and a semen assessment was performed at 3 months.

Main results and the role of chance: Mean age of the 14 patients who were treated with cc was 38.5 years. After 1 and 3 month, total serum testosterone level increased in all patients from a mean of 7.8 nmol/l (3.8–10.8) to 22.5 nmol/l (15.6–29.1) and 23.2 (14.3–30.1), respectively. Total sperm count doubled in 6/14 patients (baseline sperm concentration 8.8 ± 3.2 million/ml, post treatment sperm concentration 22.2 ± 7.4 million/ml).

Limitations, reason for caution: This is a retrospective review with a small sample size. The potential use and safety of cc in male infertility caused by idiopathic secondary hypogonadism to improve natural pregnancy rates needs to be evaluated in a prospective trial with an adequate sample size.

Wider implications of the findings: Male infertility can be successfully treated with in vitro fertilization and intracytoplasmic sperm injection which is invasive and expensive. It is important to develop new treatments that specifically target the cause of infertility to increase the chance of a natural pregnancy. In selected infertile male patients with secondary hypogondism, cc could represent an alternative to more invasive treatment. Use of cc to increase endogenous testosterone level may have a positive impact on patients' quality of life.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Lausanne University Hospital.

Trial registration number: NA.

Keywords: clomiphene citrate, male secondary hypogonadism

P-127 The results of intracytoplasmic sperm injection (ICSI) using testicular spermatozoa among presumed histopathology in testicular biopsy from non-obstructive azoospermia (NOA)

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Study question: What are sperm retrieval rates (SRR) by micro dissection testicular sperm extraction (micro-TESE), fertilization rate, and embryonic development among patients with presumed Sertoli cell only syndrome (SCOS), maturation arrest (MA), and hypospermatogenesis (HS) in testicular biopsy in NOA couples?

Summary answer: The SRR in SCOS and MA was lower than patients with HS. However, good fertilization and embryonic development were achieved without significant differences even in presumed SCOS in those couples whom spermatozoa were obtained.

What is known already: Fertilization and pregnancies can be obtained with spermatozoa recovered from the seminiferous tubules. Sertoli cell only syndrome (SCOS), maturation arrest (MA), and hypospermatogenesis (HS), and, with or without focal spermatogenesis are the commonest histological patterns of patients with non-obstructive azoospermia (NOA). To our knowledge, no study has specifically examined the results of intracytoplasmic sperm injection (ICSI) in SCOS, MA, and HS patients.

Study design, size, duration: We performed a retrospective study based on a reproduction center in Japan and evaluated 221 patients with NOA who underwent micro-TESE between September 2013 and December 2014 in this study.

Participants/materials, setting, methods: We identified patients in whom SCOS, MA, and HS were reported at pathological examination. At the same session of micro-TESE, surgically obtained small tissue specimens were sent to the histopathology laboratory. Two pronuclei (2PN) oocytes, blastocysts development, good-quality blastocysts, biochemical pregnancies and clinical pregnancies rates were examined.

Main results and the role of chance: Spermatozoa were retrieved in 103 of 221 (46.6%) patients with NOA in whom micro-TESE was performed. In 221 patients with histopathology reports, 150 (67.9%), 32 (14.5%), and 39 (17.6%) patients had presumed SCOS, MA, and HS, respectively. SRR in SCOS (44/150 = 29.3%) and MA (20/32 = 62.5%) were lower than patients with HS (39/39 = 100%) patients ($p < 0.001$, respectively). 2PN oocytes, blastocysts development, and good-quality blastocysts rates were 57.2%, 43.1%, and 38.9% in SCOS, 59.2%, 40.8%, and 42.9% in MA, and 62.7%, 44.4%, and 34.5% in HS. Biochemical pregnancy and clinical pregnancy rates were 31.4% (16/51) and 25.5% (13/51) in SCOS, and 30.4% (7/23) and 30.4% (7/23) in MA, and 36.7% (18/49) and 26.5% (13/49) in HS (no significant differences, respectively).

Limitations, reason for caution: The participants are limited to the NOA patients. We excluded obstructive azoospermic (OA) patients, >39 years age of the spouses at the time of ICSI cases in this study, and the patients including with azoospermia factor (AZF) a and AZFb deletion.

Wider implications of the findings: The SRR in patients with SCO was lower than NOA caused by other factors. However, our fertilization and pregnancy rates in patients with SCOS are similar with other histopathological pattern of patients with NOA once successful sperm retrieval is achieved. It is useful to obtain reliable embryonic information from NOA patients and to

encourage micro-TESE for NOA patients even with severe dysregulation of spermatogenesis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproduction Clinic Osaka.
Trial registration number: NA.
Keywords: non-obstructive azoospermia, Sertoli cell only syndrome, maturation arrest, hypospermatogenesis, ICSI

POSTER VIEWING

EARLY PREGNANCY

P-128 Epidemiological survey and risk factor analysis of recurrent spontaneous miscarriage in infertile women at large infertility centers

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Study question: What is the prevalence of recurrent spontaneous miscarriage (RSM is defined as ≥ 2 spontaneous miscarriages and ≤ 20 weeks' gestation, following the definition of American Society for Reproductive Medicine in 2008) among infertile women with a history of spontaneous miscarriage at large infertility centers in China?
Summary answer: 200 out of 751 (26.63%) infertile women with a history of spontaneous miscarriage(SM) had actually experienced RSM were recruited from 6 large infertility centers (>150 assisted reproductive technology cycles per month)including 3 from general hospitals and 3 from maternity hospitals.
What is known already: Spontaneous miscarriagerate after assisted reproductive technology (ART) was reported as 12.2% in China. Many more patients with a history of SM were observed in the miscarriage group than in the non-miscarriage group (33.33% vs 7.92%). A small study with 43 patients showed that 25.6% of infertile patients with SM had experienced ≥ 2 times of SM. Thus, an analysis of differences in the various factors in infertile women between RSM group and single SM group may provide information to allow prevention of RSM in infertile women.
Study design, size, duration: This is a multi-center, cross-sectional epidemiological study. Based on an estimated the primary endpoint of 25.6%, 750 patients produced a two-sided 95% confidence interval with a precision equals to 0.033 considering 10% invalid questionnaire. All the patients were recruited from March to November in 2013.
Participants/materials, setting, methods: A total of 757 infertile women with at least one SM history (£ 20 weeks gestation) were recruited. Six of them were excluded due to noncompliance with the inclusion or exclusion criteria. Demographic data, medical history and evaluation of infertility factors of 751patients were analyzed.
Main results and the role of chance: Among 751 patients, there were 200 infertility patients with RSM, accounting for 26.63% (95% CI: 23.50%,29.95%) and 155 (20.64%), 34 (4.53%) and 11 (1.46%) experienced twice, 3 times and at least 4 times SM. In the RSM group, 31.5% (63/200) patients experienced at least one SM between 12–20 weeks. Whereas when all miscarriages were considered together, 15.90% (162/1019) occurred between 12 and 20 weeks. It has been reported from other studies that the rate of SM after 12 weeks in the general pregnant population is 4.4%. Among all the analyzed factors, multivariate logistic regression indicated that age, low educational background, concomitant endocrine disorder, uterine factor and immune factor were associated with RSM independently ($p < 0.05$).
Limitations, reason for caution: This study was a cross-sectional investigation partially based on retrospective medical history collection. There might be bias for either site or patient selection. Furthermore, some independent risk factors were found but the causality could not be defined clearly.
Wider implications of the findings: As more the 25% of infertile women with a history of SM experienced RSM, these women require a good risk assessment of the independent RSM factors, monitoring or prevention treatment if necessary. In clinical practice, more attention is paid to the pregnant women before 12 weeks of gestation, while, this study results shows that women at 12~20 weeks gestation also need close monitoring.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Abbott Established Pharmaceuticals Division in China.
Trial registration number: NA
Keywords: recurrent miscarriage, recurrent spontaneous miscarriage, risk factor, Epidemiology, infertility

P-129 Expression of GnRHR in Fallopian tube implantation sites from women with ectopic pregnancy

Abstract withdrawn by the author

P-130 Influence of Body mass index (BMI) on risk of early pregnancy loss after high-quality embryo transfer in PCOS patients

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Study question: Debate exists regarding the effect of BMI and PCOS on the outcome of pregnancies after assisted reproduction technology, therefore, this study accessed the effect of Body mass index (BMI) on early pregnancy loss after high-quality embryo transplantation in PCOS patients.
Summary answer: For PCOS patients conceived after high-quality embryos transplant, raised BMI is significantly associated with higher early pregnancy loss rate, especially biochemical pregnancy loss rate after IVF treatment, and BMI increased significantly along with abnormity of basis sex hormones and insulin resistance, which may lead to early pregnancy loss.
What is known already: Obesity is associated with PCOS and a higher risk of pregnancy complications, and there is evidence that obese women who conceive spontaneously are at an increased risk of miscarriage. Debate exists on whether miscarriage risk would increase for patients with PCOS when embryo quality and BMI were taken into account.
Study design, size, duration: A retrospective analysis was implemented on 453 women who were diagnosed with PCOS and accepted the in vitro fertilization and high quality embryo transplantation (IVF - ET) in reproductive center between January 2011 and September 2013.
Participants/materials, setting, methods: Patients were grouped according to their BMI at the time of accepting ET. The main outcome measure was the early pregnancy loss rate. Confounding variables examined included female age, number of spontaneous abortion, endometrial thickness at transplant day, previous miscarriage, whether cryo-thawed cycles, whether blastocyst, and basic sex hormone. Patients received ICSI, ever had got laparoscopic ovarian drilling, one of couples have chromosome abnormality, or had uterine malformation were excluded.
Main results and the role of chance: Basic LH values of normal BMI group ??(11.45 ± 5.9) was significantly higher than that of high BMI group (8.64 ± 4.34), and index of FBG, FINS, HOMA-IR rose with the increase of BMI, with significant difference between every two groups (<0.05). After transplanted with quality embryo, the obese group has significantly higher early pregnancy loss rate (29.63%) and biochemical pregnancy loss rate (20.37%) than that of normal-weight group, while its implantation rate (42.57%) was significantly lower than that of normal-weight group (57.80%, $P < 0.05$). Logistic regression analysis showed that for patients with PCOS after high quality embryos transplantation, basic androgen level, age, and BMI was related to

early pregnancy loss positively, while endometrial thickness at transplant day negatively correlated with early pregnancy loss.
Limitations, reason for caution: A larger size sample, multi-center study should be carried out to carry out to ensure this conclusion.
Wider implications of the findings: This information should be used in counselling patients on their productive consequences of raised BMI prior to transplant their high-quality embryos. Other clinical variables need more concerns and deep research to avoid EPL. Appropriate measures should be taken to offer effective weight management services for obese women intending to embark on IVF treatment
Study funding/competing interest(s): Funding by hospital/clinic(s) – The People's Hospital of Henan Province.
Trial registration number: NA.
Keywords: PCOS, early pregnancy loss, BMI, insulin resistance

P-131 High and low factor V activity, but not factor V Nara and Hong Kong mutations, might be associated with recurrent miscarriage

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Study question: Is the factor V (FV) Nara (W1920R) mutation, Hong Kong (R306G) mutation or the plasma levels of FV activity associated with recurrent miscarriage (RM)?
Summary answer: None of the patients and controls had either homozygotes or heterozygotes for the FV Nara or Hong Kong mutations. Mean values of plasma FV activity level were not significantly different. However, the prevalence of patients with low or high FV activity levels was significantly higher than that of controls.
What is known already: FV Leiden (R506Q) mutation, a major cause of activated protein C resistance (APCR) and deep venous thrombosis (DVT) is well-known as a risk factor of RM. The FV Nara mutation, FV Hong Kong mutation and R2 haplotype of FV gene are associated with APCR and DVT.
Study design, size, duration: The frequency of FV Nara and Hong Kong mutation and FV activity were compared between 88 patients with unexplained RM and 95 fertile controls. The frequency of 16 SNPs of R2 haplotype were compared between patients with high and low FV activities.
Participants/materials, setting, methods: We conducted genomic analysis by direct sequencing to determine the presence of the FV Nara, Hong Kong mutation and 16 SNPs reported as FV R2 haplotype. The FV activity levels were determined by a one-stage clotting assay based on the prothrombin time using FV-deficient plasma and factor assay-controlled plasma.
Main results and the role of chance: Both the FV Nara and Hong Kong mutations were not detected in patients and controls. The mean plasma FV activity levels in the patients and controls were 105.0% and 102.5%, with not statistically significant difference. The prevalence of patients with low FV activity levels (<5th percentile) or high FV activity levels (>95th percentile) was significantly higher. In the lower FV activity group homozygous minor types were identified in 13 of 16 SNPs. Two heterozygous and one homozygous states were found at each of Arg 1299 and Gly 2194. Homozygous states of both Ser 1240 (tct) (minor type) and Val 1736 (gtg) (minor type) were found in 4 patients.
Limitations, reason for caution: The sample size was relatively small. The mean age of patients was significantly lower than that of controls. The influence of age on the FV activity level was speculated to be small, because the correlation was not significant in the present study.
Wider implications of the findings: The distribution of FV activity in patients may differ from that in controls. Some of the SNPs including R2 haplotype were seemed to relate to low level of FV activity in some patients with RM. Our results suggested that both low and high level of FV activity might be associated with RM in part of the patients. Further study is needed to examine the association between FV activity and RM.
Study funding/competing interest(s): Funding by national/international organization(s) – The Ministry of Health, Labour and Welfare of Japan.
Trial registration number: NA.
Keywords: recurrent miscarriage, coagulation factor V, FV Nara mutation, FV Hong Kong mutation, FV R2 haplotype

P-132 S protein, factor VIII and D dimer levels comparison in normal pregnancy and under-treatment with low molecular weight heparin

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Study question: To analyze the changes in F VIII, SP (S protein) and DD (D Dimer) levels in pregnant women with documented thrombophilia under LMWH and without thrombophilia and the anticoagulant effect of the LMWH (Low Molecular Weight Heparin)
Summary answer: LMWH treatment shows a stronger effect in the first and the second trimester as much as in SP as in DD, not showing the same behavior on FVIII concentration.
What is known already: Pregnancy represents a physiological prothrombotic state. Factor VIII shows a progressive increase during pregnancy development. S Protein decreases from the first weeks. D Dimer levels increase significantly during the 3 trimesters.
Study design, size, duration: Prostective, case control, diagnostic test. A 162 patients were involved, 66 normal pregnancy patients and 96 with confirmed thrombophilia, between march 2012 and July 2014.
Participants/materials, setting, methods: *66 pregnant without thrombophilia were included:* 25 in first trimester (GIa), 22 second trimester (GIb), 19 third trimester (GIc) 96 pregnant using LMWH: 30 in first trimester (GIIa), 32 second trimester (GIIb) 34 third trimester (GIIc). We measured FVIII with coagulation method, DD with fluoroimmunoassay and SP with coagulometric method
Main results and the role of chance: FVIII GIa 110% (56–154), GIb 137% (80–266), GIc 144% (80–310), *p*: 0.0013; GIIa 118% (58–182), GIIb 137% (70–280), GIIc 201(112–320), *p*: *0.0010.* **SP** GIa 63% (37–94), GIb 53% (38–85), GIc 50% (36–83), *p*: NS. GIIa 80% (30–106), GIIb 63 (32–103), GIIc 45% (28–72), *p*: 0.0010. **DD** GIa: 473 (154–954), GIb 784 (401–1680), GIc 1301 (622–2294) $p < 0.0001$. GIIa 125 (100–621), GIIb 460 (159–1180), GIIc 1115 (311–2270) $p < 0.0001$. **FVIII** GIa (106) vs GIIa (118): *p* NS, GIb (137) vs GIIb (137) pNS, GIc (144) vs GIIc (201) *p*: 0.0167. **SP** GIa (63)vs GIb (80) *p* 0.048. **DD**: GIa (473) vs GIIa (125) $p < 0.0001$, GIb (784) vs GIIb (460) *p* 0.0003.
Limitations, reason for caution: We consider that is important that each center create their own table of values for these parameters.
Wider implications of the findings: LMWH treatment shows a stronger effect in the first and the second trimester as much as in SP as in DD, which is an important parameter to perform a correct treatment and follow up of these patients. It is useful to adequate the dose of heparin in order to prevent obstetrical complications.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital Nuestra Señora de la Misericordia, Origen, Salud Reproductiva, Fundación para el Progreso de la Medicina.
Trial registration number: This is not a RCT study.
Keywords: thrombophilia, pregnancy

P-133 Chorionic villous vascularization related to the uterine pathology in early pregnancy miscarriages

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Study question: What is the determining factors in the chorionic villous vascularization in early miscarriage specimens?
Summary answer: There were no significant differences in chorionic villous vascularization depeding on either types of miscarriage-embryonic, yolk sac or empty sac miscarriage- or chromosomal status of aborted fetus- euploid or aneupoid. However, uterine pathology was a significant factor in the chorionic villous vascularization.
What is known already: At least half of first trimester miscarriages are due to embryopathogenesis associated with chromosome abnormalities resulting in miscarriages. Absent or decreased chorionic villous vascularization is frequently present in these miscarriage specimens.

Study design, size, duration: For this retrospective study, 134 slides of miscarriage tissue of less than 10 weeks of gestation were collected between July 2013 and October 2014 from a private fertility center.
Participants/materials, setting, methods: Chorionic villous vascularization was determined using a previously published histological classification (Grade 0, unknown; I, normal; IIA, mild hypoplasia; IIB, severe hypoplasia and III, avascular). Uterine pathology was defined as uterine synechia, adenomyosis and myoma with endometrial compression. The vascularization scores of chorionic villi were compared with the ultrasound findings, corresponding chromosome results, and the presence of uterine pathology.
Main results and the role of chance: There were 98 embryonic miscarriages, 18 yolk sac miscarriages and 18 empty sac miscarriages. Chromosome results were obtained in 79 of the 134 miscarriages; 54.4% were noneuploid. Twelve cases (9%) were insufficient villi for evaluation, and 37 (27.6%) were classified as normal, 57 (42.5%) as mild hypoplasia, 25 (18.7%) as severe hypoplasia, and 3 (2.2%) as avascular. Uterine pathology was detected in 25 cases (18.7%). The vascularization score did not differ between embryonic, yolk sac or empty sac miscarriages or euploid or noneuploid miscarriages. It was not related to the gestational age at miscarriage, maternal age, or retention time. However, uterine pathology was a significant factor in the chorionic villous vascularization (p = 0.015 for chi-square test). The rate of preexisting uterine pathology was higher in the avascular villi compared with that in the normal villi (66.7% versus 10.8%, p = 0.054 for Fisher's exact test).
Limitations, reason for caution: The number of samples was limited and the study population was heterogenous.
Wider implications of the findings: Uterine pathologies may impede chorionic villous vascularization. Further study is warranted to determine whether uterine surgery can improve the vascularization.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding in this study.
Trial registration number: NA.
Keywords: miscarriage, chorionic villous vascularization, uterine pathology

P-134 Initial serum ß-hCG levels in clinical pregnancies resulting from the transfer of a single vitrified-thawed blastocyst are higher compared to single fresh blastocyst transfers

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Study question: Our objective was to compare serum ß-hCG levels following transfer of a single fresh or a single vitrified-thawed blastocyst and to determine the predictive value of serum ß-hCG levels for pregnancy outcomes.
Summary answer: Our study suggests that initial serum ß-hCG values are higher after the transfer of a single fresh blastocyst compared to a single vitrified-thawed blastocyst transfer resulting in a clinical pregnancy, even after adjusting for confounding variables.
What is known already: Several studies evaluated the predictive levels of serum ß-hCG levels after embryo transfers in an IVF setting. Most of them report serum ß-hCG values after the transfer of single or multiple embryos, and none have compared the predictive value after the transfer of a single fresh versus vitrified blastocyst.
Study design, size, duration: Retrospective analysis of 1144 single blastocyst cycles with a positive ß-hCG results on day 16 after oocyte collection and equivalent calculated day 16 (frozen embryos) between December 2008–2013. Logistic regression determining the association of potential factors and ROC analysis assessing the predictive value of ß-hCG for a clinical intrauterine pregnancy were performed.
Participants/materials, setting, methods: We analyzed covariates including maternal and paternal age, embryo quality, assisted hatching, total dose of FSH, LH, micromanipulation and treatment protocol. Multiple gestations resulting from SET were excluded. Pregnancy outcome included a biochemical pregnancy, ectopic pregnancy, clinical intrauterine pregnancy and live birth.
Main results and the role of chance: There were 801 positive ß-hCG results following the transfer of a single fresh blastocyst and 343 others following the transfer of a single vitrified-thawed blastocyst (650 and 233 clinical intrauterine pregnancies respectively). Mean ß-hCG levels of clinical intrauterine pregnancies were significantly higher following a single vitrified blastocyst than a single fresh blastocyst transfer (383 ± 230 and 334 ± 192; p = 0.01). This difference remained (p = 0.04) after adjusting for the confounding variables. The threshold value predicting a clinical pregnancy for a vitrified blastocyst was 137 IU/L (sensitivity 83%, specificity 68%). For a fresh blastocyst transfer, the predictive threshold value was 111 IU/L (sensitivity 90%, specificity 60%).
Limitations, reason for caution: The limitation of the study is in the retrospective nature of the study. Pregnancy outcome was recorded as part of routine prospective patient follow-up by telephone surveillance and documented in the computerized patient chart. Medical charts were reviewed for patients that delivered at our hospital.
Wider implications of the findings: Transfer of more than one embryo makes it impossible to evaluate the true contribution of the embryo in the very early stages of development. Our study shows that among clinical pregnancies, the initial maternal serum ß-hCG values are significantly higher following the transfer of a single vitrified-thawed blastocyst than a fresh blastocyst. This information might assist clinicians in counseling patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: SET, pregnancy, blastocyst, vitrified, ß-hCG

P-135 Endocannabinoids and decidual remodeling: COX-2 oxidative metabolism as a key regulator

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Study question: We previously described anandamide (AEA)-effect on rat decidual cell apoptosis. Besides its importance in early pregnancy, cyclooxygenase-2 (COX-2) regulates AEA levels. In this study we investigated the role of COX-2 oxidative metabolism in AEA-induced cell death and hypothesized the impact of endocannabinoid disruption on decidual remodeling.
Summary answer: Collectively, our findings suggest that COX-2 has a key role for AEA-induced decidual apoptosis. COX-2 metabolism regulates endocannabinoid levels required for uterine tissue remodeling, and integrates the intracrine uterine stromal crosstalk underlying decidualization.
What is known already: Decidualization is a process crucial for the establishment of pregnancy. COX-2 is restricted to blastocyst surroundings, and disruption of its expression in mice results in decidualization impairment. Endocannabinoids and prostaglandins are lipid mediators involved in endometrial receptivity. Anandamide, the main endocannabinoids, induces apoptosis in rat decidual cell. COX-2 is responsible for the oxidative metabolism of AEA into prostamides. Low levels of AEA allow implantation and alterations of its levels are associated with miscarriages.
Study design, size, duration: Rat decidual tissue from d10 of pregnancy was used to prepare decidual cell cultures. Selective COX-2 inhibitors and a FAAH inhibitor (URB597) were co-incubated with AEA and cell viability assessed after 24 h. The time course of AEA-induction of COX-2 expression (1, 6 and 24 h), in presence and absence of URB597, was analysed.
Participants/materials, setting, methods: To study the effect of AEA or of its oxidative metabolites, cell viability (MTT and LDH release assay) and morphology (Giemsa staining) were analyzed; quantification of prostamides (LC-MS-IT-TOF); caspase -3/-7 and -9 activity, membrane mitochondrial potential alteration ($\Delta\psi m$), generation of ROS (Fluorometry) and Cox-2 expression (Western Blot) were performed.
Main results and the role of chance: The major features of AEA-apoptotic pathway in decidual cells were reversed by selective COX-2 inhibitors (Celecoxib; R-Flurbiprofen). Endogenous levels of prostamide E2 (PME2) in decidual tissue were detected (8.05 pmol/g) and incubation with AEA resulted in a 7-fold increase. PME2 induced cell viability loss in a dose-dependent manner, associated with chromatin condensation, and an increase by 17% of caspase -3/-7 activity. The apoptotic intrinsic pathway was confirmed by a drop in $\Delta\psi m$ by 18%, an increment by 24% of caspase -9 activity, and generation of ROS by 28% in comparison to control. AEA induced a rapid increase of COX-2

activity and expression, sustained in the presence of URB597, a FAAH inhibitor, through a mechanism involving activation of p38 kinase and NF-?B.
Limitations, reason for caution: For ethical reasons, the studies of endometrial tissue remodeling in humans are limited. The rat, like human, exhibits a highly invasive type of placentation, and hence is an acceptable model for studying the mechanisms of decidualization, but caution is required in the translation of results.
Wider implications of the findings: A network of different mediators orchestrates pregnancy, among which endocannabinoids and prostaglandins are prominent. Taking into account that COX-2 spatial-temporal expression indicates that this enzyme is tightly regulated during early pregnancy, we shed light into the role of this enzyme in coordinating the endocannabinoid and eicosanoid systems. COX-2 inhibitors are widely used drugs. Hence, this study is clinically relevant since COX-2 modulation may lead to impairment of decidualization, which predisposes to pregnancy complications, including miscarriage.
Study funding/competing interest(s): Funding by national/international organization(s) – The authors thank Fundação para a Ciência e Tecnologia (FCT) for the grant attributed to Almada M (SFRH/BD/81561/2011) and Fonseca BM (SFRH/BPD/72958/2010).
Trial registration number: NA.
Keywords: Anandamide, COX-2, Decidualization, Apoptosis

P-136 3-fold increased risk of miscarriage in women with a normal BMI undergoing IVF/ICSI if periconceptional TSH level is greater than or equal to 2.5 mU/L

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Study question: Is the periconceptional thyroid stimulating hormone (TSH) level in women undergoing in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) correlated with pregnancy outcome in terms of implantation, biochemical pregnancy, clinical pregnancy, miscarriage, live birth and pregnancy complications?
Summary answer: In women with a normal body mass index (BMI) who underwent IVF/ICSI, a periconceptional TSH level of ≥ 2.5 mU/L is correlated with a 3.8-fold increase in risk of miscarriage.
What is known already: Hypothyroidism, and even subclinical hypothyroidism, is associated with a number of adverse reproductive outcomes. The relevance of screening for thyroid function in women undergoing IVF treatment is still controversial, especially since there is no consensus regarding what the treatment threshold should be. Available data is conflicting. To further address this issue, we evaluated the relation between pregnancy outcome and periconceptional TSH levels after IVF/ICSI.
Study design, size, duration: A monocentric retrospective cohort study was performed in patients who underwent a fresh embryo transfer after an IVF/ICSI treatment between January 2007 and September 2011. This study was approved by the local Ethics Committee.
Participants/materials, setting, methods: We included 1128 IVF/ICSI cycles of 626 patients with male subfertility as primary cause of subfertility. Data were analysed in a univariate (Mann-Whitney U-test & Fisher's Exact test) and a multivariate model (multiple logistic regression; adjusting for age ≥ 36, smoking, previous pregnancy/deliveries, > 1 prior miscarriage, number/quality of embryos transferred).
Main results and the role of chance: Outcomes were similar amongst women with TSH < 2.5 mU/L and TSH ≥ 2.5 mU/L. However, women with TSH ≥ 2.5 mU/L had more miscarriages (26.1% of pregnancies established on ultrasound scan versus 13.0%; $p = 0.040$). Interestingly, when considering only women with a normal BMI (18.5–25 kg/m^2) in the multivariate analysis, TSH ≥ 2.5 mU/L was correlated with a more than tri-fold increase in risk of miscarriage ($p = 0.030$, OR = 3.758, 95% CI (1.133–12.468)). In this model adjusting for age ≥ 36, previous pregnancies, previous deliveries, > 1 prior miscarriage, number and quality of embryos transferred, we found other independent risk factors for miscarriage, such as maternal age ≥ 36 ($p = 0.013$, OR = 5.850, 95% CI (1454–23.541)) and having experienced more than one previous miscarriage ($p = 0.028$, OR = 15.448, 95% CI (1.336–178.663)).
Limitations, reason for caution: This study was monocentric and retrospective in design. Only a single measurement of periconceptional TSH was obtained, furthermore free T4, T3 and thyroid antibodies were not available for most cycles.
Wider implications of the findings: Our findings support the recommendation that treatment with levothyroxine should be initiated if serum TSH level are ≥ 2.5 mU/L in order to prevent miscarriages in women with normal BMI undergoing IVF/ICSI. However, prospective studies are needed to examine the effect of substitution therapy on pregnancy outcome in this group of women.
Study funding/competing interest(s): Funding by national/international organization(s) – L. Dhaenens is a holder of a PhD grant of The Agency for Innovation by Science and Technology in Flanders (IWT). The authors declare that they have no conflicts of interest.
Trial registration number: BE670201111456.
Keywords: TSH, miscarriage, pregnancy outcome, IVF/ICSI

P-137 Transgenic overexpression of anti-Müllerian hormone (AMH) causes miscarriage in adult mice

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Study question: Does high AMH expression cause miscarriage?
Summary answer: The post-pubertal overexpression of AMH in female mice causes a large reduction in fertility relative to wild-type females. There is evidence that the effect is caused by miscarriage as foetuses were observed in mid-pregnancy but transgenic dams rarely gave birth.
What is known already: Maternal AMH levels decline as pregnancy progresses which may be necessary for progression of pregnancy. AMH is known to affect processes leading to ovulation but there is also a possibility of actions in other organs as both the pituitary and uterus express AMH receptors. The roles of uterine and pituitary AMH receptors have not been identified.
Study design, size, duration: The study consisted of 12 mice; 6 wild type control mice and 6 transgenic AMH-overexpressing mice (AMH^{Tg}). The study was conducted over a period of 5 months.
Participants/materials, setting, methods: The study utilised a transgenic mouse (AMH^{Tg}) with overexpression of AMH beginning at puberty. Virgin wild-type and AMH^{Tg} female mice underwent 3 matings with wild-type males. Litter size was assessed from matings 1 and 2 and foetuses were examined on the third mating at 10 days post-coitus.
Main results and the role of chance: The majority of wild-type dams gave birth to least one litter in the first two mating periods but only a single litter was observed from five AMH^{Tg} dams. Foetuses were found to be present in AMH^{Tg} dams at 10 days post-coitus but ~40% showed signs of degeneration. Less than 5% of foetuses displayed signs of degeneration in wild-type dams. The mean number of surviving foetuses was significantly different between wild-type (10.3 ± 0.99) and AMH^{Tg} (4.3 ± 0.99) dams ($p = 0.003$).
Limitations, reason for caution: The AMH transgene was produced in the nervous system under control of the neuron-specific enolase promoter leading to high levels of AMH in the brain and blood. Ongoing studies aim to determine whether the miscarriage phenotype arises from excess AMH signalling in the brain, uterus or ovary.
Wider implications of the findings: The majority of research into AMH has focused on its ability to act as a biomarker of ovarian function and its influence on reproductive senescence. This study suggest that AMH may also affect reproductive fitness in young females during the period of peak reproductive fitness. These findings may improve the understanding of clinical conditions such as recurrent miscarriage, for which effective treatments remain elusive.
Study funding/competing interest(s): Funding by national/international organization(s) – Health Research Council of New Zealand.
Trial registration number: NA.
Keywords: anti-mullerian hormone, miscarriage

P-138 Detecting multiple gestations by MALDI ToF Mass spectral profiling of maternal urine: a non-invasive approach for predicting twins and triplets following positive pregnancy test

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Study question: Can the non-invasive analysis of early first trimester urine by MALDI ToF MS distinguish between multiple and singleton pregnancies?
Summary answer: Algorithms exploiting mass/charge variability in the spectra generated from early first trimester urine can accurately detect multiple pregnancies from singleton pregnancies.
What is known already: Detecting a twin or triplet pregnancy is usually established in the 5–6th week of pregnancy following demonstration of multiple embryonic sacs by ultrasound. The analysis of protein markers to indicate pregnancy disorders have been used for decades but proteomic analysis of the serum and urine are relatively new approaches to identifying twins. This study represents the first to correlate the spectral analysis of urine by MALDI ToF MS to identify multiple from singleton pregnancies.
Study design, size, duration: Prospective pilot study examining 117 urine samples from women testing positive for pregnancy and attending ART center in the USA. Urine from 6–10 weeks gestation was analysed by MALDI ToF MS and subsequently correlated with singleton or multiple gestations. Samples were collected and analyzed between March and December 2014.
Participants/materials, setting, methods: Urine samples were obtained from women who conceived spontaneously, after IUI, or after ART, and shipped frozen to the analytical laboratory. Once thawed, samples were subjected to matrix assisted laser desorption ionization (MALDI), time of flight (ToF) mass spectrometry (MS) either as neat urine or diluted 10–1000 in dH_2O.
Main results and the role of chance: Mass spectral data were examined in the region of 6,000–14,000 m/z following MALDI ToF MS of urine from pregnant women. Spectral data was normalized and quantitative characteristics of the profile were compared between outcomes: ongoing singleton pregnancies (N = 111) and ongoing twin or triplet pregnancies (N = 6). Algorithms exploiting the m/z variability were designed to predict outcome with > 98% accuracy. Diagnostic decisions were formulated using a decision tree made up of only three m/z based cut-off criteria.
Limitations, reason for caution: Small number of samples. Not all samples were followed to term.
Wider implications of the findings: Unlike traditional immunoassay based and ultrasound approaches, MALDI ToF MS of very early maternal urine represents an accurate, rapid, and non-invasive method of determining pregnancy outcome and success in women who are trying to conceive. Rapid and non invasive early diagnosis of multiple gestations represents a valuable new tool in the management of women undergoing ART and spontaneous pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by commercial/corporate company(ies) – Virginia Center for Reproductive Medicine, USA, and MAP Diagnostics, London, UK.
Trial registration number: NA.
Keywords: pregnancy, MALDI-ToF, mass spectrometer, multiple pregnancy

P-139 The effect of medical versus surgical treatment of spontaneous abortion on subsequent in vitro fertilization cycles

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Study question: To evaluate the effect of dilatation and curettage (D&C) and misoprostol as treatments for spontaneous abortion (SA) on in vitro fertilization (IVF) parameters in the subsequent IVF cycle.
Summary answer: D&C and misoprostol are both effective treatments for IVF patients with SA without an adverse effect on subsequent IVF treatment outcome.
What is known already: Evacuation of the uterus is usually quicker and more effective with surgical rather than medical management. Major complications are rare in each of these methods and the infection rates are similar. No significant differences were found regarding future conception rates and outcomes in the subsequent pregnancy among the general population. To the best of our knowledge, the effect of misoprostol vs. D&C on IVF treatments has not been evaluated previously.
Study design, size, duration: Retrospective, cohort study. Database from 3 academic reproductive centers on 73 women who had SA and treated with either misoprostol or D&C during IVF treatment was collected from 2010–2014. The data contained the patients' baseline demographic and hormonal characteristics. IVF cycle parameters before and after SA were evaluated.
Participants/materials, setting, methods: Women treated for SA after IVF treatment. The SA was treated with either D&C or misoprostol and the patient underwent a subsequent IVF cycle within 12 months of the SA.
Main results and the role of chance: A total of 73 women were included in the study, 32 treated with misoprostol and 41 underwent D&C for uterine evacuation. One patient in the misoprostol group underwent D&C due to incomplete evacuation. There were no significant differences in maternal age, BMI, gravity, parity, duration of infertility and basal FSH between the groups. The maximum hCG level and gestational age at diagnosis were also similar between the two groups. There were no differences in the interval to the subsequent IFV cycle according to the mode of abortion. No significant differences were found in either group regarding the IVF parameters (endometrial thickness, number of eggs retrieved and fertilized, and quality and amount of embryos transferred). The pregnancy and abortion rates were comparable between the groups.
Limitations, reason for caution: The weakness of the current study is its retrospective nature and the small number of patients. However, our results support the safety of larger, prospective studies
Wider implications of the findings: Many infertile patients wish to undergo another treatment as soon as possible after failed pregnancy. Therefore, there is a need to identify treatment for SA that does not affect the ovarian response to IVF and that allows the shortest interval before the next IVF treatment. We found no advantage of D&C vs. misoprostol in all aspects of IVF treatment. These findings allow the physician to individualize treatment according to the specific needs of each patient.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Meir medical center
Trial registration number: NA.
Keywords: spontaneous abortion, dilatation and curettage, IVF, lag period, misoprostol

P-140 A non-invasive approach for the early prediction of spontaneous abortion following positive pregnancy test by MALDI ToF Mass spectral profiling of maternal urine in early pregnancy

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Study question: Can the non-invasive analysis of early first trimester urine by MALDI ToF MS predict ongoing pregnancies in assisted reproduction cycles?
Summary answer: Algorithms exploiting mass/charge variability in the spectra generated from early first trimester urine can accurately predict pregnancy outcomes.
What is known already: Human chorionic gonadotropin has been used as a marker in pregnancy for over one hundred years. However, in the last few decades hCG glycovariation has been detected by more discriminating immunoassays and most recently subtle glycovariations have been observed using mass spectrometry on the urine of woman in early pregnancy. This study represents the first to correlate the spectral analysis of urine by MALDI ToF MS with the ongoing status of pregnancy.
Study design, size, duration: Prospective pilot study examining 121 urine samples from women testing positive for pregnancy and attending ART centre in the USA. Urine from 6–10 weeks gestation was analysed by MALDI ToF MS and subsequently correlated with ongoing singleton pregnancy outcomes. Samples were collected and analyzed between March and December 2014.
Participants/materials, setting, methods: Urine samples were obtained from women who conceived spontaneously, after IUI, or after ART, and shipped frozen to the analytical laboratory. Once thawed, samples were subjected to matrix assisted laser desorption ionization (MALDI), time of flight (ToF) mass spectrometry (MS) as neat urine or diluted 10–1000 fold in dH_2O.
Main results and the role of chance: Mass spectral data were examined in the region of 6,000–14,000 m/z following MALDI ToF MS of urine from pregnant women. Spectral data was normalized and quantitative characteristics of the profile were compared between outcomes: ongoing pregnancy (n = 111), and subsequent spontaneous abortion (n = 10). Algorithms exploiting the m/z

variability were designed to predict outcome with > 99% accuracy and only one false negative. Diagnostic decisions were formulated using a decision tree made up of only five m/z based cut-off criteria.
Limitations, reason for caution: Small number of samples. Not all samples were followed to term.
Wider implications of the findings: Unlike traditional immunoassay based and ultrasound approaches, MALDI ToF MS of very early maternal urine represents an accurate, rapid, and non-invasive method of determining pregnancy outcome and success in women who are trying to conceive. Early diagnosis of the chance of SAB potentially represents a valuable new tool in the management of women with recurrent miscarriage and infertility of unexplained origin.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by commercial/corporate company(ies) – Virginia Center for Reproductive Medicine, MAP Diagnostics.
Trial registration number: NA.
Keywords: pregnancy test, predicting SAB, urinalysis, mass spectrometry

P-141 Decoy receptor 3 ameliorates recurrent spontaneous abortion by directly counteracting local inflammation and downregulating Th17 cells

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Study question: What is the modulatory role of decoy receptor 3 (DcR3) in recurrent spontaneous abortion (RSA)?
Summary answer: The DcR3 is important in counteracting local inflammation and downregulating Th17 cells
What is known already: T cells play a central role in immune system, including immunoregulation and immunostimulation. The peripheral blood and decidua in unexplained recurrent spontaneous abortion patients revealed increased prevalence of T helper 17 (Th17) cells, suggesting an important role of The 17 cells in the pathogenesis of RSA.
Study design, size, duration: Our study is the animal study. We used hydrodynamic-based intravenous (IV) administration of DcR3 plasmid into female CBA/J mated male DBA/2 as the abortion-prone model to elucidate the physiological role of DcR3 on RSA.
Participants/materials, setting, methods: We assessed abortion rate in the abortion-prone model. DcR3 expression was analyzed and its effects on abortion rate were evaluated. Flow cytometry was performed to evaluate the expression levels of T cell subsets.
Main results and the role of chance: DcR3 significantly reduced abortion rate in pregnant female CBA/J mice on d13.5. The expression of IL-17-producing CD4 T cells was lower in the uterus in DcR3-treated mice. Our data suggest that DcR3 has potential as a suppressor of uterus inflammation in the abortion-prone model, which may be attributed to either direct inhibition of uterus inflammation or suppression of abortive Th17 cells.
Limitations, reason for caution: This study is mainly performed in the abortion-prone model. Application of DcR3 in unexplained recurrent spontaneous abortion patients needs further studies.
Wider implications of the findings: This study provides a therapeutic effect of DcR3 in the abortion-prone model, suggesting its potential for treating women RSA.
Study funding/competing interest(s): Funding by hospital/clinic(s) – TSGH-C101-163; CMNDMC10304.
Trial registration number: All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) in Taiwan.
Keywords: recurrent spontaneous abortion (RSA), Decoy receptor 3 (DcR3), Th17 cells, Abortion-prone model

P-142 Soluble HLA-G as a non-invasive biomarker from ovulation to early pregnancy in assisted reproduction

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Study question: Human leukocyte antigen (HLA)-G role as biomarker of ovulation induction protocol response and pregnancy course.
Summary answer: sHLA-G plasma levels showed a possible use as a biomarker for women with a higher ovulation induction protocol response and favorable pregnancy course.
What is known already: The artificial induction of ovulation and hyperovulation greatly increased the efficiency of oocyte retrieval in assisted reproduction, but specific follow-up biomarkers are still unknown. HLA-G molecules are expressed by cytotrophoblast cells as membrane bound and soluble isoforms (mHLA-G, sHLA-G), with immune-inhibitory functions as ligand of immune-inhibitory receptors (ILT2, ILT4, KIR2DL4). They were detected in the plasma of pregnant women with increased levels during the first trimester and associated to the clinical outcome.
Study design, size, duration: Eighteen women undergoing in vitro embryo transfer were divided into pregnant (6) and non-pregnant (12), based on β-hCG > 5 mIU/ml and viable intrauterine pregnancy. Plasma samples were taken before ovulation induction, 2 days before and at oocyte collection, at oocyte embryo transfer, after 7 days from transfer and at pregnancy test.
Participants/materials, setting, methods: Plasma samples were analyzed for sHLA-G levels with HLA-G specific enzyme immunosorbent assay with MEM-G9 as capture antibody and anti-beta2microglobulin as detection antibody. The data obtained were compared by T student paired test, Mann Whitney U test and Sperman correlation test (StatView statistical software).
Main results and the role of chance: In pregnant women, there were significant initial higher plasma sHLA-G levels in comparison with non-pregnant women ($p = 0.042$) and a significant increase from the day of oocyte retrieval to that of embryo transfer ($p = 0.04$). These differences were mainly associated with HLA-G5 isoform. In addition, in the viable pregnancy group, plasma sHLA-G was higher at transfer in comparison with non-pregnant women ($p = 0.03$). A trend to correlation was observed between plasma sHLA-G and serum β-hCG (human chorionic gonadotropin) in pregnant women ($r = 0.46$, $p = 0.048$).
Limitations, reason for caution: This study is based on a limited number of subjects. It will be necessary to confirm these data on a larger cohort of subjects.
Wider implications of the findings: These observations are in agreement with the important role of HLA-G molecules during pregnancy. However, this is the first study demonstrating that for a successful pregnancy, a high initial plasma sHLA-G level and a significantly increase from the day of oocyte retrieval to that of embryo transfer are required. We suggest a role as biomarker of ovulation induction protocol response and pregnancy course.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Procrea SA.
Trial registration number: NA.
Keywords: HLA-G, assisted reproduction, pregnancy

P-143 Perinatal results after IVF/ICSI. A prospective study

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Study question: Increased risk for obstetric and neonatal complications after IVF/ICSI is due to techniques or due to multiple pregnancy and/or advanced maternal age?
Summary answer: Compared to naturally conceived pregnancies, a higher rate of unfavorable obstetrics and neonatal complications after IVF/ICSI was detected in a sample of women with singletons pregnancies, age-matched, and attending to the same tertiary care center
What is known already: After IVF/ICSI techniques an increased risk for a number of obstetric and neonatal complications compared to naturally conceived pregnancies, has been reported. A potential negative impact of micromanipulation techniques, extended culture systems, and medications used in the context of IVF/ICSI has focus on research in this field, but other problems inherent in the infertile couple, pregnancy or delivery management, high rate of multiple pregnancies and advanced maternal age could also explain these worse results.

Study design, size, duration: Prospective, observational, study. Two hundred eighty couples studied between 2012 January and 2014 January.
Participants/materials, setting, methods: One hundred forty couples with single pregnancy conceived via IVF/ICSI were referred to the Department of Obstetrics and Gynecology of Cabueñes Hospital, Gijon, Spain in the early gestational weeks and other 140 couples with single pregnancy naturally conceived age-matched and attending to the same Department were included in the study.
Main results and the role of chance: Maternal parameters as age; BMI; gravity; pregnancy complications such as pregnancy-induced hypertension, preeclampsia, premature rupture of membranes, cervical insufficiency, and premature uterine contractions; and number of hospitalizations; and fetal parameters as gestational age; birth weight; pH of the umbilical artery; APGAR after one minute; APGAR after 1 and 5 minutes; congenital malformation; admission of the newborn to a neonatal intensive care unit; and death of the infant were evaluated. There were no statistical differences between both groups in maternal basal parameters. Women with IVF/ICSI showed statistically significantly more frequent pregnancy complications, such as gestational diabetes ($p < 0.001$), preeclampsia ($p = 0.007$), IGR ($p < 0.001$), premature rupture of membranes and premature birth ($p < 0.001$), cesarean section ($p = 0.028$) and fetal malformations ($p = 0.03$).
Limitations, reason for caution: Increased risk for birth defects, compared to naturally conceived pregnancies (OR: 2.8) must be taken carefully due to the short sample size. Other possible study limitation is that IVF/ICSI procedures were performed in different centers with different controlled ovarian stimulation protocols.
Wider implications of the findings: Increased risk for obstetric and neonatal complications after IVF/ICSI is not related to multiple pregnancy or advanced maternal age.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CABUEÑES HOSPITAL.
Trial registration number: NA.
Keywords: FIV-ICSI, perinatal results

P-144 The effect of Tokishakuyaku-san on the expression of transcription factors T-bet/GATA-3 in human decidual NK cells in early pregnancy

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Study question: To study if the traditional herbal medicine Tokishakuyaku-san has any effect on the expression of transcription factors T-bet/GATA-3 of human decidual NK cells in early pregnancy.
Summary answer: The in vitro experiments found that Tokishakuyaku-san down-regulated the expression of T-bet mRNA and up-regulated the expression of GATA -3 mRNA of human decidual NK cells in early pregnancy.
What is known already: Tokishakuyaku-san has been used empirically in the treatment of immunity-related recurrent miscarriage, but the mechanisms underlying its effectiveness is still largely unknown. Our previous study suggested that Tokishakuyaku-san influenced the expression of transcription factors T-bet/GATA-3 of human peripheral blood mononuclear cells and might play a role in shifting the Th1/Th2 balance toward Th2 polarization. In this study, we examined whether Tokishakuyaku-san modulates the expression of T-bet/GATA-3 in decidual NK cells from early pregnancy.
Study design, size, duration: We enrolled 12 healthy women aged under 35 years who were in 8–9 weeks of pregnancy and chose to terminate the unwanted pregnancy using surgical abortion in our University hospital. The study was approved by the Hospital Ethical Committee, and informed consent was obtained from each patient.
Participants/materials, setting, methods: The decidual NK cells were isolated from decidual tissues obtained from the women included and cultured with various concentrations of Tokishakuyaku-san for 24 h. The cell proliferation was evaluated by CCK-8 assay. The mRNA levels of T-bet and GATA-3 in decidual NK cells were detected by real-time PCR.
Main results and the role of chance: Cell proliferation of decidual NK cells was not changed by treatment with 10 or 100 μg/mL of Tokishakuyaku-san but was significantly inhibited with incubation of Tokishakuyaku-san at the concentration of 1000 μg/mL ($P < 0.05$). The amount of T-bet mRNA was significantly decreased while the GATA-3 mRNA increased after treatment with 10 mg/mL and 100 mg/mL of Tokishakuyaku-san ($P < 0.05$ vs the controls).
Limitations, reason for caution: The limitation is that we investigated the effect of Tokishakuyaku-san on transcription factors T-bet/GATA-3 expression in decidual NK cells only in vitro.
Wider implications of the findings: Considering the ratio of expression of T-bet and GATA-3 reflected changes in the Th1 and Th2-specific cytokines, the down-regulatory effect of Tokishakuyaku-san on T-bet mRNA and stimulatory effect on GATA-3 of decidual NK cells may suggest that Tokishakuyaku-san play a role in shifting the balance of Th1/Th2 toward Th2 polarization at feto-maternal interface, which might have therapeutic potential in alloimmune related miscarriage where Th1 response was pathologically enhanced.
Study funding/competing interest(s): Funding by national/international organization(s) – The National Natural Science Foundation of China (81330018), and Grants of Science & technology department of Sichuan Province (2014KJT062-2014SZ).
Trial registration number: NA.
Keywords: Tokishakuyaku-san, T-bet, GATA-3, decidual NK cell

P-145 Marked improvement in the success rate of medical management of early pregnancy failure (EPF) in clinical practice after dose optimization and clinician education

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Study question: Can the success rate of medical management of EPF with Mifepristone and Misoprostol in clinical practice be optimized by formally educating clinicians about the treatment and outcome measures and by adopting a protocol of 200 mg Mifepristone orally followed by a single dose of 800 mcg Misoprostol vaginally after 48 h?
Summary answer: We observed a marked increase in the success rate of treatment (85%), closely approaching that reported under study settings. For these results, we credit the implementation of a novel institutional protocol for medical management of EPF and the formal education of clinical practitioners about the standardized procedure and expected outcomes.
What is known already: EPF is a very common pregnancy complication. Recent studies have supported medical management as a valuable non-invasive alternative to D&C/D&E due to its high success rate, low complication rate, and patient acceptability. However, the variability in medications, dosages, routes of administration and definitions of successful treatment makes it difficult to interpret the published data. Success in clinical practice and in publications from non-research settings is often far below that quoted in the literature.
Study design, size, duration: We retrospectively identified 76 patients with EPF (missed abortion or anembryonic gestation) who were treated according to the adopted protocol over a one year period. Exclusion criteria were complete or incomplete abortion, multiple gestation, gestational age over 13 weeks by ultrasound, and contraindications for Mifepristone or Misoprostol.
Participants/materials, setting, methods: We abstracted data on sonographic results, bleeding, gynecologic, obstetric, and medical history. Unique to our study is the ability to collect data on time to passage of tissue from medication administration, amount of bleeding, and need for analgesics since patients were admitted to an inpatient day-unit for administration of misoprostol.
Main results and the role of chance: We observed a significant increase in the overall success rate of medical management following modification of the institutional protocol and formal clinician education from about 60% to 85% ($p < 0.001$). Clinicians were instructed to diagnose failed treatment by the continued presence of a gestational sac, endometrium thickness > 30 mm, and/or clinical symptoms of persistent heavy bleeding. Also a standardized time to follow up and re-evaluation was defined. Neither gestational age (<9 weeks versus ≥9 weeks) nor diagnosis (missed abortion vs. anembryonic gestation) were predictors of successful treatment. Of successfully treated patients, almost all (96%) passed the pregnancy within 8 hours of receiving vaginal misprostol. Patients reported acceptable pain and bleeding. No serious side effects, such as need for antibiotics or blood transfusion, occurred.
Limitations, reason for caution: The study is limited by its retrospective design. We evaluated data collected in a real life clinical setting influenced by

patients' wishes, treating physicians' decisions and preferences. However, this can also be seen as a strength, reporting results for daily clinical practice, independent of regulated study protocols.

Wider implications of the findings: Our results show that following the revision of the protocol and formal physician education, the subsequent 85% success rate was comparable to that reported in interventional studies. Our results support the use of medical management as a valuable non-invasive alternative to surgery in routine clinical practice. Our findings underscore the importance of formally educating caregivers regarding the expected findings, patterns of bleeding and pain to optimize treatment and to likewise improve patient understanding and expectations.

Study funding/competing interest(s): Funding by University(ies) – Medical University of Innsbruck.

Trial registration number: NA.

Keywords: misoprostol, mifepristone, early pregnancy failure, missed abortion, medical management

P-146 Quality of life for curettage versus expectant management in women with incomplete evacuation of the uterus after treatment with misoprostol for miscarriage: the misorest trial

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Study question: Does, in women with incomplete evacuation of the uterus after misoprostol treatment for first trimester miscarriage, curettage affects quality of life, compared to expectant management?

Summary answer: In women with an incomplete evacuation of the uterus, curettage and expectant management result in comparable quality of life.

What is known already: Misoprostol treatment for first trimester miscarriage is non-invasive and cost-effective. However, incomplete evacuation of the uterus occurs in about 30% of women treated with misoprostol. Additional curettage is often performed while expectant management is probably equally effective. In order to inform women with an incomplete evacuation of the uterus, we compared quality of life after curettage and expectant management.

Study design, size, duration: Between June 2012 and June 2014, we conducted a multi-center randomized controlled trial (RCT) with a prospective cohort study alongside it. The study was positioned in the consortium form women's health research, and in this study 27 Dutch hospitals participated.

Participants/materials, setting, methods: Women were randomized within 24 hours after sonographic identification of incomplete evacuation of the uterus. Women who refused randomization were asked for follow-up in the cohort. Curettage was performed within three days. Patients answered quality of life related questionnaires HADS, SF-36, RI-10 and EQ5-D at baseline 2, 4 and 12 weeks.

Main results and the role of chance: We randomized 59 women (curettage $n = 30$, expectant management $n = 29$), while 200 women were followed in a prospective cohort study according to the treatment they preferred (curettage $n = 70$, expectant management $n = 130$). Response rate to the questionnaires was 70% in the RCT and 80% in the cohort. The SF-36 showed that for both mental and physical health, women managed expectantly did slightly better but differences were not statistically significant. Similarly, anxiety and depression were slightly more present immediately after treatment in the curettage group (36% vs 30%) to improve to 21% vs 17% after 12 weeks. Both groups improved over time on all dimensions and differences reduced. EQ5D at 12 weeks showed similar scores in both groups. None of the differences were at any moment statistically significant.

Limitations, reason for caution: Randomization was difficult due to preference of treatment. Response-rate to the quality of life related questionnaires at baseline, 2, 4 and 12 weeks was around 75%. Missing data were equally distributed in different treatment groups. In the final analysis, data will be imputed.

Wider implications of the findings: In women with incomplete evacuation of the uterus, expectant management and curettage leads to similar quality of life. It is important to take this into account when counseling women for treatment of a first trimester miscarriage. This might lead to a further implementation of misoprostol treatment for first trimester miscarriages.

Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by ZonMw, a Dutch organization for Health Research and Development. Project number 80-82310-97-12066.

Trial registration number: Dutch Trial Register NTR3310, http://www.trialregister.nl/

Keywords: Miscarriage, misoprostol, incomplete evacuation, expectant management, quality of life

P-147 Time to conception (TTC), cumulative conception rate (CCR), and time to live birth (TTLB) in 189 women with recurrent early pregnancy loss (REPL) in an academic RPL program

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Study question: What are the TTC, CCR, and TTLB in women with REPL, defined as 2 or more pregnancy losses of less than 10 weeks size?

Summary answer: Mean TTC was 2.3 ± 2.0 menstrual cycles. Mean CCRs were 84% and 96% at 3 and 6 months. Mean TTLB was 46.1 ± 10.4 weeks. Women with REPL appear to be fertile or perhaps 'superfertile', conceiving easily without assisted reproductive technology (ART) and having a high likelihood of a successful pregnancy outcome.

What is known already: In fertile couples, Gnoth (2003) reported a CCR of 88% at 6 months. In recurrent miscarriage, Kaandorp (2014) reported a CCR of 56% at 6 months; median TTC and TTLB was 21 and 102 weeks. In RPL patients with structural chromosomal rearrangements, Desjardins (2012) reported TTC of 1.7 cycles. In women with pregnancy loss, Sapra (2014) reported TTC of 0–3 cycles in 69% and 4–6 cycles in 25%.

Study design, size, duration: This observational cohort study is a secondary analysis of an existing dataset (Boots et al., 2014). All patients were seen in consultation by a single provider (MDS) in an academic RPL Program between July 2004 and June 2012. IRB approval and written consent by all patients were obtained.

Participants/materials, setting, methods: ·Inclusion: REPL patients with subsequent spontaneous pregnancy(ies) following timed intercourse to luteinizing hormone (LH) surge. Pregnancy defined as positive serum human chorionic gonadotropin (hCG) 1–2 days after missed menses. Monitoring: ultrasound q1–2 weeks from 6–10 weeks. TTLB: cumulative TTC x 4 weeks + gestational weeks.

Main results and the role of chance: Cohort: 189 patients with 788 pregnancies prior to RPL consultation consisting of 12% ($n = 94$) livebirths, 41% ($n = 323$) embryonic miscarriages, 36% ($n = 285$) pregnancy losses < 6 weeks, and 11% ($n = 86$) other. Following RPL consultation: The mean maternal age was 35.5 ± 4.0 years at the end of each subsequent pregnancy. There were 263 subsequent spontaneously conceived pregnancies, resulting in 48% ($n = 125$) livebirths, 26% ($n = 68$) embryonic miscarriages, 25% ($n = 66$) pregnancy losses < 6 weeks, and 1% ($n = 4$) other. Mean TTC was 2.3 ± 2 cycles. CCRs were 49%, 84%, and 96% at 1, 3, and 6 months, respectively. Mean TTLB was 46.1 ± 10.4 weeks. 41 patients had prior pregnancies conceived through ART. This subgroup had 52 subsequent spontaneously conceived pregnancies with 37% ($n = 19$) livebirths. Mean TTC was 2.3 ± 1.8 cycles. Mean TTLB was 50.7 ± 17.1 weeks.

Limitations, reason for caution: Whether these encouraging pregnancy outcomes can be replicated outside of an academic, protocol-driven RPL Program needs further study. Direct comparisons of TTC, CCR, and TTLB in spontaneously and ART-conceived pregnancies require identical definitions of 'pregnancy' and 'pregnancy loss', consistently including or excluding pregnancy loss < 6 weeks.
Wider implications of the findings: The diagnosis of recurrent pregnancy loss causes significant distress as couples plan subsequent pregnancies. Based on this study, counseling should include reassurance that the cumulative conception rate is comparable to fertile couples, therefore, assisted reproductive technology is not indicated, unless there is concomitant infertility. In addition, subsequent live births have been shown to be obtainable in a short period of time, following a recurrent pregnancy loss consultation and close monitoring until 10 weeks of gestation.
Study funding/competing interest(s): Funding by University(ies) – University of Illinois at Chicago - no direct financial support.
Trial registration number: NA.
Keywords: recurrent pregnancy loss, time to conception, time to live birth, cumulative conception rate

P-148 Our experience in ovarian ectopic pregnancy: ultrasound, clinical and therapeutical correlation

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Study question: The aim of this study was to evaluate the accuracy of transvaginal ultrasound (TVS) and color Doppler (CD) examination in the diagnosis of Ovarian pregnancy (OP), its surgical treatment and clinical correlation
Summary answer: OP should be suspected in every ectopic pregnancy(EP) if: significant hemoperitoneum and abdominal pain are seen in very early pregnancy. Diagnostic can be reached by combining clinical symptoms, BhCG titters, ultrasound results and confirmed by laparoscopy and/or histology. Early diagnosis allows conservative ovarian treatment, preserving fertility in these women.
What is known already: OP has a reported incidence of 1/6000 to 1/40000 pregnancies. Patients still experience circulatory collapse, with a maternal death rate of 4–10%. Diagnosis has been based on Spielberg's criteria established over 100 years ago: The tubes of the affected side must be intact, the gestational sac must occupy the ovary, the ovary must be joined with the uterus by the utero-ovarian ligament and histology must show ovarian tissue in the chorionic villi.
Study design, size, duration: Descriptive, clinical study, since 1999 to 2014
Participants/materials, setting, methods: TVS were performed in 21534 first trimester pregnancies. 7 OP were detected and prospectively evaluated. Ovarian Ring (OR), presence of free fluid were considered. CD evaluated vascularization in OR and was correlated with B-hCG titters. Amenorrhea, type of pain, vaginal bleeding, surgical treatment, pathologist and follow up were registered.
Main results and the role of chance: The incidence of OP was 0.32‰. Diagnosis was made from 4.5 weeks of amenorrhea. OP was associated to intrauterine device in three cases, to IVF in two, to intrauterine insemination in one, and to benign teratoma in the other. TVS revealed absence of intrauterine sac, presence of OR in ovary. Hemoperitoneum was significant in 6 cases (85.71%) and scarce in 1 (4.28%). The diameters of the OR were between 10 and 33 mm. CD showed in 6 cases trophoblastic flow in OR. B-hCG titters ranged between 800 and 3200 mU/ml. Six patients (85.71%) presented severe abdominal pain and in one was middle (4.28%). Laparoscopy confirmed diagnosis and OR resection was performed. Pathologist reported ovarian tissue in 6 cases. Follow up showed normal evolution.
Limitations, reason for caution: Transvaginal sonography is of diagnostic value in differentiating an ovarian pregnancy from a tubal ectopic pregnancy, but still it is operator dependent and requires considerable experience in this rare condition of ectopic pregnancy.
Wider implications of the findings: The last Spielberg's criteria is not always possible due to conservative treatment of the ovary. Although the histology did not meet the exact definition of OP in all cases, laparoscopic findings and clinical follow-up could not be explained by any other diagnosis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IFER.
Trial registration number: NA.
Keywords: Ultrasound and color Doppler, Ectopic pregnancy, Reproductive Surgery

P-149 Comprehensive chromosome screening (CCS) improves IVF outcome in recipients suffering from repeated implantation failure (RIF) and recurrent pregnancy loss (RPL)

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Study question: Is CCS useful to increase egg donation results in RPL and RIF patients?
Summary answer: CCS applied as a therapeutic tool could improve implantation and live birth rates of in-vitro fertilization treatments mainly in RPL and RIF patients.
What is known already: Embryo chromosomal aneuploidy is the most common cause of unsuccessful pregnancy after IVF in RPL and RIF patients. The development of CCS has offered valuable insight into the chromosomal status of human gametes and preimplantation embryos. Applied as a therapeutic tool, it could improve implantation and live birth rates of in-vitro fertilization treatments and provide a means of attenuating pregnancy loss in recurrent pregnancy loss patients and increase implantation rate in recurrent implantation failure patients.
Study design, size, duration: A retrospective study was performed. We included the array-CGH results of 312 embryos (211 RIF and RPL and 101 controls) from 60 RIF and RPL patients and 31 controls performing CCS cycles in 2013 and 2014 at Instituto Bernabeu. Alicante. Spain.
Participants/materials, setting, methods: After discarding known causes, we define RIF if a total of four cleaved good quality embryos transferred failed to implant. RPL was two or more miscarriages. ArrayCGH analysis of the biopsied trophoecthoderm on day5, was performed using Agilent-SurePrintG3-8x60K. The main outcome measures were biochemical pregnancy, implantation rate and ongoing pregnancy.
Main results and the role of chance: Results from D5-CCS were obtained in the 99.5% of the biopsied embryos (310/312). To summarize, the average of biopsed embryo per egg donation cycle was 3.4. The positive beta-HCG was 58.5% and the implantation rate was 43.5%. The miscarriage rate was 10%. The aneuploidy rate in Day 5 embryos from recipients was 30.1% and the majority (70%) showed aneuploidy only in one chromosome. An increase in the implantation rate in RIF and RPL patients to be equal to control patients was reported (41.3% vs 47.1%, $p = 0.578$). Previous results in egg donation RIF and RPL recipients in 50 cycles performed before without CCS showed an implantation rate of 25% ($p < 0.05$).
Limitations, reason for caution: The use of CCS to increase the IVF results mainly in patients suffering from RPL and RIF is a promising tool. The present work shows that euploid embryo selection helps us to improve the results in these patients. Nevertheless, prospective studies are needed to conclude it.
Wider implications of the findings: This investigation reveals a high aneuploidy rate in a population of recipients suffering from RIF and RPL. Application of CCS in these patients is a promising tool. CCS in embryos from an egg donor program could be beneficial as the euploid selection and allowing single-embryo-transfer without decreasing the baby-take-home rate.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Conflicts of interest none declared. This work has been supported by a grant from Rafael Bernabeu Foundation.
Trial registration number: NA.
Keywords: CCS, RIF, RPL

P-150 First genetically confirmed monozygotic dichorionic diamniotic livebirth from day 5 blastocyst single embryo transfer – a new model for twinning and a 10yr retrospective analysis

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Study question: Monozygotic dichorionic (MZ-DC) twinning has historically been believed to occur with splitting of the embryo within 72 h of fertilisation. We have, however, evidenced that it can occur later in development and undertook an investigation of the incidence and possible mechanism.
Summary answer: Monozygotic dichorionic (MZ-DC) twinning is not uncommon after single blastocyst transfer. We theorise the mechanism may involve splitting of the embryo as the inner cell mass hatches, with potential involvement of altered zonal lysis and apoptosis.
What is known already: Approximately 1 in 3 monozygotic twin pregnancies present as dichorionic and diamniotic (DCDA). This has generally believed to have been a result of splitting in the 72 h following fertilisation. However, a literature review of twinning studies in IVF reveals occurrences of MZ-DC twins post single embryo transfer on day 5 without specific investigation into the phenomenon.
Study design, size, duration: We present the first case where monozygosity of a DCDA pregnancy has been confirmed by genetic testing rather than presumed by virtue of single embryo transfer (SET). A retrospective analysis of monozygotic pregnancies from 4780 single embryo transfers 2004–2014 and a literature review was performed.
Participants/materials, setting, methods: A couple underwent PGD treatment with embryo biopsy on day 3 and transfer of a single hatching early blastocyst on day 5. A pregnancy scan at 7 + 6 weeks confirmed DCDA twins and genetic analysis confirmed monozygosity. Clinic data was checked for previous occurrences, as was scientific literature.
Main results and the role of chance: This case report supports a late mechanism for MZ-DC twinning through subdivision of the blastocyst. The retrospective analysis revealed 26 sets of MZ twins, 4 of which were MZ-DC and gender concordant. However zygosity is not genetically confirmed and concurrent natural conception cannot be completely excluded. There are 2 earlier case reports which visualised subdivision of the blastocyst prior to embryo transfer and resulted in MZ-DC pregnancies but zygosity was not genetically confirmed in these cases either. In our case study there were no obvious morphological indications that the blastocyst would subdivide.
Limitations, reason for caution: Only the case study presented has had zygosity genetically confirmed. Discovering the true incidence of MZ-DC twinning is complicated by the number and stage of embryos transferred and the need for genetic confirmation of monozygosity.
Wider implications of the findings: The historical model for twinning is incorrect in supposing that MZ-DC twinning only occurs within 72 h of fertilisation. With increasing moves to single embryo transfer at the blastocyst stage it is incumbent upon us to develop a deeper understanding of the mechanisms behind monozygotic twinning.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CARE Fertility Nottingham.
Trial registration number: NA.
Keywords: monozygotic, dichorionic, twinning

P-151 Accuracy of serum biochemical markers in predicting outcome for women with threatened miscarriage – a systematic review and meta-analysis

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Study question: Can serum biochemical markers be used in clinical practice to predict outcome in women with threatened miscarriage?
Summary answer: Various biochemical markers including serum progesterone, beta HCG, PAPP-A, CA 125 and oestradiol have been used to predict outcome of threatened miscarriages. This systematic review and meta-analysis has shown that serum CA 125 and oestradiol have the highest sensitivity and specificity for predicting miscarriage in women with threatened miscarriage
What is known already: Miscarriage is the most common complication in early pregnancy. About 20% of pregnant women present with threatened miscarriage of which about half miscarry. Many biochemical markers have been widely investigated for predicting miscarriage, however, they are not being used in routine practice due to lack of evidence regarding their accuracy.
Study design, size, duration: We conducted a systematic review and meta-analysis of the literature to predict miscarriage in women with threatened miscarriage. Literature search was done using Medline, Embase and the Cochrane Library for relevant citations. Overall, 15 studies were eligible for the systematic review with a sample size of 1147 women.
Participants/materials, setting, methods: Prospective observational studies for women with threatened miscarriage with fetal heart beat visible on ultrasound scan were included in the review. Exclusion criteria were studies including asymptomatic pregnant women, those with pregnancy of unknown location and women with history of infertility, recurrent miscarriage and multiple pregnancies.
Main results and the role of chance: The summary chart of QUADAS (Quality assessment for diagnostic accuracy studies) showed risk of bias in 80% of studies for index test, 35% of studies for reference standard and flow and timing of the studies. Also there were concerns for applicability in 65% of studies for patient selection, 10% of studies for index test and 15 % of studies for reference standard. Amongst the various biochemical markers (serum progesterone, beta HCG, PAPP-A, CA 125 and serum oestradiol), serum CA125 (n = 6 studies) showed the highest sensitivity (CA 125 = 0.92, 95% CI = 0.71, 0.98) and specificity (CA125 = 0.89, 95% CI = 0.75, 0.95) for predicting miscarriage in women with threatened miscarriage.
Limitations, reason for caution: A major drawback is that the threshold level for biochemical marker used has not been specified in majority of the studies, which restricts the applicability. Since the number of studies in the meta-analysis is small, the results need to be interpreted with caution in the absence of high quality studies.
Wider implications of the findings: The biochemical markers of serum progesterone, beta HCG, PAPP-A, CA 125 and oestradiol can be used alone or in combination to predict miscarriage in women with threatened miscarriage. Our study suggests that serum Ca125 should be explored further as a marker to predict miscarriage in a larger cohort of women.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University Hospitals of Leicester.
Trial registration number: NA.
Keywords: threatened miscarriage, biochemical markers

P-152 How low is too low? – Cycle day 28 estradiol levels and pregnancy outcomes

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Study question: How can a low estradiol level at the time of a first positive pregnancy test after IVF be utilized to predict pregnancy outcomes?
Summary answer: In IVF cycles with a positive hCG, low estradiol levels on cycle day 28 are associated with increased rates of ectopic and biochemical pregnancies and decreased live birth rates. Among pregnancies with low estradiol levels, an hCG <50 mIU/mL is predictive of poorer outcome.
What is known already: The use of luteal estradiol levels to predict pregnancy has been well-studied. Significant differences in estradiol levels 6 days post-LH surge have been found between conception and non-conception cycles. Late luteal phase increases in estradiol have been associated with increased implantation and pregnancy rates, whereas declines are associated with poor cycle outcomes. However, data regarding the value of estradiol level, particularly a low level, in the context of a positive pregnancy test is limited.
Study design, size, duration: This was a retrospective cohort study. 5471 IVF pregnancies achieved at our center between January 2007 and December 2012 were analyzed.
Participants/materials, setting, methods: Cycles resulting in a day 28 hCG >5 mIU/mL were included. Cycles utilizing estradiol for luteal support were excluded. Primary outcome was live birth rate. Secondary outcomes included biochemical, ectopic, and miscarriage rates. Groups were sub-stratified by day 28 hCG. Student's t-test and chi-squared test were used.
Main results and the role of chance: Cycles were stratified by day 28 estradiol level. 1: <50 pg/mL, 2: 51–100 pg/mL, 3: >100 pg/mL. The groups were similar in terms of age, BMI, percentage of blastocyst transfers, and number of embryos transferred. Comparing groups 1 and 3, results were as follows: biochemical 66.5 vs. 7.31% (OR 25.2, 95% CI 20.9-30.4; $p < 0.01$), ectopic 6.2 vs. 0.66% (OR 9.92, 95% CI 6.17-15.9; $p < 0.01$), live birth 15.4 vs. 77.4% (OR 0.05, 95% CI 0.04-0.07; $p < 0.01$). Similar results were observed when

comparing groups 1 and 2 and groups 2 and 3. After sub-stratification of group 1 by hCG level, the odds of biochemical pregnancy was 12.7 times greater when hCG was <50 mIU/mL (95% CI 6.5–24.9; $p < 0.01$) and the odds of a life birth was 0.067 times less (95% CI 0.03–0.1; $p < 0.01$).

Limitations, reason for caution: This was a retrospective study. We did not control for IVF stimulation protocols or peak estrogen levels, although significant differences in peak estradiol levels were only noted between groups 1 and 3 (1633 vs. 1724, $p = 0.02$).

Wider implications of the findings: Low estradiol levels early in IVF pregnancy are associated with poorer pregnancy outcomes. Estradiol can be used alone or in conjunction with hCG levels to predict the odds of a live birth. Clinicians can utilize this data to counsel patients and help them manage expectations about the likelihood of a successful pregnancy.

Study funding/competing interest(s): Funding by University(ies) – Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine – Weill Cornell Medical College.

Trial registration number: NA.

Keywords: IVF, pregnancy outcomes, Day 28 Estradiol

P-153 Embryo selection impact on early pregnancy loss

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Study question: Objective of the study was to compare early pregnancy loss rate between conservative embryo cultivation/selection and time-lapse system based morphokinetic monitoring system groups.

Summary answer: Embryo selection using time-lapse morphokinetic analysis could decrease early pregnancy loss rate and improve success of infertility treatment.

What is known already: One of the noninvasive embryo selection methods is time-lapse based morphokinetic evaluation of the embryonic development. Time-lapse morphokinetic assessment of embryonic development is safe and reliable method improving reproductive outcome and clinical pregnancy rate on more than 10% using Meseguer algorithm of embryo classification. Time-lapse morphokinetic assessment is based on embryo classification according to their implantation rate excluding morphologically normal embryos with discordant cleavage timing.

Study design, size, duration: Embryo development was retrospectively analysed between two study group. In the study 327 patients undergoing infertility treatment were recruited receiving autologous or oocyte donation cycles (poor responders, genetic disorders) between 2013 and 2014. Time-lapse based morphokinetic monitoring system was provided for patient with experience of negative previous treatment outcome.

Participants/materials, setting, methods: Study group included 130 embryo transfers selected using time-lapse morphokinetics analysis. Control group included 388 embryo transfers cultivated/selected conventually using traditional static observations method. Prior to treatment cycle all patients were evaluated and treated for uterine cavity pathology (except uterine septae) by two demential ultrasound or/and hysteroscopy, endocrinopathies, thrombophilias.

Main results and the role of chance: We have found no statistically significant difference in age, endometriosis, polycystic ovary syndrome, poor responders status, previous ART success, oocytes retrieved, oocyte quality, day 5 single embryo transfer (not more than 2 embryos per transfer), biochemical pregnancy rate (50.4% study group vs 51.4% control group) clinical pregnancy rate (49% study group vs 45% control group) or previous ART treatment experience. Embryos cultivated using time lapse morphokinetic analysis were achieved by oocyte fertilisation by olygospermic partner sperm ($p = 0.04$) and PICSI ($p = 0.01$) more often (criterion of time-lapse morphokinetic analysis choice). 27.5% of control group pregnancies were lost before 12 weeks of pregnancy, but time-lapse morphokinetic analysis group early pregnancy loss rate was decreased – 6.1% ($p = 0.01$).

Limitations, reason for caution: The study is a one centre study and is limited due to small patient groups. Patients are not randomly selected due to clinical environment.

Wider implications of the findings: Further multi centre randomised clinical trials are needed to highlight the impact of time-lapse morphokinetic embryo selection on early pregnancy loss in different patients groups undergoing infertility treatment.

Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Riga.

Trial registration number: NA.

Keywords: miscarriage, morphokinetics analysis, time lapse

P-154 4G/5G polymorphism of PAI-1 as risk factor fro recurrent miscarriage in Greek population

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Study question: The aim of this study was to investigate whether the 4G/5G polymorphism of plasminogen activator inhibitor-1 (PAI-1) is associated with increased risk for recurrent miscarriage in Greek population.

Summary answer: The 4G/4G genotype has been found to be associated with higher rate of recurrent miscarriage.

What is known already: PAI-1 is principal inhibitor of tissue plasminogen activator (t-PA) and major regulator of fibrinolysis. PAI-1 seems to play important role in the initial steps of embryo implantation. 4G variant has been shown to be associated with increased risk of venous thrombosis. Several other reports have linked the 4G allele with complications in pregnancy, although these associations remain controversial. Recent meta-analysis found significant association between 4G/5G polymorphism of PAI-1 and the risk of recurrent miscarriage.

Study design, size, duration: Prospective case – control study of the prevalence of 4G/5G polymorphism of PAI-1 was performed from April 2007 till January 2014. We evaluated 197 patients with recurrent miscarriage and 92 healthy parous women with at least two live births and no history of miscarriages or terminations as control.

Participants/materials, setting, methods: Peripheral blood was collected from 197 patients with recurrent miscarriage defined as above two consecutive pregnancy losses and 92 parous women who attended for the regular smear test the gynaecology outpatient department. Following DNA extraction, real-time PCR was performed for the detection of the polymorphism 4G/5G of PAI-1.

Main results and the role of chance: Both groups had the same demographic characteristics apart from the age, number of miscarriages and parity. The 4G/4G genotype is statistically more frequent in women with recurrent miscarriage compared to control (41.6 vs 20.7%, OR: 2.06, 95% CI: 1.32-3.21, p-value <0.001). Statistical significance was also found for the presence of 4G allele in women with recurrent miscarriages (60.1 vs 39.1%, p-value <0.001). However, the frequency distribution of 4G/5G genotype is similar in both groups (37.1 vs 37%). When we only include patients with history of more than three consecutive recurrent miscarriages the statistical significance for the 4G/4G genotype is maintained (37.6 vs 20.7%, OR: 1.55, 95% CI: 1.06-2.29, p-value = 0.013).

Limitations, reason for caution: In the group of recurrent miscarriages we included women with history of two or more miscarriages, although, in the subgroup analysis where patients with three or more consecutive miscarriages were included, the statistical significance was maintained. We also did not measure the PAI-1 levels in view of performing genotype-phenotype correlations.

Wider implications of the findings: The available data on the association of 4G/5G polymorphism of PAI-1 with increased risk of recurrent miscarriages are still controversial with conflicting findings in the different ethnic populations. This is the first study to associate the 4G/5G polymorphism of PAI-1 with recurrent miscarriages in the Greek population. Our results demonstrate a strong correlation between recurrent miscarriages and 4G/4G genotype. We propose the 4G/5G polymorphism of PAI-1 as important marker of recurrent pregnancy loss.

Study funding/competing interest(s): Funding by University(ies) – 1st Obstetrics and Gynaecology Department of University of Athens.

Trial registration number: NA.

Keywords: recurrent miscarriage, PAI-1 polymorphism, fibrinolysis

P-155 Endocannabinoids impact in trophoblast turnover: 2-arachidonoylglycerol induces reactive oxygen/nitrogen species generation and cell death in primary cultures of cytotrophoblasts

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Study question: The placenta development requires a tight regulated proliferation, differentiation and apoptosis of cytotrophoblasts. Is the major endocannabinoid 2-arachidonoylglycerol (2-AG) part of the signaling network involved in cytotrophoblast cell death?
Summary answer: 2-AG induces a decrease in human cytotrophoblast cell viability and a dramatic increase in reactive oxygen/nitrogen species (ROS/RNS) generation. The types of cell death include necrosis for higher concentrations while for lower concentrations other mechanisms, such as apoptosis, may be involved.
What is known already: The endocannabinoid system is implicated in pregnancy events such as implantation and decidualization. Our group has shown the presence of 2-AG metabolic machinery in cytotrophoblast cells and revealed that this endocannabinoid induces apoptosis in the choriocarcinoma cell line- BeWo cells, a cytotrophoblast cell model and in decidual cells.
Study design, size, duration: Human term placenta (n = 5) were used to obtain primary cultures of cytotrophoblasts. After 12 h of adhesion, cells were treated with 2-AG for 24 h at different concentrations (1–50 mM). At the end of incubation time, the impact of 2-AG treatment versus control was assessed by parameters related to cell death.
Participants/materials, setting, methods: Term placentas were collected from normal pregnancies (24–36 years old).To evaluate cell viability, we performed MTT assay and measured LDH released from damaged cells, as a biomarker for cellular cytotoxicity and cytolysis. ROS/RNS generation and mitochondrial membrane potential were evaluated by fluorimetry; morphological changes were observed by Höechst/Giemsa staining.
Main results and the role of chance: Treatment of cell cultures with 2-AG resulted in oxidative stress-related challenges with massive reactive oxygen and nitrogen species generation. In addition, MTT results indicate that 2-AG induced a cytotrophoblast viability loss reflected in the decrease of NAD(P)H-dependent cellular oxidoreductase mitochondrial enzymes activity. These findings were supported by Höechst and Giemsa staining analysis. The highest concentrations caused LDH release suggesting a necrotic process of cell death.
Limitations, reason for caution: Although our data were obtained in human primary cytotrophoblasts, they result from *in vitro* studies. Thus, caution is required in their translation for *in vivo* physiology.
Wider implications of the findings: This study supports the importance of cannabinoid signalling in placenta, more specifically during cytotrophoblast cell turnover. Deregulation of cannabinoid signalling may be implicated in alterations of oxidative state of cytotrophoblasts and may contribute to the pathophysiological mechanisms of some pregnancy complications. Thus, the study of the effects of endocannabinoids on maternal-fetal interface may be relevant for the understanding of the underlying mechanisms in pregnancy pathologies and contribute to future treatments for infertility.
Study funding/competing interest(s): Funding by national/international organization(s) – The authors thank Fundação para a Ciência e Tecnologia for the Post-doctoral grant of Bruno Fonseca (SFRH/BPD/72958/2010).
Trial registration number: NA.
Keywords: 2-arachinoylglycerol, endocannabinoids, placenta, cytotrophoblasts, cell death

P-156 The immunophenotype of women with recurrent pregnancy loss

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Study question: The purpose of the study was to estimate the alterations in the immunophenotype of women with unexplained pregnancy failures in comparison with healthy women.
Summary answer: The purpose of this study was to estimate selected parameters of the immune system in patients with recurrent pregnancy loss and healthy, fertile women with the history of successful pregnancies. Because it is known that T and NK cells are responsible for the foreign antigen recognition and the adaptive immune response, we studied the populations of T cells and the markers of activation of T lymphocytes. Furthermore, we studied the populations of B cells – especially B-1 cells which are responsible for the production of autoantibodies.
What is known already: Approximately 15–20% of all pregnancies result in miscarriage, usually in the first trimester of pregnancy. The prevalence of recurrent spontaneous abortion (RSA), defined as three or more consecutive losses, is approximately 1–2%. About 40–60% repetitive abortions have no identifiable cause.
Study design, size, duration: The study population included 14 nonpregnant women with the history of recurrent pregnancy miscarriages (defined as 3 or more consecutive, unexplained first trimester spontaneous abortions with the same partner), 25–34 years old (mean – 28.92). A complete medical, surgical, and social history was obtained from all patients evaluated in this study. All couples also had peripheral blood chromosome assessment using standard banding techniques. All patients underwent hysterosalpinography. Luteal phase defects were excluded. The patients with the presence of autoantibodies and infections were excluded.
Participants/materials, setting, methods: The diagnostic thrombosis tests have been performed such as: anticardiolipin antibodies, lupus anticoagulant, activated partial tromboplastin time, prothrombin time, platelet level. In their previous history women did not have neither arterial nor venous thromboembolic events. All patients with a normal profile of the investigations were classified as unexplained repetitive aborters. The control group comprised 18 non-pregnant healthy, fertile women (with the history of successful pregnancies) within the same age range of 26–35 years old (mean – 27.42) were studied. Women with infections (viral and bacterial) or autoimmune diseases were excluded from the control group. The study design was accepted by the local Ethics Committee. Informed consent was obtained from the women before their participation in the study.
Main results and the role of chance: We found that the percentage of T CD 4^+ lymphocytes, $CD3^-16/56^+$ cells, and T CD 8^+11b^- cells was significantly higher in patients with recurrent pregnancy loss in comparison with healthy women. The percentage of B-1 $CD19^+5^+$ lymphocytes was higher than 1.5 % in women with unexplained habitual miscarriages and was also significantly higher in comparison with healthy women. The percentages of B CD 19^+ and T CD 8^+11b^+ lymphocytes were significantly lower in women with pregnancy failures when compared to controls. The ratio of T $CD4^+$: T CD 8^+ was also significantly higher in patients with recurrent pregnancy loss in comparison with healthy women. The percentage of T CD 3^+ lymphocytes and T CD 8^+ cells did not differ in both studied groups. Furthermore, we found higher expression of CD 25 antigen on T CD 3^+ and T CD 4^+ lymphocytes in the study group when compared to controls. The expression of CD 25 antigen and HLA-DR molecule on T CD 8^+ did not differ in the study group when compared to controls. Similarly, the expression of HLA-DR antigen on T CD 3^+ and T CD 4^+ lymphocytes did not differ in the group of women with reproductive failures in comparison with the control group.
Limitations, reason for caution: Chromosomal aberration is the principal cause of fetal loss during the early stage of gestation. The limitations of our study is a lack of chromosomal analysis of the abortion tissue material in previous pregnancies of patients with recurrent pregnancy loss.
Wider implications of the findings: In conclusion, our results suggest that there are alterations in the immunological parameters in women with the history of RSA. These changes can be involved in the etiopathogenesis of recurrent pregnancy loss.
Study funding/competing interest(s): Funding by University(ies) – Medical University of Lublin, Poland.
Trial registration number: There was no trial study.
Keywords: immunology, pregnancy, recurrent pregnancy loss

P-157 Is T cell exhaustion involved in the pathogenesis of idiopathic secondary recurrent miscarriage?

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Study question: Are immunologic factors involved in the pathogenesis of idiopathic secondary recurrent miscarriages (isRM) of unknown cause?
Summary answer: IsRM patients show immunologic changes that resemble a condition of abnormal T cell function termed T cell exhaustion. Lower levels of $CD3^+$, $CD8^+$ and regulatory T cells but higher proportions of activated $CD3^+$, $CD4^+$ and $CD8^+$ T cells and decreased in-vitro response (proliferation) to various mitogen stimuli are present.
What is known already: Immunologic diagnostic is of growing interest in the context of recurrent miscarriage, especially in patients with isRM. Several studies described immunological parameters that differ in affected patients compared to healthy controls and might be associated with RM. However, results are discussed controversially and limitations of recent studies often are the small size of study populations and inhomogeneous definitions of RM.
Study design, size, duration: N = 276 couples with ≥3 RM were recruited between 10/2011 and 12/2014. A standardized diagnostic procedure including screening for anatomical, endocrinologic, autoimmune, haemostatic, genetic and immunologic disorders was performed. N = 44 patients with ≥3 consecutive RM after a successful pregnancy without abnormalities in the diagnostic were identified. Immunologic diagnostic was compared to n = 31 controls.
Participants/materials, setting, methods: Diagnostic was performed in non-pregnant patients and controls in the mid-luteal phase. Peripheral blood levels of lymphocyte subpopulations, cytokines and neopterin were determined using four-color fluorescence flow cytometry, ELISA, and Luminex technique. In-vitro proliferation was studied using a lymphocyte transformation test with different mitogens and pooled allogeneic stimulator cells.
Main results and the role of chance: IsRM patients showed significantly lower absolute numbers (/mL) of CD45+ (p = 0.032), CD3+ (p = 0.027), CD8+ (p = 0.045) T-lymphocytes, lower proportions of regulatory T cells (CD4 + CD25 + FOXP3 + CD127-; p = 0.028) but higher proportions of activated CD3 + DR+ (p = 0.001), CD4 + DR+ (p < 0.001) and CD8 + DR+ (p = 0.010) lymphocytes and a lower in-vitro stimulation of lymphocytes with phytohaemagglutinin (PHA, p = 0.004), pokeweed mitogen (PWM, p = 0.029) and CD3 mAb (p = 0.048) than healthy controls. Further the analysis of the cytokine assays revealed lower levels of TGF-b2 (pg/ml; p = 0.048) in isRM patients.
Limitations, reason for caution: As screening was performed after isRM it cannot be proven whether the immunologic disorders were cause or consequence of the miscarriages.
Wider implications of the findings: This study increases the understanding of the pathophysiology of RM and assists to establish immunologic diagnostic that helps to identify targets of future immunomodulatory therapies. We suggest T cell exhaustion, induced by a persistent antigen in the maternal circulation, as a potential mechanism involved in isRM. The clearance of the antigen or the repair of the T cell abnormality might be a potential treatment option for isRM.
Study funding/competing interest(s): Funding by University(ies) – Ruprecht-Karls University Heidelberg.
Trial registration number: A trial registration number was not required due to the retrospective study design.
Keywords: recurrent miscarriage, immunologic disorders, T cells, cytokines

P-158 Effect of thyroid autoantibodies per se on pregnancy outcomes in euthyroid women undergoing IVF/ICSI

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Study question: Does thyroid autoantibodies (ATA) per se impair IVF/ICSI outcomes?
Summary answer: Thyroid autoantibodies per se doesn't impair IVF/ICSI outcomes in patients without subclinical hypothyroidism (SCH).
What is known already: The incidence of SCH and the level of ATA was high in infertile women, and they were always co-existed and may have adverse effect on pregnancy outcomes. However, two recent meta-analysis hold opposite opinion about the effect of ATA on pregnancy outcomes in IVF/ICSI patients.
Study design, size, duration: We undertook a systematic review and meta-analysis of the literature published until April 2014. PubMed, Embase, and the Cochrane trial register were searched. The study was conducted according to the MOOSE guidelines.
Participants/materials, setting, methods: We used the software package stata 12.0 for statistical analysis. The risk ratio (RR) with a 95% confidence interval (CI) was calculated using the random/fixed effects model for binary data variables. We displayed the results from the meta-analysis as forest plots.
Main results and the role of chance: Six studies evaluating 2531 women were included in our study, four studies evaluating 1825 women were left after excluding patients with SCH. In euthyroid women not excluding SCH, women with positive ATA was associated with higher risk of miscarriage (pooled RR = 1.638, 95% CI 1.228–2.185, P = 0.001) compared to women with negative ATA, and the pregnancy rate and delivery rate were similar between groups. In euthyroid women excluding SCH, the pregnancy rate was similar in ATA positive women compared to ATA negative women (pooled RR = 0.993, 95% CI 0.853–1.155, P = 0.923). The miscarriage rate was similar in ATA positive women compared to ATA negative women (pooled RR = 1.445, 95% CI 0.970–2.154, P = 0.070). The delivery rate was similar in ATA positive women compared to ATA negative women (pooled RR = 0.935, 95% CI 0.785–1.112, P = 0.446).
Limitations, reason for caution: The study with relatively small number of women included in this review may not have been sufficient to recognise small differences between groups. In addition, unequal TSH level in one of included studies maybe a underlying influencing factor.
Wider implications of the findings: Based on the findings of the current review, it can be concluded that the apparent miscarriage risks resulting from ATA became weaken, even disappeared after excluding the effect of SCH and ATA per se may not impair IVF/ICSI outcomes. However, the results must be interpreted with caution because of limited sample size, bordline P value about miscarriage and methodological weaknesses of the included studies.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by grants from the Major State Basic Research Development Program of China (973 Program; no. 2012CB944902).
Trial registration number: NA.
Keywords: thyroid autoantibodies, subclinical hypothyroidism, miscarriage, ART

P-159

Abstract withdrawn by the author

P-160 Elevated concentrations of circulating serum soluble LH/hCG-Receptor (sLHCGR) and its complex (LH-sLHCGR) are associated with early pregnancy loss (EPL) after blastocyst transfer

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Study question: Does the circulating complex of sLHCGR/LH-LHCGR remain stable in individuals over time, and does it influence the establishment or course of pregnancy after blastocyst transfer in a programme of IVF?
Summary answer: The sLHCGR concentrations prior to fertility treatment (PT) and on the day of embryo transfer (ET) showed close correlation, suggesting that soluble receptor levels remain stable during the menstrual cycle and fertility treatment.

A significant proportion of women of reproductive age (ca 14%) show elevated concentrations of sLHCGR. These women show positive pregnancy rates which do not differ from women with 'normal' or 'low' concentrations. However, the incidence of EPL in these women was significantly higher than those with 'normal' or 'low' concentrations.
What is known already: The measurement of sLHCGR and its complex has been explored in limited circumstances in IVF so far. However, recently published reports suggest that prenatal testing of sLHCGR and hCG-LHCGR in early human pregnancy (first trimester) significantly contribute to the detection of miscarriage, Down's, preeclamsia.
Study design, size, duration: The study was a prospective exploration of a potential association of extreme concentrations of sLHCGR with pregnancy

outcome after blastocyst transfer in a programme of IVF. Follicular phase serum samples were evaluated in 166 successive blastocyst embryo transfers effected between August 2013 and June 2014.

Participants/materials, setting, methods: All women (n = 166; mean age = 35.5 years) underwent blastocyst transfer effected in a single centre, and 108 of them were frozen embryo transfers mostly during natural cycles. Positive pregnancy test was determined 17 days after ovulation trigger or LH surge by serum bHCG concentrations >5 IU/L. A clinical pregnancy was confirmed by ultrasound identification of a fetal heart at 8 weeks. EPL was confirmed when a positive pregnancy test was followed by a bleed and decline in bHCG prior to the 8-week scan.

The serum sample was collected and stored at -20°C until assay in batches, prior to knowledge of treatment outcome. The assay was a specialist ELISA assays performed in a dedicated laboratory. The sLHCGR and its complex concentrations were expressed as pmol/ml or as multiplicity of median (MoM) values.

Main results and the role of chance: The serum concentrations of the complexes (sLHCGR and its complex) showed good stability over time with pre-treatment and pre-embryo transfer samples showing close correlation (r^2 = 0.99).

The median circulating concentration value was 2.8 pmol/ml (one MoM) showing a non-normal distribution varying from undetectable to an excess of 150 pmol/ml (>53 MoM), and a pragmatic value of ≥28 pmol/ml (≥10 MoM) was used to describe the 'elevated' group (the highest 12% of cases). From the 166 embryo transfers 96 (58%) resulted in a positive pregnancy test. There was no association between sLHCGR concentrations and incidence of positive or negative pregnancy test. Non-continuing pregnancy was confirmed in 34 of the 96 positive tests, and 24% of these cases showed elevated sLHCGR values. In contrast, only 6% of the continuing pregnancies showed elevated values (Fisher's exact test: p = 0.01).

Limitations, reason for caution: A larger series of test evaluations are required to confirm this initial observation, and that series is being undertaken.

Wider implications of the findings: There are numerous reasons for pregnancy failure after embryo transfer, but this first step towards identifying a circulating factor undermining the establishment of clinical pregnancy is a potentially important finding, with implications for recurrent EPL, as high concentrations appear to be patient specific.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Glasgow Centre for Reproductive Biology, UK and Origin Biomarkers, UK.

Trial registration number: NA.

Keywords: early pregnancy loss, soluble LHCG Receptor

P-161 Genotyping analyses for polymorphisms of PD-1 gene and CTLA4 gene in patients with recurrent pregnancy loss

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Study question: Are PD-1(programmed Cell Death-1) gene and CTLA4 gene polymorphisms risk factors for recurrent pregnancy loss (RPL) and do the risk alleles have any influence on further miscarriage?

Summary answer: There was a significant difference in frequency of the SNP of the PD-1 gene of the RPL group and the controls group; however the live birth rates of the next pregnancy in patients with and without the risk allele were determined to be 84.6 and 81.7%, showing no significant difference.

What is known already: Because PD-1 controls excessive activity of T cells and causes immune tolerance, disfunction of PD-1 is a cause of autoimmune disease such as systemic lupus erythematosus, and in cases of RPL, decreased regulatory T (Treg) cells have been reported. PD-1 ligand (PD-L1) expresses highly at the placenta and the promoter activity of PD-1 differs between -606G and -606A alleles, the -606G allele being associated with higher promoter activity than the -606A allele.

Study design, size, duration: Genotype analysis was performed on SNPs in PD-1 gene and CTLA4 gene on 264 patients who were diagnosed with unexplained RPL between 2007 and 2012 and on 181 fertile controls. Next, the subsequent live birth rate in the 264 patients was compared with and without the risk allele.

Participants/materials, setting, methods: Genomic DNA was extracted from peripheral blood samples. All genotyping was carried out using TaqMan PCR assays. We calculated the crude OR and 95% confidence intervals (95% CI) in the frequencies of the alleles between patients and controls. Next, we calculated the live birth rates of next pregnancy in patients.

Main results and the role of chance: There was a significant difference in frequency of the SNP of PD-1 gene of the RPL group and the controls group (p = 0.014, OR = 1.21 95% CI; 1.04-1.40), however there was no difference in the SNP of CTLA4 gene. From our cohort study, the live birth rates of patients with the risk allele of PD-1 gene were determined to be 84.6% and those without to be 81.7%, with no significant difference after excluding cases with an abnormal embryonic karyotype. Product of conception (decidua and villus tissue) was tested by immunohistochemistry on cases resulting in miscarriage and having a normal embryo in the next pregnancy and showed little difference in the expression of PD-L1 with or without risk allele of PD-1 gene.

Limitations, reason for caution: Our study includes the patient who had two miscarriages.

Wider implications of the findings: In this study, it was shown that decreased PD-1 activity was related to miscarriage whereas the expression of PD-L1 didn't change. It was shown that genetic polymorphism was related to the immune tolerance which was the cause of the miscarriage. This study gives support to Treg cells playing an important role in the induction of immune tolerance and that steroids could be employed in the treatment of RPL caused by abnormal immune tolerance.

Study funding/competing interest(s): Funding by national/international organization(s) – The Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology of Japan/We have no conflict of interest.

Trial registration number: NA.

Keywords: recurrent pregnancy loss, PD-1, immune tolerance, CTLA4

P-162 The expression of LAMß3 and MFAP5 increases in pregnant women endometrial cells during implantation window

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Study question: Are the expression of lamininb3 (LAMβ3) and microfibril-associated protein 5 (MFAP5) in endometrial cells during implantation window of pregnant group different from those of non-pregnant group?

Summary answer: LAMβ3 and MFAP5 expressed highly in endometrial cells of pregnant women than those of non-pregnant women during implantation window.

What is known already: LAMβ3 and MFAP5 are important components of extracellular matrix (ECM) and express up-regulated in the LH + 7 samples compared with LH + 2 during the implantation window.

Study design, size, duration: We compared the mRNA expression and protein production of LAMβ3 and MFAP5 in endometrial cells of pregnant women with those of non-pregnant women following IVF. Eighteen endometrial samples cultured in vitro in each group were selected for our detection.

Participants/materials, setting, methods: Thirty-six women were divided into two groups according to their clinical pregnancy outcome: eighteen in pregnant group and eighteen in non-pregnant group. Endometrial samples were collected from them in the 60 day after the dominant follicle disappeared by the transvaginal ultrasound of their natural cycle. The endometrial cells were cultured in vitro. Their morphological characteristics were observed under inverted microscope. The expression of LAMβ3 and MFAP5 were analyzed using quantitative real-time PCR and immunochemical staining.

Main results and the role of chance: The endometrial cells were cultured in 36 endometrium samples, 18 in pregnant group and 18 in non-pregnant group. Their morphological characteristics were similar between the two group. Quantitative real-time PCR showed that the expression level of LAMβ3 and MFAP5 in endometrial cells were significantly higher in pregnant group than that in non-pregnant group (P < 0.05). Immunochemical detection of LAMβ3 and MFAP5 protein showed that they were strongly positive in endometrial glandular epithelial cells and stromal cells in pregnant group while weakly positive in non-pregnant group (P < 0.05).

Limitations, reason for caution: The independent and mutual role of the two genes in endometrial cell signaling needs to investigate further.

Wider implications of the findings: Our study finds that women with a higher expression of LAMβ3 and MFAP5 in endometrial cells during implantation window may have better IVF-ET outcome. LAMβ3 and MFAP5 could be considered as biomarkers of endometrial receptivity.
Study funding/competing interest(s): Funding by national/international organization(s) – This project was funded by National Natural Science Foundation Project (81471520), State Scholarship Fund (2011911033), Beijing Natural Science Foundation Project (5122015), and Beijing Project of Training High-level Medical Technical Personnel in Health System. The authors declare no conflict of interest.
Trial registration number: NA.
Keywords: endometrial receptivity, lamininß3, microfibril-associated protein 5, implantation window

P-163 Endometrial lgr7 expression and implantation failure: which role for relaxin system?

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Study question: To compare the endometrial LGR7expression in samples obtained from women with proven fertility to samples obtained from women with history of implantation failure
Summary answer: An analysis of the decreased expression of the endometrial receptor LGR7 in women with implantation failures, both IVF failure and RPL
What is known already: Implantation failure is considered a major cause of infertility not only in women with recurrent pregnancy loss (RPL), but also in otherwise healthy women with unexplained infertility. In a previous study, we have suggested that RLX plays a crucial role in endometrial preparation for implantation. Other studies showed that RLX levels were impaired in women with early pregnancy loss and that granulosa cells.
Study design, size, duration: Three groups of patients referring to the Fertility Clinics of the Second University of Naples were enrolled for this prospective observational study. An endometrial biopsy was planned to be done on every patient in the secretory phases of the menstrual cycle through hysteroscopic examination; determination of LGR7 expression was performed with PCR analysis.
Participants/materials, setting, methods: 69 women with regular ovulatory cycle and normal uterine cavity divided into three groups: 23 patients with recurrent pregnancy loss (Group A), 23 patients with unexplained infertility undergone at least 3 cycles of failed IVF reporting good oocyte and embryo quality (group B), 23 patients with proven fertility (group C).Endometrial biopsy were obtained in the secretory phases (from day +3 to +5); determination of LGR7 expression was performed with PCR analysis immunohistochemistry.
Main results and the role of chance: A significative difference between groups was noticed ($p = 0.02422$). Mean and median values were 1.10 and 1.12 for group A, 0.94 and 0.95 for group B, 1.70 and 1.68 for group C. Multiple comparison test showed a remarkable difference between group B vs C and A vs C. Immunohistochemistry confirmed a lower endometrial expression of LGR7 protein in women with a history of implantation failure.
Limitations, reason for caution: In the control group the time elapsed between the last pregnancy and the sample collection and the possible occurrence of confounding factors were not considered. Moreover, ours was performed in non-conception cycles, and cyclic expression of endometrial factors is unrelated to a subsequently ensuing pregnancy. Chromosomal embryos abnormalities have not been evaluated.
Wider implications of the findings: Our work underscores the important role played in endometrium by RLX – through its receptor LGR7 – in ensuring successful implantation. The decreased expression of the endometrial receptor LGR7 in women with implantation failures, suggests that RLX may play a crucial role during the "window of implantation" – For this reason, our study may open new possibilities for studying and understanding the mechanisms of embryo implantation.
Study funding/competing interest(s): Funding by University(ies) – Second University of Naples.
Trial registration number: Trial registration was not required.
Keywords: Relaxin receptor, endometrial biopsy, window of implantation, implantation failure

P-164 Relationship between maternal age and numerical abnormalities of fetal chromosomes in spontaneous abortion during the first trimester

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Study question: To investigate whether the relationship between maternal age and numerical abnormalities of chorionic villus chromosomes in spontaneous abortion during the first trimester show a different pattern among different pregnancy ways, different chromosomes and different numbers of abnormal chromosome.
Summary answer: Advanced maternal age is a high risk factor to conceive aneuploidy fetuses in natural conception (NC) and *in vitro* fertilization (IVF), but not in intracytoplasmic sperm injection (ICSI). Abortuses with multiple aneuploidy occur predominantly in advanced maternal age and different biological structure of chromosome shows different susceptibility to aging.
What is known already: Approximately 10–15% of pregnancies end in miscarriage and the major factor contributing to abortion is numerical abnormalities of fetal chromosomes. The prevalence of aneuploidy rises dramatically with increasing maternal age. As the increasing use of assisted reproductive technology (ART), it has generated a concern about its safety. However, it is not clear the plot patterns of maternal age to aneuploidy rate among different pregnancy ways, different chromosomes and different numbers of abnormal chromosome.
Study design, size, duration: Retrospective cohort study. A total of 55 cases of NC, 147 cases of IVF and 85 cases of ICSI suffered from spontaneous abortion during the first trimester were enrolled in this study. The productions of conception were collected as materials.
Participants/materials, setting, methods: Aneuploidy was analysis by multiplex ligation-dependent probe amplification (MLPA) method. According to the maternal age, each group was further divided into 4 subgroups: ≤29, 30 ~ 34, 35 ~ 39 and ≥40. The relationship between maternal age and aneuploidy was compared among different pregnancy ways, different chromosomes and different numbers of abnormal chromosome.
Main results and the role of chance: Maternal age of abortuses with aneuploidy was higher than that without aneuploidy in NC and IVF ($P < 0.05$), but not in ICSI. Abortus' aneuploidy rate was increase with maternal age in NC and IVF but not in ICSI, while statistical difference was observed in IVF ($P = 0.002$). An increase in abnormal rates of chromosomes 15, 20, 21 and 22 was noted with increasing maternal age. Abnormal rates of chromosome 16 were similar in different subgroups. Sex chromosome's abnormal rate of ≥40 years subgroup decreased and without 45, XO in this subgroup. Maternal age of abortuses with multiple aneuploidy was higher than that with single aneuploidy ($P = 0.039$), and the incidence of multiple aneuploidy in ≥40 years subgroup was significantly higher than other three subgroups ($P < 0.05$).
Limitations, reason for caution: It's not valid to detect haploidy and polyploidy by using MLPA methods. This research is just based on analysis of 6 loci per chromosome by adopting 3 different MLPA kits. Therefore, imbalances related to structural rearrangements may be less reliably detected.
Wider implications of the findings: Except advanced maternal age, additional mechanisms may be also involved in causing chromosome abnormality of fetus in ICSI. Abortuses with most of the small chromosomes' aneuploidy occur predominantly in advanced maternal age, whereas abortuses with Turner syndrome are more common in the young gravidas. Effect of maternal age on Trisomy 16 does not exist. It may bring insight into the nature of aneuploidy and exploring the biological mechanisms underlying these pathological processes.
Study funding/competing interest(s): Funding by national/international organization(s) – the Basic Research Program of Shenzhen (no. JCYJ20120829150019349).
Trial registration number: NA.
Keywords: aneuploidy, advanced maternal age (AMA), spontaneous abortion, assisted reproductive technology (ART), chorionic villus

P-165 The prognostic impact of HY restricting HLA class II alleles in secondary recurrent pregnancy loss, a confirmatory study

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Study question: Is maternal carriage of HLA class II alleles known to present male specific (HY) antigens associated with a poor prognosis for live birth in secondary recurrent pregnancy loss (SRPL) after the birth of a boy?
Summary answer: Maternal carriage of the HY restricting HLA class II alleles HLA-DRB1*15; -DRB1*07 and DQB1*05:01/02 are associated with a lower chance of cumulative live birth in SRPL after a first-born boy, in a dose-response related manner.
What is known already: SRPL is defined as three or more pregnancy losses after the birth of a child. We have shown that maternal carriage of HLA class II alleles HLA-DRB1*15 and HLA-DQB1*0501/02 is associated with a decreased chance of live birth in the first pregnancy after referral among women with SRPL after a boy. These HLA alleles are known to restrict CD4+ cell immunity against male-specific HY antigens. In 2013, HLA-DRB1*07 was reported also to present HY-antigens.
Study design, size, duration: A prospective cohort study was carried out on women with SRPL after the birth of a boy ($N = 151$) or a girl ($N = 135$) referred between 2005 and 2013, follow-up ranged from 2 to 9 years. HLA-DRB1*15; -DRB1*07; -DRB3*0301; -DQB1*05:01/02 were considered putatively important, in concordance with published studies.
Participants/materials, setting, methods: HLA class II typing was performed by PCR-SSO on DNA from peripheral blood drawn at first consultation at the Danish RPL Unit at Rigshospitalet.

We dichotomized cumulative outcome as 'live birth' or 'no live birth' for c^2 testing with risk estimates.
Main results and the role of chance: Of the 286 women, 151 had a first-born boy, and 135 had a first-born girl. 126 women carried one HY-restricting HLA allele and 60 carried two. Cumulative live birth rate for all patients was 63%, and there was no significant difference according to the sex of the first-born child (OR for live birth: boy vs. girl. 0.87; 95% CI 0.52; 1.42). Live birth rate for women with first-born boys decreased with increasing number of HY-restricting alleles (N (%): 40 (76%); 38 (57%); 15 (48%), OR: 0 (reference) vs. 1: 0.43 (0.19; 0.94); 0 vs 2: 0.31 (0.12; 0.78). We did not show this for women with first-born girls: Live birth rates: 34 (73%), 37 (63%), 17 (59%); OR 0.64 (0.28; 1.47), 0.52 (0.20; 1.44), respectively.
Limitations, reason for caution: A longer follow-up period would add to the merits of the study.
Wider implications of the findings: We found that maternal carriage of HY-restricting HLA class II alleles HLA-DRB1*15; -DQB1*05:01/02 and –DRB1*07 decreased the chance of live birth among women with SRPL after a boy in a dose-response related manner. This extends our previous findings to cumulative outcome and adds –DRB1*07 as prognostically negative. Our findings support the hypothesis that an aberrant immune response to the semi-allogenic fetus by the mother's immune system plays an important role in SRPL after a boy.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Funding by national/international organization(s) – University Hospital Copenhagen, Rigshospitalet; University of Copenhagen, Den Obelske Familiefond; Maersk Foundation.
Trial registration number: NA.
Keywords: Recurrent pregnancy loss, HY-restricting HLA class II alleles, Prospective cohort study, Reproductive immunology

P-166 Crucial role of hypoxia inducible factor 2alpha in the pregnant uterus

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Study question: It has been shown that hypoxia inducible factor 2α(Hif2α), one of major transcriptional factors induced by low oxygen tension, is strongly induced in the mouse decidua during early pregnancy. Does it play an important role in early pregnancy? And if so, what is its role?
Summary answer: Our findings suggest that uterine Hif2α plays a critical role in early pregnancy by optimizing progesterone-dependent decidualization and supporting progesterone-independent fetal and placental growth.
What is known already: Although Hif2α is predominantly expressed in the decidualizing stroma in mice, its functions in the pregnant uterus remain unclear.
Study design, size, duration: A mouse model with the deletion of uterine Hif2α was used in this study.
Participants/materials, setting, methods: Uterus-specific Hif2α null ($Hif2a^{loxP/loxP}Pgr^{cre/+}$; Hif2α cKO) and control ($Hif2\alpha^{loxP/loxP}$) mice were generated by crossing Hif2α-floxed mice with Pgr-cre mice. Embryo implantation, decidualization, placental and fetal growth, and litter size in these mice were evaluated after mating with wild-type fertile male mice.
Main results and the role of chance: Although the control mice demonstrated normal litter size, Hif2α cKO females showed infertility. In addition, Hif2α cKO mice showed early pregnancy loss with compromised decidualization. Moreover, the administration of progesterone improved decidualization in the Hif2α-deleted uterus, and however, it could not recover early pregnancy loss in Hif2α cKO females.
Limitations, reason for caution: We performed all the analyses using a mouse model. Further investigations using human cells are needed to confirm the similar cellular effects of Hif2α in the human endometrium.
Wider implications of the findings: These findings provide for molecular evidence for the essentiality of Hif2α in the pregnant uterus.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.
Trial registration number: NA.
Keywords: hypoxia inducible factor, embryo implantation, decidualization, uterus, progesterone

P-167 Pregnancy of unknown location (PUL): prevalence and clinical outcome

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Study question: To examine the prevalence and clinical outcome of pregnancy of unknown location
Summary answer: One in ten women attending for early pregnancy scans had PUL. While >90% of them had conservative management, 3.6% needed laparoscopy and 1.8% of them had methotrxate.
What is known already: With the development of sensitive urine pregnancy tests and modern high-resolution ultrasound, an increasing number of women are being diagnosed with ectopic pregnancies and PULs. A vast majority of these women are offered medical and expectant management, however some may still require significant surgical intervention for diagnosis and treatment. The data on the clinical outcomes in these women vary widely among various studies, and is dependent upon many factors.
Study design, size, duration: A single centred, retrospective analysis of 1443 consecutive women who had early pregnancy ultrasound scans over a period of four months. Cases with PULs were identified and their management was reviewed.
Participants/materials, setting, methods: All women with a positive pregnancy test and no evidence of an intrauterine or extrauterine pregnancy on ultrasound were included as PUL. The data was collected from 112 cases of PULs.
Main results and the role of chance: The overall prevalence of PUL in our population is 10.7% (154/1443) with 87.5% (1262/1443) intrauterine pregnancies and 1.8% (28/1443) ectopic pregnancies. Of the 112 PUL cases followed up for a mean of 7 days (range: 2–49 days), 69 (61.6%) cases were identified as failing PULs, 32 (28.6%) were intrauterine pregnancies and 2 (1.8%) were ectopic pregnancies. 9 (8%) were diagnosed to have persistent PULs, of which 3 treated with surgical evacuation of uterus, 4 with laparaoscopic management and 2 with Methotrexate. There were no serious adverse event with regards to significant bleeding requiring transfusion.
Limitations, reason for caution: A significant proportion of women had incomplete follow up and was therefore excluded from the data analysis.
Wider implications of the findings: Utilisation of high-resolution ultrasound and serial serum surveillance with hCG estimations will enable the early pregnancy units to manage PULs efficiently and safely in most cases.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None declared.
Trial registration number: NA.
Keywords: pregnancy of unknown location, ectopic, ultrasound, early pregnancy

P-168 Peri-conceptional Progesterone treatment in women with unexplained recurrent miscarriage, A randomized double-blind controlled trial

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Study question: Is peri-conception progesterone effective in preventing miscarriage in patients with history of recurrent unexplained miscarriages.
Summary answer: Peri-conceptional progesterone in women with unexplained recurrent miscarriage is not only effective in reducing miscarriages but also preterm birth and lowering pregnancy complications with no observed maternal or peri-natal complications.
What is known already: Progesterone is necessary for successful implantation and the maintenance of pregnancy. This benefit of progesterone could be explained by its immmunomodulatory actions in inducing a pregnancy-protective shift from pro-inflammatoryTh-1 cytokine responses to a more favorable anti-inflammatory Th-2 cytokine response and or postulated that decidualization of endometrial stromal Cells serve as sensors of embryo quality upon implantation.
Study design, size, duration: Two hundred and eighty patients of recurrent unexplained miscarriages were randomised using sealed envelopes.
Participants/materials, setting, methods: The study group ($n = 140$) received progesterone (400 mg pessaries, twice daily), started soon as possible at luteal phase and continued after a positive pregnancy test till 28 weeks of gestation, compared to the control group ($n = 140$) who received placebo of the same time and duration. Main outcome measures were miscarriage rate and the rate of continuation of pregnancy beyond 20 weeks of gestation.
Main results and the role of chance: There was improvement in the clinical pregnancy rate with reduction of miscarriage among the study group compared to control group. The rate of miscarriages among the progesterone group is significantly lower than the placebo one (12.1 vs. 22.8%, $p < 0.02$). The rate of preterm delivery is lower (15.7 vs. 26.4%, $p < 0.001$) and the patients completing 36 weeks gestation is significantly higher, (71.4 vs. 32.1%, $p < 0.0001$). There were no observed maternal, fetal or neonatal complications observed.
Limitations, reason for caution: First it did not study the long term effects of the progesterone on the infantile, childhood and adulthood of the offspring; second it needs large population of study with more focusing on the probable mechanisms of progesterone in prevention of the miscarriages.
Wider implications of the findings: This is the first study which use of the vaginal rout progesterone in the peri conception period in recurrent unexplained miscarriages with higher patients acceptability and minimal adverse effects on the mother and the fetus with potential beneficial effect in reducing the miscarriage rate.
Study funding/competing interest(s): Funding by University(ies) – Assuit University.
Trial registration number: ClinicalTrials.gov Identifier: NCT01670929.
Keywords: progesterone, periconception, miscarriage, randomised controlled trial

P-169 Increased expression of Plasminogen-Activator-Inhibitor Type 1 (PAI-1) in the sera of patients with recurrent miscarriages (RM) and anti-trophoblast antibodies (ATAB)

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Study question: To study potential mechanisms how ATAB may interfere with early gestation, we compared the expression of pro-inflammatory cytokines and acute-phase-proteins in the sera of ATAB- positive and -negative RM patients and healthy controls.
Summary answer: ATAB-positive sera of women suffering from recurrent miscarriages display significantly increased expression of Plasminogen-Activator-Inhibitor Type 1 (PAI-1) compared to sera of ATAB-negative RM patients and healthy controls.
What is known already: Reproductive failure including RM has been suggested to correlate with antibodies that cross react with HLA-negative syncytiotrophoblasts. We found that 17% of women with 2 or more miscarriages and 34% of women with 3 or more miscarriages express anti-trophoblast antibodies (ATAB) [1]. The mechanism, how ATAB interfere with early gestation remains currently unknown. Successful early pregnancies require an intricate balance of regulatory cytokines and acute-phase-proteins possibly disrupted in ATAB-positive RM patients.
Study design, size, duration: Sera of patients with a history of idiopathic RM were screened for ATAB. The presence of ATAB was detected as described earlier [1]. Sera of each 18 RM patients with ATAB and of 18 RM patients without ATAB were pooled and termed ATAB-positive or ATAB-negative. Pooled sera of 10 healthy control individuals with at least one pregnancy carried to term and without miscarriages served as controls. All pools were investigated with respect to their cytokine expression level.
Participants/materials, setting, methods: The expression levels of 36 different cytokines, chemokines and akute-phase proteins were investigated with a human cytokine array (Panel A, R&D Systems) in all 3 sera pools. Detected semi quantitative differences were further analysed with an enzyme-linked immunosorbent assay (ELISA).
Main results and the role of chance: ATAB-positive sera of RM patients show increased expression of Plasminogen-Activator-Inhibitor Type 1 (PAI-1) compared to sera of ATAB-negative RM patients and healthy controls in the human cytokine array. ELISA results confirmed a significant higher expression level of PAI-1 in ATAB-positive sera of RM patients compared to ATAB-negative sera and controls ($p = 0.027$).
Limitations, reason for caution: The identification of PAI-1 as differentially expressed in ATAB-positive RM patients compared to healthy controls is a first hint at how ATAB may interfere with early gestation. Further investigations are required to sufficiently elucidate the pathomechanism of RM in patients with ATAB.
Wider implications of the findings: PAI-1 is the main inhibitor of tissue plasminogen activator and urokinase, which both convert plasminogen to plasmin, thus promoting fibrinolysis. Increased activity of PAI-1 and consecutive hypofibrinolysis may result in elevated intervillous fibrin deposition leading to lower placental perfusion in RSA patients with ATAB.
Study funding/competing interest(s): Funding by University(ies) – Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Klinikum der Ludwig-Maximilians-Universität, Campus Großhadern, Munich, Germany.
Trial registration number: NA.
Keywords: recurrent miscarriage, anti-trophoblast antibodies, early gestation, PAI-1

P-170 Patient-centered care for couples faced with early pregnancy complications: a systematic review of the literature

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Study question: What is the perspective of couples faced with early pregnancy complications, defined as miscarriage, recurrent miscarriage or ectopic pregnancy, on current healthcare and is there a need for improvement in patient-centered care?
Summary answer: Patient-centered care for (recurrent) miscarriage should be improved, regarding seven of the eight dimensions of patient-centeredness. Twenty-four specific care aspects were reported by couples to be important. For each of these care aspects healthcare performance was experienced as problematic. Data on patients' perspective on ectopic pregnancy care was not available.
What is known already: Early pregnancy complications affect the physical and emotional wellbeing of intended parents. Research in the field of (recurrent) miscarriage and ectopic pregnancy has so far mainly focused on improving accuracy of diagnostic tests and safety and effectiveness of therapeutic management. An overview of care aspects regarding values, preferences and needs important to couples and an identification of which of these care aspects performs well or poorly in current healthcare is missing.
Study design, size, duration: This systematic literature review followed a predefined protocol replicated by two reviewers. In December 2014, five electronic databases were searched for empirical studies on couples' perspective on care

for early pregnancy complications. Both quantitative and qualitative data was eligible, which implied performing a meta-synthesis rather than a meta-analysis.
Participants/materials, setting, methods: Data was extracted on couples' value clarification and service quality assessment and dichotomized in important/non-important and problematic/non-problematic, respectively. The eight dimensions of patient-centered care (**www.Pickerinstitute.org**) served to organize the reported care aspects varying from strengths to maintain to high priority targets for improving patient-centered care.
Main results and the role of chance: The search yielded 3,630 publications, of which 21 were eligible. Couples' perspective of care was examined by interviews and/or questionnaires. All 21 studies focused on (recurrent) miscarriage care and none on ectopic pregnancy. Twenty-four care aspects were important to couples according to at least one study and for all care aspects healthcare performance was reported problematic by at least one study. The 24 care aspects covered all but one (i.e. emotional support) of the eight dimensions of patient-centered care. For 16 care aspects, all studies agreed on the importance to couples and on the reported problematic performance. The most frequently reported care aspect that required high priority improvement was 'treating individual human beings experiencing a significant live event rather than a common condition' (n = 12 studies).
Limitations, reason for caution: The methodology of the included studies was often insufficiently reported and their quality varied. All but one study only included the female partner of the couple confronted with (recurrent) miscarriage and therefore data on males' perspective is limited.
Wider implications of the findings: The quality of healthcare depends on its safety, effectiveness, timeliness, efficiency, equity and patient-centeredness. Our systematic literature review identified care aspects likely to require improvement of current early pregnancy care. Further research is required to develop and test improvement strategies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Not applicable.
Trial registration number: NA.
Keywords: early pregnancy, patient-centered care, patient satisfaction, systematic review

POSTER VIEWING

EMBRYOLOGY

P-171 Late (blastocyst) morphokinetic parameters are better predictors of live birth compared to early (cleavage) ones: retrospective study of 212 single blastocyst transfers following mild IVF

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Study question: Which morphokinetic markers are predictive of live birth in a cohort of unselected infertile patients undergoing single blastocyst transfer following mild ovarian stimulation?
Summary answer: The late morphokinetic parameter observed at expanded blastocyst stage (tEB2: time until reaching 160 mm of expansion) was highly predictive of live birth following single blastocyst transfer. Among previously described early (cleavage-stage) morphokinetic parameters only cc2a (t3-t2) was found to be associated with clinical outcome albeit at a lower statistical significance.
What is known already: Published reports (Campbell, 2013) are still scarce on the predictive value of late (blastocyst-stage) morphokinetic parameters. Most TLM (time-lapse monitoring) studies involved cleavage-stage embryos and the prediction models developed so far only included early morphokinetic parameters (Meseguer 2011, Rubio 2014). Our series is unique because it has involved single blastocyst transfers exclusively. Therefore all resulting implantations could be taken into account without relying on known implantation data (KID) only or excluding any transferred embryos.
Study design, size, duration: A 2-year, retrospective study performed between October 2012 and September 2014 in a single private infertility centre. Pregnancies were followed-up until live birth or at least until 20 weeks of gestation. Embryos were cultured with a TLM incubator (EmbryoScope, Unisense Fertilitech, Aarhus, Denmark).
Participants/materials, setting, methods: Early (PNf, t2-t9), late (start of blastulation, time until reaching expanded blastocyst size of 130 (tEB1) and 160 um (tEB2), respectively) and interval (cc2 a-b, s2 and s3) morphokinetic parameters were scored according to recently published consensus criteria. Data were analysed by choosing quartiles with the highest implantation rates.
Main results and the role of chance: 212 consecutive single blastocyst transfers from 144 infertile patients (median age 38.1 ± 4.1 years, range: 28-47) undergoing natural cycle IVF or minimal ovarian stimulation. The overall live birth rate of the cohort was 34% (72/212). Compared to non-implanted ones, blastocysts that resulted in live birth needed on average -4.7 and -5.7 h less to reach the previously defined stages of expansion (tEB1 and 2). Implantation rates for tEB2 were significantly different (43 and 23%, p = 0.0025) for the lower and higher two quartiles, respectively. For cc2a the difference was statistically less significant (44 and 27%, p = 0.027).
Limitations, reason for caution: Our unselected patient cohort was biased towards advanced-aged, poor-prognosis patients who undergone mild IVF treatment coupled with single blastocyst transfer exclusively. This might limit generalizibility to other less infertile populations or to centres which use different treatment protocols.
Wider implications of the findings: Late morphokinetic parameters (especially those that incorporate a defined size of blastocyst expansion) might prove to be superior too early ones in predicting clinical outcome. Our findings are in line with the previously published studies of Campbell et al which have suggested that late morphokinetic markers (tSB and tB) could predict both euploidy status and clinical outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kobe Motomachi Yume Clinic.
Trial registration number: NA.
Keywords: time-lapse monitoring, blastocyst culture, minimal ovarian stimulation, single embryo transfer, in-vitro fertilization

P-172 The spatiotemporal dynamics of centrosomes and the genome during first cleavage in human tripronuclear zygotes in vitro

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Study question: Most tripronuclear (3PN) zygotes obtained through assisted reproductive technology (ART) present an abnormal cleavage pattern, such as multipolar mitosis. To reveal the underlying mechanism of this abnormality, we analyzed the behavior of centrosomes and genome segregation during the first cleavage of human 3PN zygotes using live-imaging techniques.
Summary answer: We established three-dimensional (3D) centrosome-tracking datasets during the first cleavage using human 3PN zygotes. Not every centrosome participated in genome segregation during this process in those zygotes retaining four centrosomes. In 3PN zygotes retaining two centrosomes, each centrosome moved in a different direction during genome segregation.
What is known already: Some 3PN zygotes derived from conventional *in vitro* fertilization (c-IVF) divide into three cells via a tripolar spindle (Plachot and Crozet, 1992; Balakier, 1993). We previously demonstrated that 3PN zygotes derived from intra-cytoplasmic sperm injection (ICSI) had two centrosomes, whereas of those derived from c-IVF, almost all had four centrosomes and they showed a Y-shaped metaphase plate and tripolar spindle in syngamy.
Study design, size, duration: Donated 3PN zygotes derived from c-IVF and ICSI were used in this study (n = 5). We visualized the centrosomes and histone H2B of these zygotes, then performed live imaging from zygote stage to first cleavage. The obtained datasets were used to analyze the dynamics of centrosomes and histone H2B.
Participants/materials, setting, methods: We constructed a plasmid to express histone H2B using commercially available plasmids from Evrogen. We also modified pCAG-PACT-mKO1, gifted by Dr. Matsuzaki (RIKEN CDB), and synthesized mRNA using an mMESSAGE mMACHINE Kit (Ambion). Images were obtained using CV1000 (Yokogawa) and tracking datasets were generated using R program and ImageJ.

Main results and the role of chance: In this study, centrosomes appeared just before the pronucleus disappeared, and assembled around the genome at syngamy. All 3PN zygotes derived from c-IVF ($n = 3$) demonstrated tripolar division after first cleavage, and commonly showed four centrosomes with a genome divided into 3–4 components after syngamy. The four centrosomes moved in different directions following genome segregation during the first cleavage, with most centrosome leading the genome components during the cleavage, while some were detached from genome segregation. The centrosomes and genome were not always distributed to all blastomeres after the cleavage. As a result, one embryo had a blastomere containing no centrosome and no genome. The 3PN zygotes derived from ICSI ($n = 2$) showed even cleavage with bidirectional genome segregation by two centrosomes.
Limitations, reason for caution: Our sample size was only small in this study, and further similar studied are needed to confirm these results. The limitation in study design size lies in the time required to obtain sufficient fluorescent signals to analyze, due to temporal differences between mRNA injection and translation.
Wider implications of the findings: In this study, we successfully tracked the spatiotemporal dynamics of centrosomes and genome segregation during first cleavage in human zygotes. Our techniques proved very useful in establishing characteristic morphological patterns in abnormal zygotes such as polyspermy. In future studies, we will investigate the mechanism by which four centrosomes participate in the division of a triploid genome during the first cleavage.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Mio fertility clinic.
Trial registration number: NA.
Keywords: Live-cell imaging, tripronuclear zygote, centrosome, multipolar mitosis, 3D tracking

P-173 An automated time-lapse embryo selection algorithm is correlated with embryo implantation potential; clinical validation of Eeva

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Study question: Is there a correlation between the three EEVA (early embryo viability assessment) predictive categories: High, Medium, Low and implantation rates?
Summary answer: Yes, the three EEVA predictive categories are correlated with implantation rates
What is known already: A preliminary analysis of a published morphokinetic algorithm (Wong2010) was designed for blastocyst prediction. The algorithm was used for embryo selection by an automated time-lapse technology based on the combination of two parameters:P2 = t3-t2 (time to 3 cell-time to 2 cell) and P3 = t4-t3 (time to 4 cell-time to 3 cell) to predict blastocyst formation rate. The algorithm classifies embryos as having High, Medium or Low probabilities of becoming a blastocyst. Our objective is to correlate these categories with implantation.
Study design, size, duration: Retrospective, cohort study. This study includes 2198 embryos from our oocyte donation program undergoing ICSI from October 2013 to November 2014 of which 774 were transferred.
Participants/materials, setting, methods: University-affiliated infertility clinic. Patients were cultured in a standard incubator using a non-invasive test that combines time-lapse image analysis with an automatic cell-tracking software, EEVA (Early Embryo Viability Assessment).
Main results and the role of chance: We observed a direct relationship between morphokinetic categories and implantation potential. Results were only referred to those embryos with known implantation ($n = 518$) (number of gestational sacs matched with number of embryos transferred).

When categorizing according to EEVA: 146(28%) embryos were labelled as High, 123 (24%) were labelled as Medium and 155 (30%) as Low. The rest of embryos ($n = 94$ (18%)) were not suitable for grading by the software. Implantation rates of all embryos despite day of transfer were HIGH 50% (CI 95% 41.79–58.21); MEDIUM 39.84% (CI 95% 31.06–48.61) and LOW 33.55% (CI 95% 26.03–41.06), being statistically significant ($p = 0.036$).

A further analysis depending on the day of embryo transfer was performed: **Transfer Day3** implantation rates were: HIGH 45.13% ($n = 113$); MEDIUM 32.95% ($n = 88$) and LOW 27.50% ($n = 120$) ($p = 0.040$). **Transfer Day5** implantation rates were: HIGH 67.74% ($n = 31$); MEDIUM 57.14% ($n = 28$) and LOW 53.13% ($n = 32$).
Limitations, reason for caution: The retrospective nature of this study may be a reason for caution; however it is representing a consistent amount of embryos analysed by EEVA test and the starting point of future prospective studies to demonstrate the clinical usefulness of this test in our clinic.
Wider implications of the findings: Our study has demonstrated that embryo selection in the automated time-lapse supported by the use of a two variable morphokinetic model is related with reproductive outcome. The observed relationship with the implantation potential reflects a direct link between the parameters provided by the automatic system and embryo quality. A larger sample size will be mandatory to demonstrate a significant relationship and prospective randomized studies to exhibit the potential improvement that this clinical tool may offer.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: Morphokinetic parameters, time-lapse, embryo, implantation

P-174 Analysis of clinical outcome achieved with 126 poor quality blastocysts obtained on day-7 of culture in 944 Preimplantation Genetic Screening cycles

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Study question: The aim of this study was to evaluate the development and implantation potential of poor quality blastocysts obtained on day-7 of culture in Preimplantation Genetic Screening (PGS) cycles performed with trophoectoderm biopsy and array comparative genomic hybridization (aCGH).
Summary answer: The blastocysts obtained on day-7 are often discarded, due to their poor morphological quality. Our study demonstrates that these blastocycts have the same probability of being euploid, as those showing morphologically higher quality and they lead to a good implantation rate; therefore they should be considered for cryopreservation and transfer.
What is known already: Historically, the more widespread employed selection strategy to identify embryos with higher implantation potential, has been based on morphological criteria. However, several studies highlighted a weak correlation between this conventional method of embryo evaluation and their ploidy status. Recently, other embryo selection strategies, both invasive and non-invasive, are under evaluation. To date, the most efficient way to identify euploid blastocysts seems to be the trophectorderm biobsy followed by the genetic test.
Study design, size, duration: From January 2012 to December 2014, 944 PGS cycles with trophoectoderm biopsy and aCGH were performed. The development of 126 poor quality blastocysts, obtained on day-7 of culture, was analyzed. Mean female age was 37.2 ± 4.46 years old.
Participants/materials, setting, methods: Blastocysts were biopsied on day-5, day-6 or day-7 and immediately vitrified, waiting for the genetic results. Sometimes, the blastocysts obtained on day-5 were transferred on day-6 of the fresh cycle. The euploid balstocysts formed on day-6 or day-7 were transferred after thawing in a subsequent natural cycle.
Main results and the role of chance: A total of 7384 oocytes were injected and 5820 (78.8%) of them fertilized; 5742 (98.7%) embryos formed on day-3 and other 210 embryos, previously frozen without genetic analysis, were thawed and cultured until the blastocyst stage, in order to perform a trophectoderm biopsy. A total of 3076 (56.2%) blastocysts were obtained (1914 on day-5, 1036 on day-6 and 126 on day-7); 2983 of them were biopsied (1871 on day-5, 996 on day-6 and 116 on day-7). After the genetic analysis, 977 blastocysts (32.8%) resulted euploid. Of the 116 blastocysts analyzed on day-7, 32 (27.6%) were euploid even if 53.1% ($N = 17$) of them showed a poor morphological grade. Seventeen day-7 blastocysts were transferred and 8 (47.1%) of them implanted.
Limitations, reason for caution: More data, including take home baby rate and perinatal outcomes on babies born, are necessary. There is still a considerable disagreement if morphological evaluation of the embryos can be representative of their ploidy status.

Wider implications of the findings: The findings of this study show that also the poor quality and slower blastocysts, that would have been discarded before reaching the full blastocyst stage, can be euploid and implant, improving cumulative pregnancy rate. Even if blastocyst morphology, developmental rate and ploidy status seem to show a weak association, there is still a lack of knowledge regarding their viability and PGS remain the safer technique to minimize the risk of transferring chromosomally abnormal embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No specific funding was obtained for this study. None of the authors have any competing interests to declare.
Trial registration number: NA.
Keywords: preimplantation genetic screening, balstocyst biopsy, blastocyst morphology

P-175 Effect of embryo culture media on percentage of males at birth

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Study question: Does culture medium influence the percentage of males at birth?
Summary answer: G5™, Global, G5™ PLUS, and Quinn's Advantage Media are associated with different percentages of males at birth within the ICSI cycle group; the percentage of males of G5™ is significantly higher than Global, G5™ PLUS, and Quinn's Advantage Media.
What is known already: Male and female embryos have different physiologies during pre-implantation development. Manipulating the energy substrate and adding growth factors have a differential impact on the development of male and female embryos.
Study design, size, duration: This was a retrospective analysis of the percentage of males at birth, and included 4,411 singletons born from fresh embryo transfer cycles between January 2011 and August 2013.
Participants/materials, setting, methods: Only singleton gestations were included. Participants were excluded if pre-implantation genetic diagnosis, donor oocytes, and donor sperm were used. The database between January 2011 and August 2013 was searched with unique medical record number, all patients were present in the database with only one cycle. Demographics, cycle characteristics, and the percentage of males in the four culture media groups were compared with analysis of variance or c^2 tests. Multivariable logistic regression was performed to determine the association between the sex at birth and culture media after adjusting for other confounding factors, including parental age, parental BMI, type of infertility, parity, number of embryos transferred, number of early gestational sacs, cycles with testicular sperm aspiration (TESA)/percutaneous epididymal sperm aspiration (PESA)/testicular sperm extraction (TESE), number of oocytes retrieved, cycles with blastocyst transfers, and gestational age within ICSI group.
Main results and the role of chance: Within the IVF group, the percentage of males at birth for G5™, Global, Quinn's, and G5™ PLUS media were comparable ($p > 0.05$); however, within the ICSI group, there was a lower percentage of males in the Global (47.16 vs. 56.05%; $p = 0.003$), G5™ PLUS (47.69 vs. 56.05%; $p = 0.005$), and Quinn's media (44.97 vs. 56.05%; $p = 0.009$) compared with the G5™ media. The percentage of males at birth in the Global, G5™ PLUS, and Quinn's media were comparable ($p > 0.05$). Multivariable logistic regression indicated that culture media (G5™ vs. Global, G5™ PLUS, and Quinn's) were significantly associated with the sex at birth ($p = 0.008$) after adjusting for parental age, parental BMI, type of infertility, parity, number of embryos transferred, number of early gestational sacs, cycles with TESA/PESA/TESE, number of oocytes retrieved, cycles with blastocyst transfers, and gestational age.
Limitations, reason for caution: This study was not a RCT and that allocation of treatment cycles over the four media was not completely at random. Cigarette smoking was not included in the current study because this confounding factor was not registered in our database. Moreover, intra-variability of sperm selection between the five embryologists may directly affect the percentage of males;
Wider implications of the findings: Our study suggests that human embryogenesis responds differently to G5™, Global, G5™ PLUS, and Quinn's Advantage Medium. This finding can be generalized to other commercial culture media.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Science Foundation of China.
Trial registration number: NA.
Keywords: sex ratio, culture media, ICSI

P-176 Comparison of dynamic culture and dynamic culture with specialized surfaces culture method in poor responders

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Study question: In poor responders, conventional static culture (SC) method was used as a control group. We compared dynamic culture (DC) group that using micro-vibrator and dynamic culture with co-culture (DCC) group that using cumulus cell as a specialized surfaces. Embryonic development rates and pregnancy rates were compared among the groups.
Summary answer: The total pregnancy rates were significantly increased when the DC was applied to the embryonic culture in poor responders. There were no differences in the total pregnancy rates between DC group and DCC group. The blastocyst development rates were improved when DCC was applied to surplus embryos in poor responders.
What is known already: Advanced culture platforms that are classified as new static culture platforms, dynamic culture platforms, specialized surfaces have been developed in the current ART fields. The beneficial effects of micro-vibration have been reported already in human IVF. Furthermore, new trends have been attempted to try combining each culture platforms. We are looking forward to find out the effects of combination of dynamic culture and specialized surfaces.
Study design, size, duration: We conducted a review between January 2011 and November 2014. Cycles of conventional SC group, DC group and DCC group were 857, 804 and 484 cycles respectively. Number of surplus embryos of SC group, DC group and DCC group were 592, 623, and 373 respectively.
Participants/materials, setting, methods: The zygotes were cultured in a 50? Micro-droplet for 48 h, and the embryos were subsequently selected for transfer. Some surplus embryos were cultured for blastocyst development. A micro-vibrator was set at a frequency of 42 Hz, 5 s/30 min duration for embryo development. Autologous cumulus cell was used for co-culture.
Main results and the role of chance: There were no differences in the average age, number of oocytes, fertilization rate, zygote number, embryo transfer number among the groups. The total pregnancy rates were significantly higher in the DC group (175/804 = 21.8%) and DCC group (105/484 = 21.7%) than in the SC group (142/857 = 16.6%). There were no differences in the total pregnancy rates between DC group and DCC group. And, there were no differences in the blastocyst development rates between the SC group (158/592 = 26.7%) and the DC group (189/623 = 30.3%). However, the blastocyst development rate in the DCC group (141/373 = 37.8%) was higher than in the SC group and DC group.
Limitations, reason for caution: Poor responders exhibit advanced age, previous ovarian surgery or pelvic adhesions, all of which can contribute to a reduced oocytes and embryo quality in comparison with normal responders. Induction protocols for various poor responders are routinely utilized to maximize the numbers of eggs in this hospital.
Wider implications of the findings: The embryonic development rate was improved in DC method in poor responders. Especially, the blastocyst development rates were significantly increased when DCC was applied to surplus embryos in poor responders. And, the cumulative pregnancy rate in the DCC group is expected to be higher than in the SC group and the DC group because of significantly higher blastocyst development rates. Further investigations are necessary to determine the effects of combination of the culture platforms.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maria Fertility Hospital.
Trial registration number: NA.
Keywords: static culture, dynamic culture, spercialized surfaces

P-177 Pregnancy outcome of frozen warmed blastocysts developed from multinucleated cleavage stage embryos

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Study question: This study aimed to ascertain whether frozen-warmed blastocysts arising from embryos identified as having one or more multinucleate

blastomeres (MNB) during development, were capable of giving rise to a clinical pregnancy and live birth following frozen transfer.
Summary answer: Good quality blastocysts which had developed from embryos with MNB at cleavage stages, had a high rate of survival following vitrification and warming and also gave rise to a high proportion of clinical pregnancies as well as live births following frozen embryo transfer.
What is known already: Embryos with MNB are frequently observed during preimplantation development. Such embryos have previously been associated with a low pregnancy and implantation rate, compromised embryo development and potential increased chromosomal abnormalities during *in vitro* studies. MNB embryos are therefore often regarded as abnormal and excluded from clinical use. However, some retain the capability of developing into good quality blastocysts and occasionally give rise to a live birth, following transfer in a fresh cycle.
Study design, size, duration: Retrospective study carried out over a four year period. All embryos which developed into good quality blastocysts were vitrified and stored. In subsequent frozen embryo transfer cycles pregnancy and implantation rate was compared for blastocysts in which MNB had been identified and a control group with no evidence of MNB.
Participants/materials, setting, methods: All embryos which were not replaced or frozen on day 3 were cultured to the blastocyst stage of development using Vitrolife sequential medium in a low oxygen atmosphere. Good quality blastocysts were frozen using a vitrification protocol (Irvine). Blastocysts were warmed and replaced in a frozen embryo transfer cycle.
Main results and the role of chance: 141 blastocysts arising from MNB embryos were vitrified and stored. So far, 17 MNB blastocysts have been thawed. Fifteen have been replaced in a single embryo transfer (SET) resulting in an implantation rate (IPR) of 73% (11/15 foetal sacs) and a clinical pregnancy rate (CPR) of 53% (8/15 foetal hearts). Five gave rise to a healthy live birth with 3 pregnancies ongoing. This compares with a control group of 127 thawed and transferred non-MNB blastocysts, with IPR and CPR of 49% (62/127) and 39% (50/127) respectively following SET. In addition, in two separate double embryo transfers, an MNB blastocyst was transferred with an accompanying non-MNB blastocyst, resulting in two twin clinical pregnancies and two live births of healthy dizygotic twins in each case.
Limitations, reason for caution: Under normal circumstances, blastocysts arising from embryos without MNB are used first for fresh and frozen embryo transfer procedures. Therefore, due to practical constraints, the number of embryos in the MNB study group is limited and a larger cohort study is required to obtain robust long term outcome data.
Wider implications of the findings: Although the number of transfers in the study group is small, the data presented provides evidence that embryos with multinucleated blastomeres, which then develop into good quality blastocysts, are capable of implanting at a rate comparable with the control group and can give rise to a normal live birth. This suggests that such embryos should be assessed in relation to their developmental capacity rather than their cleavage stage morphology and should not be routinely discarded.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by Edinburgh Reproductive Endocrine Centre.
Trial registration number: NA.
Keywords: multinucleated blastomere, blastocyst, pregnancy rate

P-178 Effect of storage duration of cryopreserved blastocysts on pregnancy and neonatal outcome: a retrospective single-center cohort study of 6,117 blastocysts vitrified by open vitrification system

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Study question: Does the storage duration of blastocysts vitrified by open vitirification system influence the pregnancy and neonatal outcomes?
Summary answer: The storage duration of blastocysts vitrified by open vitirification system caused no negative influence on pregnancy and neonatal outcomes.
What is known already: It has been known that storage duration of embryos cryopreserved by slow freezing and closed vitrification system had no negative impact on survival, implantation and live birth rates, neonatal outcomes and congenital malformations in babies born. However, a large cohort study on the effect of storage duration in the case of embryos vitrified by open vitrification system has not been evaluated.
Study design, size, duration: A retrospective cohort study of 6,117 autologous single vitrified-warmed blastocyst transfer (SVBT) cycles was conducted in single center from 2007 to 2012. This cohort fulfilled the following criteria: egg retrieval age 35–39 years, SVBT of day 5 blastocyst and follow-up data on pregnancy and neonatal outcome was available.
Participants/materials, setting, methods: The oocytes were retrieved by minimum stimulation or natural cycles. Blastocysts were vitrified by cryotop methods. Main outcome measures were clinical pregnancy (CPR: confirmation of gestational sac) and live birth rate (LBR) per SVBT. Secondary outcome was neonatal outcome including congenital malformation rate and gestational age.
Main results and the role of chance: Of 6,117 SVBT cycles, cryo-storage duration was classified into four groups: A: 0–3 months ($n = 4761$), B: 4–12 months ($n = 683$), C: 1–3 years ($n = 596$), and D: 4–8 years ($n = 77$). The survival rates after warming were comparable among groups. No significant differences was observed in CPR and LBR (CPR: A: 57.2%, B: 54.1%, C: 52.9% and D: 53.9% and LBR: A: 44.2%, B: 41.4%, C: 41.5% and D: 48.7%). Birth weight, gestational age and congenital malformation rates were similar in all groups.
Limitations, reason for caution: This study was limited by SVBT in minimal stimulation and natural cycle IVF. This cohort included multiparous patients (A: 14.3%, B: 18.0, C: 77.8%, D: 97.4%) and the patients who received multiple cycles. The long-term follow-up studies would be necessary.
Wider implications of the findings: Our results showed blastocyst cryo-storage duration didn't influence pregnancy and neonatal outcomes even in the case of open vitrification system. Therefore, the open vitrification system is usefully applicable as a cryopreservation devices for infertile couple in ART and long-term storage is also possible with no negative impact on IVF outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kato Ladies Clinic.
Trial registration number: NA.
Keywords: vitrification, open device, single blastocyst transfer, minimal ovarian stimulation, cryopreservation

P-179 Retrospective validation of EEVA (Early Embryo Viability Assessment) at large scale. Correlation between eeva categories and blastocyst formation rate

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Study question: Is there a correlation between the three EEVA predictive categories: High, Medium, Low and the blastocyst formation/quality rate?
Summary answer: Yes, the three EEVA predictive categories are correlated with the formation of blastocysts and with the formation of good quality blastocysts.
What is known already: The EEVA test utilizes an algorithm based on early kinetic markers defined as P2 = t3-t2 (time to 3 cell – time to 2 cell) and P3 = t4-t3 (time to 4 cell – time to 3 cell). Taking in consideration these variables, the model classifies embryos as High, Medium or Low according to their probability of becoming a blastocyst. A few groups have tested this algorithm; however a large scale validation is necessary.
Study design, size, duration: Retrospective study of 432 patients and 2198 embryos analysed between February and November of 2014.
Participants/materials, setting, methods: University-affiliated infertility clinic. Patients were cultured in a standard incubator using a non-invasive test that combines time-lapse image analysis with cell-tracking software, EEVA (Early Embryo Viability Assessment).
Main results and the role of chance: A total of 432 patients generated 2198 embryos. Out of those, 59.5% (1307/2198) developed to the blastocyst stage; however when categorizing by EEVA, the blastocyst formation rate for the three categories was: High = 80.4% (201/250); Medium = 61.1% (323/529) and Low = 52.0% (509/961); $p < 0.0001$. The rest of the embryos ($n = 458$) were not suitable for grading by the software. In addition, development to 'good quality' blastocysts (defined by inner cell mass and trophectoderm morphology) was also analysed with decreasing percentages as we moved on between categories. More specifically: High = 44.7% (90/201); Medium = 37.4% (121/323) and

Low = 32.6 % (166/509), $p = 0.014$. These results validate the ability of this objective and automated tool for blastocyst prediction and embryo selection.
Limitations, reason for caution: The retrospective nature of this study may be a reason for caution; however it represents a consistent amount of embryos being analysed by the EEVA test and the starting point of future prospective studies to demonstrate the clinical usefulness of this test in our clinic. This study is underway.
Wider implications of the findings: Blastocyst transfer has proven to improve implantation rates. This strategy may not apply when few or poor embryos are present on day 3. An automatic cell tracking system with early prediction of blastocyst formation represents an attractive alternative in these situations. The EEVA test improves embryo selection while minimizing handling and monitoring by the embryologist. To the best of our knowledge this is the largest independent data set used to retrospectively validate the EEVA test.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVI.
Trial registration number: NA.
Keywords: EEVA, blastocyst, morphokinetics

P-180 Contamination risks in human oocyte and embryos cryopreservation: open vs closed vitrifying systems

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Study question: To study the risk of contamination in open (cryotop) and closed (cryotip) vitrifying devices for oocyte and embryo cryopreservation by evaluating the presence of contaminants (bacteria and fungi) in the same medium in which they were thawed and evaluate the sterility conditions of NL2 in oocytes and embryos banks.
Summary answer: No contaminants were found in the oocytes thawing media (open device) or in embryos (closed device). No fungi were found in NL2. However, saprophytic and pathogenic bacteria were found in all NL2 containers of the oocytes and embryos bank. Periodic chemically sterilization in NL2 containers is recommended.
What is known already: The risk of disease transmission in humans during assisted reproductive technology (ART) procedures has been described. Most micro-organisms can survive storage at LN2 temperatures. The cryoprotectants used in embryo and oocyte cryopreservation also protect bacteria and viruses. During assisted reproductive procedures, cryostorage is the only situation where large quantities of biological materials of patients are kept together in a common liquid medium. Although the temperature of liquid nitrogen is -196ºC, it may transmit infective agents from one sample to the other if they are not sealed properly.
Study design, size, duration: Retrospective study of vitrifying device safety and NL2 sterility performed from January to November 2014. Every month, a sample of open and one of closed device was evaluated. The NL2 supplied by the company, that of the storage container and also the NL2 of the containers of the oocyte-embryo bank were also evaluated.
Participants/materials, setting, methods: From each of the eight bank containers, 96 thawing media of oocytes vitrified in cryotop and 96 of embryos vitrified in cryotip were evaluated for the presence of contaminants (bacteria and fungi). Both types of devices were stored in the lower canister position. The NL2 was analyzed 3 times a year. Vitek2 automated system (BioMerieux) and the corresponding susceptibility to antibiotics were used for the bacteria identification. Fungus detection was performed by evaluating the colonies morphology developed and their microscopic characteristics.
Main results and the role of chance: No bacteria or fungi were observed in any of the devitrifying media regardless of the type of device used (open or closed), nor in the NL2 supplied by the company. No fungi were observed in any of the NL2 samples tested. However, saprophytic bacteria such as: *Stenotrophomonas maltophilia, Enterobacter* spp; *Bacillus* spp were found in all NL2 containers of the oocyte-embryo bank. Mostly environmental bacteria were identified in all samples of LN2. However, in the containers bottom some agents such as: *Alcaligenes faecalis ssp faecalis-Sphingomonas paucimobilis*, *Sphingomonas paucimobilis*, *Acinetobacter baumannii* and *Chrysenomonas luteola* were isolated. *Sphingomonas paucimobilis* was resistant to 6 antimicrobial compounds and are capable of causing nosocomial infections in humans. Cryopreservation devices cryotop and cryotip were safe for oocyte and embryo banking.
Limitations, reason for caution: The fact that micro-organisms survive in LN2 is important for the potential of disease transmission to recipients by embryo transfer and for testing of samples for health certification of embryos for international movement.
Wider implications of the findings: Storage containers should be emptied and cleaned periodically due to the risk of lost straws or small particles of contaminated material that falls to the bottom of a large container. The air in the room could be also the cause of bacterial contaminations. The operators can lead to contamination by contact or peeling during processing of samples or the handling of cryogenic tanks. Periodic chemically sterilization in NL2 containers is recommended for oocyte and embryo banking.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital Universitario y Politécnico La Fe, Valencia, Spain.
Trial registration number: NA.
Keywords: oocyte and embryos vitrification, open vs closed vitrifying systems, NL2 contamination risk

P-181 Distribution of mitochondria in fresh and frozen-thawed/devitrified human oocytes at different stages of maturation

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Study question: The aim of this study was to elucidate what is the distribution of mitochondria in human mature MII (unfertilized) oocytes and immature germinal vesicle (GV) oocytes and if the cryopreservation procedure (vitrification or slow freezing) affects it.
Summary answer: In GV oocytes the pattern of mitochondrial distribution was appearing as aggregated clusters and was similar in all cells, while in MII oocytes differed and varied from aggregated clusters to smooth pattern. There was no difference in distribution of mitochondria between fresh, devitrified and frozen-thawed oocytes.
What is known already: The proper function and distribution of mitochondria in the oocyte is essential for subsequent embryo development since the early embryo is dependent only on mitochondria stored in the mature oocyte and zygote. From the literature is it known that in GV oocytes mitochondria are distributed in homogeneous clusters around the oocyte, while in MII oocytes mitochondria are more concentrated around the polar body and appear as clusters in the perinuclear ring.
Study design, size, duration: Altogether 127 oocytes were included into our study: 29 fresh oocytes (22 MII and 7 GV oocytes), 37 oocytes after vitrification/devitrification (24 MII and 13 GV), and 61 oocytes after freeze-thawing (43 MII and 18 GV oocytes) to evaluate the mitochondrial distribution and differences between the groups.
Participants/materials, setting, methods: The fresh, devitrified or frozen-thawed oocytes were stained by Mitotracker to analyze the mitochondrial distribution and by Hoechst 33258 to identify the position of genetic material and then observed under the fluorescent microscope.
Main results and the role of chance: In GV oocytes the pattern of mitochondrial distribution appeared as aggregated clusters around the oocyte and it was similar in all cells, irrespective to fresh, frozen-thawed and devitrified oocytes. Otherwise, 3 different patterns of mitochondrial distribution were observed in MII oocytes: (1) smooth pattern around the polar body with aggregated clusters in the perinuclear ring (54.5% of fresh oocytes, 54.5% of devitrified oocytes and 52.9% of frozen-thawed oocytes), (2) smooth pattern throughout the whole cell (36.4% of fresh oocytes, 27.3% of devitrified oocytes and 32.4% of frozen-thawed oocytes) and (3) aggregated clusters as in GV oocytes (9.1% of fresh oocytes, 18.2% of devitrified oocytes and 14.7% of frozen-thawed oocytes). There was no significant differences in these patterns between fresh, devitrified and frozen-thawed oocytes.
Limitations, reason for caution: It would be necessary to analyze a higher number of oocytes to be more relevant in a conclusion about no significant differences in the mitochondrial distribution between fresh, devitrified and frozen-thawed oocytes.
Wider implications of the findings: The data from literature show that mitochondria in MII oocytes are more concentrated around the polar body (smooth pattern) and appear as clusters in the perinuclear ring. Our results suggest two

additional patterns of mitochondrial distribution in MII oocytes. These two patterns might appear because the oocytes were not normal and for this reason did not fertilize. Maybe this staining could explain why the oocytes did not fertilize.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding sources and no competing interest to declare.
Trial registration number: NA.
Keywords: oocytes, mitocondrial distribution, cryopreservation, vitrification

P-182 A new strategy to diagnose embryo viability combining protearray and time-lapse technologies

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Study question: Can we develop an embryo viability diagnostic tool using a combination of biochemical fingerprint and a time-lapse morphokinetic analysis?
Summary answer: Our results suggest for the first time the utility of a combined biochemical/morphokinetic diagnostic tool to select embryos for transfer according to their implantation potential.
What is known already: Time-lapse technology and morphokinetics has been validated as a clinical tool to improve embryo selection (Rubio et al 2014). Biochemical fingerprinting by proteomics is currently available for embryo spent culture media analysis and experimental data suggest its potential as method to study embryo viability (Dominguez et al. 2014). Both technologies has not been combined yet.
Study design, size, duration: Retrospective cohort study on 21 recipients during 2014 undergoing ICSI with donor oocytes and embryo transfer on blastocyst stage. We selected 28 transferred embryos for a detailed analysis of spent culture media and morphokinetic parameters, 16 implanted embryos after single embryo transfer and 12 non-implanted.
Participants/materials, setting, methods: We analyzed six proteins in the embryo spent media: SCF, TNFR1, IFNa2, IL-6, CXCL11, GM-CSF by Luminex technology and combined with exact timings (hours) of [cell cycle duration (cc2), blastomere synchrony (s2) and exact timing of 5 blastomere cleavage (t5)] analyzed by a time-lapse incubator.
Main results and the role of chance: Logistic regression analysis by forward step likelihood selection method revealed that the presence/absence of IL-6 and the duration of the second cell cycle (cc2) were the most relevant embryo features to be used for embryo selection. We combined these two parameters to obtain a hierarchical model, this establish four categories (A/B/C/D) based on the presence of IL-6 and cc2 range between 5 and 12 h. A direct relationship was observed between morphology categories and implantation rates, those with IL-6 presence and cc2 (5-12) implanted significantly more ($p = 0.036$). Table. Results were only referred to those embryos with known implantation (KID embryos).
Limitations, reason for caution: The retrospective nature of this study may be a reason for caution. Clinical validation is mandatory to confirm the effectiveness of this study.
Wider implications of the findings: Blastocyst transfer has proven to improve implantation rates. This strategy may be applied in combination with time-lapse and proteome analysis to improve embryo selection while minimizing handling and monitoring by the embryologist. To the best of our knowledge this is the first evidence provided of the clinical combination of both technologies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVI Valencia.
Trial registration number: NA.
Keywords: embryo, proteomics, time-lapse

P-183 Value of transferring embryos formed by non-pronuclear oocytes at the time of fertilization assessment

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Study question: Is there value in transferring embryos formed by non-pronuclear (0PN) oocytes at the time of the fertilization assessment?
Summary answer: There is value in transferring 0PN embryos when there is a lack of normal 2PN embryos for transfer in fresh cycles. Supernumerary 0PN embryos after transfer in fresh cycles are worth freezing for a subsequent frozen-thawed embryo transfer cycle.
What is known already: There are few reports which describe the transfer of embryos formed by 0PN oocytes; the available data are limited and a systematic study is lacking.
Study design, size, duration: The current study was a retrospective analysis of 0PN embryo transfers in fresh and frozen-thawed cycles. In fresh cycles, transfer of cleavage-stage 0PN embryos occurred in 159 cycles (research group [FC-G]). To determine whether or not the outcomes of transfer in FC-G was related to the origin of the embryos from 0PN oocytes, a matched group (FM-G [159 cycles]) were set in the study. In frozen-thawed cycles, 39 cleavage-stage and 82 blastocyst-stage 0PN embryo transfers were chosen as the research groups (cleavage-stage embryos [TC-G]; blastocyst-stage embryos [TB-G]). To study the effect of 0PN transfer In frozen-thawed cycles, 39 cleavage-stage (cTC-G) and 82 blastocyst-stage transfers (cTB-G) were set for comparison groups. These transfer cycles were performed during the same period at the Reproductive Medical Center of Peking University Third Hospital.
Participants/materials, setting, methods: In the fresh cycles, the recruitment criteria for FM-G were as follows: 1. matched the female ages, number of oocytes retrieved, and fertilization technologies (IVF or ICSI) one-to-one with FC-G; and 2. Transfer of only 2PN cleavage-stage embryos. In frozen-thawed cycles, the recruitment criteria for cTC-G and cTB-G were as follows: 1. matching the female ages and fertilization technologies (IVF or ICSI) one-to-one with TC-G and TB-G, separately. 2. Transfer of only 2PN (cleavage-stage in cTC-G and blastocyst-stage in cTB-G) embryos was performed during the same period for the TC-G and TB-G.
Main results and the role of chance: In fresh cycles, the characteristics of patients in the FC-G were comparable with the patients in the FM-G. Implantation rate (IR) in the FC-G was lower than the FM-G (8.04% [16/198] vs. 19.50% [55/282]; $P = 0.000$). In frozen-thawed cycles, the IR in the TC-G was lower than the cTC-G (15.38% [12/78] vs. 28.24% [25/85]; $P < 0.05$), and the IR in the TB-G was comparable to the cTB-G (39.56% [36/91] vs. 48.18% [53/110]; $P > 0.05$). The low IR of 0PN embryos was related to embryos originating from 0PN oocytes. Blastocyst cultures can be a way to select better the normal 0PN embryos for transfer and achieved a satisfactory IR.
Limitations, reason for caution: Further study is warranted regarding time-lapse technology of 0PN oocytes. The security of 0PN transfer remains to be confirmed in a large patient sample with long-term follow-up.
Wider implications of the findings: There is a value to transferring 0PN embryos in fresh or frozen-thawed cycles that result in pregnancy and live births. The transfer of 0PN embryos is safe based on the current results, and blastocyst cultures give rise to normal 0PN embryos for transfer and a satisfactory outcome.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by the National Natural Science Foundation of China for Young Scholars (grant nos. 81200437 and 81300483). The authors have no conflicts of interest to declare.
Trial registration number: The study was approved by the Ethics Committee of Peking University Third Hospital (reference no. 20080612) and all patients signed written informed consent.
Keywords: 0PN, embryo transfer, implantation rate

P-184 Blastomere nuclearity: the influence of blastomeres with no apparent nuclei on blastocyst formation and quality

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Study question: Are embryos presenting at least one blastomere with no apparent nucleus on the second or third day of development more likely to fail to develop into blastocysts?
Summary answer: The presence of at least one blastomere with no apparent nucleus on day 2 or day 3 reduces blastocyst formation in 22 and 24% respectively. The presence of at least one blastomere with no apparent nucleus on day 2 or day 3 reduces blastocyst hatching in 27 and 32% respectively.
What is known already: Embryos are generally selected for transfer based on their morphological appearance. In day 2 and day 3 transfers, embryos are scored according to parameters such as number of blastomeres, blastomere symmetry and fragmentation rate. Additionally, the detection of multinucleation in blastomeres is an important criterion in embryo selection. Multinucleated embryos are related to increased aneuploidy rate and lower blastocyst formation, implantation and live birth rates. However, information regarding blastomeres with no apparent nuclei is scarce.
Study design, size, duration: A total of 17,340 zygotes obtained from 2,835 intracytoplasmic sperm injection (ICSI) cycles, performed in unselected patients attending a private assisted fertilization center between January 2010 and December 2013, were prospectively analyzed.
Participants/materials, setting, methods: Embryos were morphologically evaluated on days 2, 3 and 5 of development. The presence of blastomeres with no apparent nuclei on day 2 and 3 was recorded and then associated with blastocyst formation, quality and hatching status using logistic regression.
Main results and the role of chance: A total of 1985 embryos on day 2 and 2249 embryos on day 3 showed at least one blastomere with no apparent nucleus (11.4 and 14.0%, respectively). The presence of at least one blastomere with no apparent nucleus on day 2 or day 3 of development were determinant to the decreased odds of blastocyst formation (OR: 0.76, CI: 0.69–0.84 and OR: 0.78, CI: 0.69–0.87, respectively). The presence of blastomere with no apparent nucleus did not influence trophectoderm and inner cell mass quality. The presence of at least one blastomere with no apparent nucleus on day 2 or day 3 of development were determinant to the decreased odds of blastocyst hatching (OR: 0.73, CI: 0.59–0.89 and OR: 0.68, CI: 0.54–0.86, respectively).
Limitations, reason for caution: Since nuclear formation is a dynamic process, it may be mistaken to evaluate the nuclear status relying on observations performed within a short time interval. Nevertheless, the evaluation of nuclear status using simple light microscopy has proven to be predictive of embryo developmental capacity.
Wider implications of the findings: One of the most important steps in assisted reproduction is embryo selection for transfer. Although the combination of embryo developmental rate and morphology is noninvasive and easy to perform, the usefulness in predicting pregnancy is questionable. The search continues for additional morphological markers of embryo quality. Careful nuclear observation, taking into account not only the presence of blastomere multinucleation but also the absence of nucleus, should be part of the strategies used for embryo selection.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fertility – Centro de Fertilização Assistida
Trial registration number: NA.
Keywords: blastocyst, blastomere, embryo, ICSI, nucleus

P-185 Non-invasive prediction of human embryo implantation by mass spectrometry fingerprinting

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Study question: Is mass spectrometry (MS) fingerprinting able to predict cleavage or blastocyst stage embryo implantation?
Summary answer: Mass spectrometry fingerprinting combined with multivariate statistical model is a valuable tool for selecting the embryo with the highest implantation potential on the third or fifth day of development.
What is known already: The success of assisted reproductive technologies depends on the ability to select the most viable embryo in a cohort, which remains a challenge. Recently, new approaches have been reported, such as genomic, proteomic and metabolome profiling. Metabolomics is an emerging technology that provides the overall metabolic footprint of the surrounding medium rather than measures specific nutrients and metabolites. Recently MS fingerprinting has been shown to provide a reliable approach to evaluate culture media profile.
Study design, size, duration: Culture media samples harvested from 1,322 embryos obtained from 678 patients undergoing ICSI cycles, were split into those collected and transferred on respectively: (i) day-3 and day-3 (n = 395); (ii) day-5 and day-5 (n = 488); and (iii) day 3 and day 5 (n = 439).
Participants/materials, setting, methods: Samples were classified according to the cycle's implantation rate (100, 66.7, 50, 33.3, and 0%) and were individually diluted and analysed. Mass spectra were analysed by a multivariate statistical-model. The likelihood ratios were calculated for assessing the value of performing a diagnostic test by measuring sensitivity, specificity and predictive values.
Main results and the role of chance: More than 1350 ions per sample were observed within the range of 100–1000 m/z. Regarding the calibration set (0 and 100% samples), MS was capable of predicting the embryo implantation with: (i) 100% sensitivity and 90% specificity for samples collected on day 3 and embryo transfer performed on day 3, (ii) 98% sensitivity and 61% specificity for samples collected on day 3 and embryo transfer performed on day 5, and (iii) 100% sensitivity and 79% specificity for samples collected on day 5 and embryo transfer performed on day 5. For the other implantation groups (the validation set), the MS was capable of predicting the embryo implantation with a sensibility ranging from 87 to 100% and a specificity ranging from 41.6 to 100%.
Limitations, reason for caution: The model was able to predict with high reliability embryo implantation potential, but not in a definitive way, which is plausible since implantation requires a cross talk with a receptive endometrium.
Wider implications of the findings: The MS fingerprinting represents an independent but complementary tool to morphological parameters. In fact, MS fingerprinting combined with multivariate statistical model is a valuable tool for selecting the embryo with the highest viability, when used as an adjunct to morphological criteria. Therefore, it should be included in the laboratory routine for facilitating the selection of the most competent embryo for transfer.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This research was sponsored by the Grant for Fertility Innovation from Merck Serono: GFI 2012-4.
Trial registration number: NA.
Keywords: mass spectrometry, fingerprinting, embryo, implantation, metabolomics

P-186 Spiral wave: a novel phenomenon during fertilization observed by high-resolution time-lapse cinematography

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Study question: Using high-resolution time-lapse cinematography (hR-TLC) we observed a novel phenomenon in the ooplasm during the fertilization process. As a preliminary study, we investigated the relationship between this phenomenon, which we termed the 'spiral wave', and further embryonic development.
Summary answer: In both c-IVF and ICSI zygotes the spiral wave was initiated at sperm entry and continued until the cytoplasmic flare appeared. Since the spiral wave significantly delays flare initiation, it may have a negative impact on the fertilization process.
What is known already: In previous hR-TLC studies, we confirmed that after sperm-oocyte fusion, the 2nd polar body was extruded within 3 h, followed by the appearance of the fertilization cone within 30 min. After the disappearance of the fertilization cone, the cytoplasmic flare appeared at the sperm entry point, followed by pronuclear formation. We observed that cytoplasmic granules

moved around within the cytoplasm (the spiral wave), before the appearance of the cytoplasmic flare.
Study design, size, duration: We retrospectively analyzed hR-TLC images obtained from 199 oocytes from 189 patients who consented to our study. In some images we observed a 'spiral wave' in the cytoplasm during the fertilization process. We then investigated further embryonic development in zygotes with and without the spiral wave.
Participants/materials, setting, methods: A total of 199 oocytes were candidates for c-IVF ($n = 96$) and ICSI ($n = 103$). The hR-TLC was commenced 1.5 h after insemination in c-IVF oocytes and immediately after sperm injection in ICSI oocytes. Digital images were acquired for 2 days at 2-min intervals with an exposure time of 1/20 s.
Main results and the role of chance: Of 199 oocytes, 153 (60 in c-IVF, 93 in ICSI) fertilized normally and 16 (12 in c-IVF, 4 in ICSI) fertilized abnormally. The spiral wave was observed in 52 normally fertilized zygotes (14 in c-IVF, 38 in ICSI), and 6 abnormally fertilized zygotes (2 in c-IVF, 4 in ICSI), but not in unfertilized oocytes. The spiral wave was initiated at sperm entry and continued until the cytoplasmic flare appeared. The flare appeared from 4.8 ± 1.1 h in zygotes with the spiral wave (SW+) and 4.1 ± 1.3 h in zygotes without the spiral wave (SW-). The time required for flare appearance in SW+ zygotes was significantly less than in SW- zygotes ($P = 0.014$). Further embryonic development after flare appearance was similar in both groups.
Limitations, reason for caution: We could only observe whether the spiral wave occurred in the cross-sectional direction because hR-TLC images were 2-D. We speculated that the spiral wave might be caused by alteration of the cytoplasmic microtubules. We plan to analyze these cytoplasmic events using molecular biological methods.
Wider implications of the findings: The spiral wave was only observed in fertilized oocytes, irrespective of whether fertilization occurred normally and was closely associated with a delay in the appearance of the cytoplasmic flare. This study suggests that the spiral wave is related to an aberrant fertilization process, particularly the delayed formation of the sperm aster and may have negative impacts on zygote development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Mio Fertility Clinic.
Trial registration number: NA.
Keywords: zygote, embryonic development, cytoplasmic flare, ooplasm, fertilization

P-187 Blastocyst morphology does not significantly increase the selection between euploid blastocysts in frozen embryo transfer cycles: evidences from a prospective non-selection study

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Study question: Does blastocyst morphological evaluation correlate with reproductive competence of euploid embryos during frozen transfer cycles?
Summary answer: Even if extremely poor quality blastocysts implanted at a significantly lower rate, morphological grade and timing of development to the blastocyst stage are not good indicators to improve the selection among euploid embryos.
What is known already: No studies have prospectively attempted to correlate conventional parameters of blastocyst evaluation with euploid embryo viability in frozen embryo transfer (FET) cycles. It is still unknown whether euploid blastocysts with a different morphology and developmental rate implant at a different rate. This knowledge may be useful to further enhance embryo selection during PGS cycles. In this study, the relationship between conventional parameters of blastocyst evaluation and implantation potential of euploid blastocysts is investigated.
Study design, size, duration: This is a prospective non-selection study performed between December-2013 and October-2014 including 335 single FET of euploid blastocysts. Whenever more than one euploid blastocyst was obtained within a PGS cycle, the selection of the embryo to be transferred was subjected to randomization to rule out patient's specific confounding factors.
Participants/materials, setting, methods: PGS was performed in infertile patients of advanced female age (>35 years). Trophectoderm (TE) biopsy was performed on fully expanded or hatching blastocyst. Prior to biopsy, morphology was assessed and categorized (excellent/good/average/poor quality). The developmental rate was defined according to the day of biopsy post-fertilization (day 5/ 6/ 7).
Main results and the role of chance: Mean female age was 38.2 ± 3.4. 335 SET of euploid blastocysts (200 excellent, 54 good, 51 average and 30 poor; 143 day 5, 175 day 6, and 17 day 7) were performed in 300 patients resulting in 144 ongoing pregnancies (43%, 95% CI = 37.6–48.5), 19 biochemical (10.1%; 95% CI = 6.5–16.1) and 16 miscarriages (10%; 95% CI = 5.8–15.7). Logistic regression showed that only blastocysts morphology was weakly related to implantation. In particular, only poor quality blastocysts showed a significantly lower implantation rate compared to excellent, good and average quality ones (10 vs 47%, 37 and 55%, respectively, OR = 0.15, 95% CI = 0.04-0.53, $p < 0.01$). TE morphology was a stronger predictor compared to ICM (OR = 0.39, 95% CI = 0.02–0.21 and OR = 0.1, 95% CI = -0.1 to 0.1, respectively). Timing of development to blastocyst was not associated with implantation (day 5 = 51.7%, day 6 = 37.1%, day 7 = 29.4%) when adjusting for morphology ($p = 0.07$). Morphology were not associated to biochemical and miscarriages.
Limitations, reason for caution: Even if based on prospective design and a high sample size, this study is still not powered to exclude possible associations between blastocyst morphology and biochemical pregnancies, miscarriages and neonatal outcomes.
Wider implications of the findings: These data suggest that, provided that the expanded stage is reached, all poor morphology and slower growing embryos have to be biopsied and similarly considered for FET cycles avoiding the potential for exclusion of low quality but viable embryos from PGS cycles or, in general, from IVF procedures. Future research to identify additional biomarkers of reproductive potential to be used in parallel with aneuploidy screening is needed to further enhance selection among euploid blastocysts.
Study funding/competing interest(s): Funding by hospital/clinic(s) – GENERA, Reproductive Medicine Centers, Rome, Italy.
Trial registration number: ISRCTN81216689.
Keywords: embryo selection, PGS, embryo morphology, aneuploidies, implantation

P-188 Oxygen level during human IVF embryo culture does not affect birth weight

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Study question: Does oxygen level during human IVF embryo culture affect birth weight of the resulting children?
Summary answer: Our findings indicate that oxygen level during human IVF embryo culture does not affect birth weight.
What is known already: In animal studies, and also some human studies, embryo culture or more specifically the medium in which embryos are cultured during an IVF treatment affects birth weight, probably through epigenetic programming of the embryo. Little is known on the effect of oxygen level during culture on birth weight. Most IVF laboratories use either 20% (atmospheric) oxygen level or 5% (physiological situation).
Study design, size, duration: From January 2012 to December 2013, all oocytes and embryos from the same cycle were fertilized and cultured under 5 or 20% oxygen. Cycles were randomly allocated to one incubator with 5% ($n = 363$) or three with 20% oxygen ($n = 1043$). Clinical and other laboratory procedures were similar in both groups.
Participants/materials, setting, methods: Data between the oxygen groups were compared for the first cycle of a patient only ($n = 612$) and for all cycles ($n = 1406$). Neonatal data were collected from delivery reports from the hospitals or midwife practices.

Main results and the role of chance: In the 5% oxygen group there were significantly more embryos of good morphology ($P < 0.05$). This did not result in a higher ongoing pregnancy rate, but more embryos could be cryopreserved ($P < 0.01$). After a follow-up period in which (part of) these cryopreserved embryos were transferred, the cumulative ongoing pregnancy rate was higher in the 5% compared to the 20% group (5% difference for first cycles, $P < 0.05$). This did however not result in significantly more live births. In 95 live born singletons, birth weight, birth weight corrected for gestational age and gender (Z-score) and gestational age were similar in both groups, also when analysed separately for fresh or cryopreserved embryo transfers. In the analysis including all cycles, similar pregnancy and birth weight results ($n = 222$) were found.
Limitations, reason for caution: This is a retrospective exploratory study, not powered for a predefined birth weight difference. Significantly more cryopreserved embryos are still stored in the 5% oxygen group. Their future transfer may further improve live birth rates, which may result in a significantly better outcome when compared with the 20% oxygen group.
Wider implications of the findings: Based on our findings, the oxygen level during culture does not seem to affect birth weight. Nevertheless, culture under 5% oxygen is preferred as this might improve IVF success rates, especially when used in an IVF program with embryo cryopreservation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University Medical Center Groningen
Trial registration number: NA.
Keywords: oxygen, culture, IVF, birthweight, human

P-189 Morphokinetics of embryos developed from oocytes matured in vitro and in vivo in IVM cycles

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Study question: Does oocyte in vitro maturation (IVM) influence embryo development as assessed by morphokinetics?
Summary answer: Oocyte IVM does not affect embryo development in terms of morphokinetic parameters monitored by time lapse microscopy (TLM).
What is known already: The introduction of TLM in human IVF offers novel opportunities to assess embryo development. In fact temporal parameters, such as specific cleavage times and intervals, are able the predict blastocyst development and quality, as well as embryo implantation. Hence, we pursued the goal of comparing the morphokinetic behaviour of embryos developed from oocytes matured in vitro and in vivo in IVM cycles, to the aim of assessing possible effects of IVM on embryo development.
Study design, size, duration: The morphokinetics of embryos developed from oocytes matured in vitro (CC, $n = 35$) or in vivo (EC-MII, $n = 102$) was compared following culture and image acquisition at short intervals (20 min) by adopting the Embryoscope equipment.
Participants/materials, setting, methods: Oocytes were obtained from patients undergoing FSH/hCG-primed IVM cycles. Mature oocytes were microinjected after 6 h of culture, while immature oocytes were matured in vitro for 30-32 h prior to ICSI. Embryo morphokinetics was comparatively analysed in terms of cleavage times (T2–T5 and T8) and intervals (cc2, cc3, s2, s3).
Main results and the role of chance: The morphokinetic behaviour of EC-MII and CC embryos was entirely comparable, as suggested by the absence of statistical differences in the averages of all cleavage times and intervals. In particular, the T2–T5 and T8 times (hours) were 27.0 ± 3.7, 36.6 ± 4.8, 38.6 ± 5.5, 48.5 ± 6.4 and 54.6 ± 6.0 in the EC-MII group and 26.8 ± 4.6, 35.9 ± 3.5, 38.1 ± 4.8, 48.0 ± 5.5 and 54.7 ± 7.6 in the CC group. P was 0.378, 0.449, 0.644, 0.539 and 0.973, respectively. cc2, cc3, s2, s3 intervals were also very similar, with P of 0.891, 0.585, 0.112 and 0.638, respectively.
Limitations, reason for caution: The study is based on small numbers. It should be extended to larger populations and additional morphokinetic parameters.
Wider implications of the findings: This is the first observation describing thoroughly the development of embryos derived from in vitro matured oocytes. The fact that their morphokinetics is entirely comparable to that of embryos developed from oocytes matured in vivo does not support the hypothesis that IVM affects early embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Biogenesi, Reproductive Medicine Centre.
Trial registration number: NA.
Keywords: embryos, oocyte in vitro maturation, oocyte in vivo maturation, morphokinetics, time lapse microscopy

P-190 Comparison of clinical results and morphokinetics analysis between fresh and vitrified oocytes by intracytoplasmic sperm injection with testicular sperm

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Study question: The aim of this study was to assess the clinical efficacy of vitrified oocytes and fresh testicular sperm in TESE-ICSI for azoospermic patients. How does oocyte vitrification affect embryonic development?
Summary answer: There were no significant differences in clinical results or cell kinetics between fresh and vitrified oocytes with TESE-ICSI.
What is known already: Vitrification has been reported to be a simple, cost effective, efficient method for cryopreservation of mammalian and human oocytes.

Many studies have shown more positive results with oocyte vitrification than with slow freezing procedures.
Study design, size, duration: Retrospective study. The subjects were 138 couples with 149 oocyte-retrieval cycles for TESE-ICSI from June, 2006 to September, 2014. These were divided into 2 groups: Group I (vitrified oocytes + fresh testicular sperm) in 61 cycles, and Group II (fresh oocytes + fresh testicular sperm) in 88 cycles.
Participants/materials, setting, methods: We assessed the embryo development and clinical results in the two groups including cases of obstructive azoospermia (OA), non-obstructive azoospermia (NOA) and Klinefelter's syndrome (KS). In some cases, inseminated oocytes were individually cultured in a time-lapse system (Primo Vision), and analyzed the time points of each morphokinetic event were analyzed.
Main results and the role of chance: The survival rate of oocytes after warming was 87.4% (464/531). There were no significant differences in FR (57.5%:438/703 vs. 62.3%:438/703), good quality embryo rate (37.2%:94/253 vs. 45.2%:178/394), blastocyst formation rate (48.2%:96/199 vs. 56.4%:193/342), pregnancy rate per embryo transfer (34.7%32/96 vs. 47.9%:86/181) or miscarriage rate (22.5%:7/32 vs. 26.1%:23/86) between group I and group II under OA, NOA and KS. Furthermore, cell kinetics did not difference, while there were high standard deviations (S.D.) in the times of embryonic cell divisions, and intervals in vitrified oocytes.
Limitations, reason for caution: None.
Wider implications of the findings: Vitrification of oocytes before TESE allows the use of best condition sperm without freezing for ICSI. There were no significant differences of embryo development or clinical results between fresh and vitrified oocytes with TESE-ICSI. However, the time range of embryo divisions varied in vitrified oocytes. This seems to be an effect of vitrification. The use of vitrified unfertilized oocytes should surely be recommended for wide medical treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kyono ART Clinic – Kyono ART clinic Takanawa.
Trial registration number: NA.
Keywords: ICSI, morphokinetics, vitrified oocyte, testicular sperm, azoosoermia

P-191 The development of embryos from translocation carriers presenting for pre-implantation genetic diagnosis and single blastocyst transfer

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Study question: Do translocations affect the development of embryos and the proportion of top quality blastocysts suitable for trophectoderm biopsy, comparative genomic hybridisation (CGH) and single blastocyst transfer?
Summary answer: In couples where the maternal age is <38 and at least one partner is carrying a translocation there is a significant reduction in formation

and top quality blastocyst development compared to the general patient population in the same age group presenting for IVF over a 12-month period.
What is known already: Translocations and advanced maternal age have been linked with higher yields of chromosomal abnormalities. Comparative Genome Hybridisation (CGH) enables the identification of the whole genome, offering greater detection of chromosomal rearrangements. Blastocyst formation and overall morphology has been associated with higher implantation rates, further to this single blastocyst transfer have been shown to produce better rates of ongoing pregnancies.
Study design, size, duration: In a retrospective cohort study over a 12-month period the blastocyst development and quality parameters from embryos (n = 136) of translocation carriers presenting for IVF treatment and CGH were compared with embryos (n = 2402) from the general patient population.
Participants/materials, setting, methods: Embryos were assessed on days 3, 5 and 6 of development. Cell division of inner cell mass (ICM) and trophectoderm (TE) was used to distinguish top quality blastocysts. Cleavage stage transfer, oocyte donor and patients aged ≥38 were excluded.
Main results and the role of chance: Blastocyst formation rate assessed as the identification of a blastocoel was significantly lower in the translocation carrier patient group than the general patient population group (62.5 vs. 72.3%, $p < 0.01$). Top quality blastocysts suitable for either vitrification or transfer were also significantly reduced in translocation patients (31.6 vs. 49.5%, $p < 0.05$). Average age between patient groups did not differ ($p > 0.5$), although translocation patients were found to have more oocytes collected per cycle (16 ± 3.6 vs. 11 ± 6.3, $p < 0.05$) and a higher proportion of oocytes fertilised normally (12 ± 2.6 vs. 8 ± 4.9, $p = < 0.05$).
Limitations, reason for caution: Poor sperm parameters may influence embryo development and blastocyst quality and this was not addressed in the current study. Further analysis of an increased sample size would allow for improved matching of controls between the two patient populations.
Wider implications of the findings: Translocations in patients presenting for ART can be associated with greater incidence of cellular arrest and poor development of blastocysts prior to embryo transfer. The first waves of embryonic genome activation are driven by parental factors. If a parent were to carry a chromosomal anomaly, this could lead to alteration in transcription activation, protein synthesis and cytokinesis, all of which influence embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hollywood Fertility Centre.
Trial registration number: NA.
Keywords: blastocyst, translocation, PGD, trophectoderm

P-192 "Ruffling" phenomenon prior to the first cleavage observed by high-resolution time-lapse cinematography and its impacts on human embryonic development in vitro

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Study question: Does the 'ruffling' phenomenon that occurs prior to the first cleavage in human zygotes and that we found by high-resolution time-lapse cinematography (hR-TLC) play a negative role in developing good-quality embryos using assisted reproduction technology (ART) programs?
Summary answer: Although the etiology of embryonic ruffling remains unknown, this phenomenon is closely related to an increased incidence of multinucleated blastomere (MNB) formation and reduced developmental velocity during the zygote stage, which is, in turn, associated with an increased rate of poor-quality embryos.
What is known already: In previous hR-TLC studies, we clarified the time course of development during ART from fertilization to hatched blastocyst stage, including several specific physiological events. The first cleavage started within 2.4 ± 0.6 h after the male and female pronucleus (PN) disappeared (syngamy). During this period, the ooplasm showed no movement in most zygotes; however, some zygotes showed a 'ruffling' phenomenon caused by cytoplasmic fluctuations between syngamy and commencement of the first cleavage.
Study design, size, duration: Since 2003, donated oocytes (n = 210) have been used for hR-TLC observation. Of those, normally fertilized zygotes were categorized into two groups: without ruffling [ruffling (-)] and with ruffling [ruffling (+)]. We compared the incidence of MNB, developmental velocity, and the quality of embryos between these groups.
Participants/materials, setting, methods: Of the 210 oocytes available, 101 were candidates for c-IVF and 109 for ICSI. We started hR-TLC 1 h after insemination in c-IVF oocytes, and immediately after the ICSI procedure. Digital-images were acquired for 2 days. Good-quality embryos subsequently developed to the 4-cell stage were cryopreserved for future clinical use.
Main results and the role of chance: There was no significant difference in the incidence of ruffling between c-IVF and ICSI zygotes (32.2 vs. 27.3%, respectively). The ruffling (+) group showed significantly higher numbers of MNBs, compared to the ruffling (-) group (72.1 vs. 20.2%, respectively). The time required from second polar body extrusion to syngamy in the ruffling (-) zygotes was significantly lower than that for the ruffling (+) zygotes (20.1 ± 3.0 vs. 22.4 ± 3.7 h, respectively). However, there was no difference in the time required from syngamy to the commencement of first cleavage between groups. Of the ruffling (-) zygotes, 74.7% (74/99) developed into good-quality embryos, whereas only 32.6% (14/43) of ruffling (+) zygotes developed into good-quality embryos; this difference was significant.
Limitations, reason for caution: Our findings suggested that the ruffling phenomenon might be involved in the consequence of cytoskeletal instability. However, further studies, including molecular biological analyses, are needed to further characterize this phenomenon.
Wider implications of the findings: Our study demonstrated for the first time that ruffling prior to the first cleavage is closely associated with embryonic development, and therefore, might serve as a useful observational parameter for predicting embryo quality before first cleavage. However, because ruffling was also significantly related to high incidences of MNB and poor-quality embryos, this phenomenon could reflect alterations in cytoskeletal stability that may directly influence further cytokinesis including chromosome segregation, cytoplasmic fragmentation, and further development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Mio Fertility Clinic.
Trial registration number: NA.
Keywords: hR-TLC, ruffling, syngamy, multinucleated blastomere, embryonic development

P-193 Evaluation of embryo development cultured individually or in group : a prospective single center study of 200 ICSI cycles

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Study question: Does the culture of embryos individually or in groups effect upon blastocyst formation and quality when the embryos inseminated exclusively by ICSI are analysed?
Summary answer: Although there were no significant differences on blastocyst formation rate between individual and group culture, morphologically good quality blastocyst rate was significantly higher in group culture group.
What is known already: Previous studies have shown that group culture of zygotes is superior in terms of blastocyst formation, implantation and live birth as compared with individual culture in human. These zygotes in the previous studies included different insemination background; ICSI or conventional IVF and the study to compare these two culture methods in the case of zygotes produced exclusively by ICSI has not been assessed.
Study design, size, duration: A single-center cohort study of 200 cycles to compare the IVF outcome between group and individual culture was conducted in single center. Patients aged 30–46 years whose cycles obtained 3 matured oocytes and 3 regularly fertilized embryos by ICSI were enrolled from February 2014 through October 2014.
Participants/materials, setting, methods: IVF cycles were performed by minimum stimulation protocol. These cycles were randomly divided to two groups (100 cycles each); embryos cultured in groups of 3 embryos (group culture) or embryos cultured singly (individual culture) in 20ul droplet(s) of media. The development to blastocyst stage and blastocyst quality were evaluated.
Main results and the role of chance: The rates of blastocyst formation with expansion grades of 3 or greater (Gardner criteria) were 39.7% (119/300) in group culture and 39.33% (118/300)in individual culture group and no significant difference was observed in blastocyst formation rate. The rates of morphologically good quality blastocyst with ICM and TE grades of 3BB or greater were 47.9% (57/119) in group culture and 29.7% (35/118) in individual culture

group. Morphologically good quality blastocyst rate was significantly higher in group culture group ($p < 0.05$).
Limitations, reason for caution: This study was limited in minimal stimulation IVF. Therefore, the developmental outcome in the case of culturing more than 3 embryos was not assessed. The follow-up studies on pregnancy and neonatal outcome are needed.
Wider implications of the findings: Our results showed that group culture was superior in the term of morphologically good quality blastocyst rate suggesting the beneficial effect of group culture on increasing the proportion of blastocyst used for transfer or cryopreservation. There would be a possibility that group culture will become the recommended embryo culture system in the near future.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kato ladies clinic.
Trial registration number: NA.
Keywords: IVF, ICSI, embryo culture, culture in group, culture individually

P-194 Role of Sirt3 on mitochondria biogenesis and developmental competence of human in-vitro matured oocytes

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Study question: Does Sirt3 dysfucntion result in poor development outcome of human in-vitro matured (IVM) oocytes?
Summary answer: Inefficiency Sirt3 expression induced mitochondria dysfunction, and therefore failure to wipe off reactive oxygen species that declined the developmental competence of human IVM oocytes.
What is known already: Cytoplasm immaturation in IVM oocytes impaired IVM oocytes developmental competence. Mitochondria dysfunction resulted in the accumulation of free radicals that leads to DNA mutations, protein damage, telomere shortening and apoptosis. SIRT3 has emerged as a mitochondrial fidelity protein that directs energy generation and regulates ROS scavenging proteins. We investigated the potential role of Sirt3 gene in development process of human IVM oocytes by regulating mitochondrial biogenesis.
Study design, size, duration: Total 39 in vivo matured (IVO) oocytes and 242 IVM oocytes were collected from patients in ART cycles to detect Sirt1-7 expression, mitochondria biogenesis identification, produce fertilized embryos and function detection by siRNA injection. Blastocysts from IVO, IVM, mRNA- and siRNA-injection groups were used for ES cells derivation.
Participants/materials, setting, methods: Gene expression was performed by single cell RT-PCR methods. The karyotyping were detected by aCGH and cytogenetic method. The function of genes was identified using siRNA and in vitro transcripted mRNA injection methods. Embryos were graded as published criterions. Markers of ES cells were identified using immunofluorescence methods.
Main results and the role of chance: Retrospective analysis results reveled the higher abortion rate and increased ratio of poor quality embryos in patients with IVM cycles, which resulted in the poor ART outcome. Declined Sirt3 expression and mitochondria biogenesis were identified in IVM oocytes and the subsequent fertilized embryos compared with IVO counterparts. IVM oocytes developmental competence was further decreased when Sirt3 expression was inhibited using Sirt3 siRNA injection, but can be improved after injected with in-vitro transcribed Sirt3 mRNA. Comparable derivation efficiency of embryonic stem cells can be obtained using the blastocysts from IVM with Sirt3 mRNA injection, which suggested potential improvement of IVM oocytes during post-implantation.
Limitations, reason for caution: Additional studies with a larger number of oocytes are required to confirm the present results owing to the limited oocytes in the present study. Moreover specific Sirt3 activator should be studied and could be a source to improve ART outcome of patients in IVM cycles.
Wider implications of the findings: To our knowledge, this is the first study investigating the role of Sirt3 gene on mitochondria biogenesis and developmental competence of human in-vitro matured oocytes. Together with prior animal studies, the data support the positive role of Sirt3 on promoting mitochondria biogenesis. The present study promotes an important step towards establishing the human oocyte maturation mechanism, with a view to facilitating their application in the clinic.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported in part by the Ministry of Science and Technology of China Grants (973 program; 2014CB943203 and 2011CB944504), the National Natural Science Funds for general program (31371521 and 31230047). The authors have no conflict of interest to declare.
Trial registration number: NA.
Keywords: in vitro maturation, oocyte, mitochondria biogenesis, apoptosis

P-195 Non-invasive predictive system for human pronuclear stage embryo quality assessment, combined with the time-lapse imaging and the measurement of respiration activity

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Study question: Time-lapse imaging enables consecutive observation of human embryos non-invasively. The SECM (scanning electrochemical microscopy) system enables measurements of intracellular respiration activity of embryos. We assessed the potential of pronuclear stage embryos to develop into highly-reliable stage embryos using the time-lapse and SECM methods in the field of ART.
Summary answer: Continuous monitoring of embryo morphology may provide data with a predictive value for the subsequent embryo developmental viability using morphological evaluation and time-lapse imaging, along with respiration rates. We are now able to evaluate embryos in their earliest stage: pronuclear stage.
What is known already: Time-lapse imaging presents an opportunity for optimizing embryo selection based on morphological grading, as well as providing novel morphological parameters, which may further improve accurate selection of viable embryos. The first kinetic behavior is able to be observed in pronuclear stage embryos. Combining embryo respiration activity measurement with morphological evaluation can provide new information regarding the quality of human embryos and improve selection of superior embryos.
Study design, size, duration: A prospective study was conducted from January to August 2014. A total of 152 embryos from 48 couples undergoing ICSI cycle were included in the blastocyst development analysis based on ethical considerations.
Participants/materials, setting, methods: Participants were patients of St. Luke Clinic. The study analyzed, observation points during time-lapse monitoring, the time at nuclei appearance, and at nuclei disappearance. In addition, the locations of two pronuclei at appearance, and the oxygen consumption rates were also examined.
Main results and the role of chance: The time of each embryo appearance and disappearance of the nuclei envelope was calculated after extrusion of the second polar body. The embryos in which nuclei appeared after 6 h, developed high quality blastocysts: 3BB on day 5. Moreover, the embryos in which the nuclei envelope disappeared earlier than 18h, developed high quality blastocysts. The rate of blastocyst formation was significantly higher in embryos in which there was distance between the two pronuclei in the cytoplasm at appearance, than in embryos with close pronuclei in the cytoplasm. Oxygen consumption rate showed that when embryo respiration activity at Day 1 was low, embryos developed to significantly high quality blastocysts.
Limitations, reason for caution: The limited number of embryos whose oxygen consumption rate could be measured, limited the information used to predict high quality blastocysts. A larger sample size may also demonstrate other statistically significant development parameters.
Wider implications of the findings: Morphological evaluation has been the only method to evaluate embryo quality for the last 36 years. Continuous monitoring of embryo morphology may provide data with a predictive value for the subsequent embryo developmental viability using morphological evaluation and time-lapse imaging, along with respiration rates. We are now able to evaluate embryos in their earliest stage: pronuclear stage. These will contribute to growth in the field of ART, especially regarding e-SET.
Study funding/competing interest(s): Funding by hospital/clinic(s) – St. Luke Clinic.
Trial registration number: NA.
Keywords: ART, embryo selection, oxygen consumption rate, time-lapse imaging, pronuclear stage embryo

P-196 Systematic assessment of nucleation error phenotypes by Time-lapse analysis of human embryos and their impact on embryo quality and implantation potential

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Study question: To assess the significance of nucleation error phenotypes (NEP) after the first embryo cleavage by Time-lapse imaging and to subsequently assess their impact on top quality embryo (TQE) formation and known implantation potential (KID) in a human IVF clinical setting.
Summary answer: Nucleation assessment of 1540 human embryos revealed that multinucleated 2-cell stage embryos had significantly lower TQE and KID than those that displayed no nucleation error. Binucleated and micronucleated 2-cell stage embryos were significantly more likely to develop into TQE than multinucleated embryos.
What is known already: With the advent of Time-lapse imaging, additional deselection parameters like blastomere nucleation error have been used as indicator of embryo viability. Nucleation status assessed by the presence and proportion of visible nuclei in blastomeres has been indicative of the implantation potential of the embryo. Nucleation error phenotypes include binucleation (presence of two nuclei per blastomere), multinucleation (more than 2 nuclei), and micronucleation (one or two larger nuclei surrounded by one or more smaller nuclei).
Study design, size, duration: 1540 transferred embryos cultured in EmbryoScope™ incubator between 2011 and 2014 were retrospectively analyzed. Only IVF cycles with exact traceability of transferred embryos (KID) were included. To assess formation of TQE, 1192 zygotes incubated for minimum of 42 h were retrospectively analyzed between April and December 2014.
Participants/materials, setting, methods: Nucleation status was assessed for 1540 transferred embryos and the different NEPs were annotated along with embryo development from 2 cell stage to determine KID. 1192 fertilized embryos which cleaved were subsequently annotated for their nucleation status and proportion of error to determine ability to form TQE.
Main results and the role of chance: Of 1540 embryos, KID was significantly higher in embryos that displayed no NEP compared with embryos exhibiting NEP at any stage (22.3 vs.10.5%) ($p < .00001$). There was no significant difference in KID between types of NEPs at 2-cell stage.

Of 1192 zygotes, 39.8% embryos were morphological TQE at 42 h (Istanbul consensus 2011). Of these TQE, 16.5% displayed NEP at the 2-cell stage. Those with NEP had significantly lower likelihood of developing into TQE compared with those with no NEP (29.7 vs. 44.8%) ($p = .00001$). Binucleated and micronucleated embryos at 2-cell stage were significantly more likely to develop into TQE compared with multinucleated embryos (36.0 vs. 19.2%) ($p < .01$). None of the 46 embryos that were multinucleated at 3, 4 or 5 cell stage developed into TQE.
Limitations, reason for caution: This retrospective study analyzed only the fate of transferred embryos. Therefore, information regarding the nucleation status of other embryos of poor or mediocre quality is unavailable. Information regarding to the final outcome of IVF treatment i.e., live births have not been included and a long-term assessment is therefore recommended.
Wider implications of the findings: Time-lapse imaging of embryos reveal the importance of dynamic evaluation of NEPs in predicting embryo viability as well as implantation potential. Nuclear formation is a transient process and hence single static observation will not accurately predict outcome. With the advent of Time-lapse imaging, a morphological assessment of the embryo aided by deselection parameters including cleavage anomalies and NEPs along with morphokinetics will enhance selection of top-quality embryo and should augment success rates in IVF.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Klinikk Hausken, Haugesund, Norway.
Trial registration number: NA.
Keywords: time-lapse imaging, nucleation errors, implantation, EmbryoScope, embryo grading

P-197 A clinically useful simplified blastocyst grading system

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Study question: We have devised a simplified blastocyst grading system: A: fully expanded, clear inner cell mass (ICM), cohesive trophectoderm, B: partially expanded, clear ICM, cohesive trophectoderm, C: small ICM ± irregular trophectoderm ± excluded/degenerate cells, Is it clinically useful? Can it predict IVF outcome? Is it accurate, precise and reproducible?
Summary answer: Our simplified blastocyst grading system can be used to effectively predict IVF outcome in terms of implantation, clinical pregnancy and live birth. The inter- and intra-observer variability associated with it is minimal demonstrating it is both accurate and precise. It is therefore clinically very useful.
What is known already: Embryo grading based on qualitative criteria is subjective and inevitably results in inter- and intra-observer variability. The more permutations there are in any grading system, the greater the variability. Minimising variability in embryo scoring is important because the grade of the embryo has certain connotations. The only practical way to minimize variability is to simplify the grading system. In addition to being accurate and reproducible however, a grading system must also confer prognostic information.
Study design, size, duration: A retrospective cohort of day 5 sETs between 15/06/09 and 29/06/12 was undertaken. Implantation, clinical pregnancy and live birth rates were correlated to embryo quality according to the simplified blastocyst grading system. Embryologists used the grading system to grade and determine the fate of blastocyst images on two occasions.
Participants/materials, setting, methods: 545 embryo transfers were included. Categorical data were analysed using Chi-square. Bonferroni corrections were applied for multiple comparisons. A p-value of <0.05 was considered statistically significant. Five embryologists assessed 80 still images using the simplified grading system. The Fleiss-Kappa statistic was used to describe inter- and intra-observer variability.
Main results and the role of chance: Implantation, clinical pregnancy and live birth decreased with deteriorating embryo quality according to the simplified grading system. There was a highly significant ($p < 0.01$) difference between the groups in all parameters, largely due to the highly significant ($p < 0.01$) difference in all outcomes between grade A and B blastocysts and the significant ($p < 0.05$) difference in clinical pregnancies and live births between grade B and C blastocysts. For implantation the difference between grade C blastocysts and cavitating embryos was also significant ($p < 0.05$). There was no significant difference in outcome amongst the poorer quality embryos. Inter-observer agreement was substantial for grade allocation ($K = 0.63$) and clinical decision-making ($K = 0.66$). Intra-observer agreement ranged from substantial ($K = 0.71$) to almost perfect ($K = 0.88$) for grade allocation and was almost perfect for fate determination ($K \geq 0.84$).
Limitations, reason for caution: Only a limited number of cavitating ($n = 38$) and compacting ($n = 15$) embryos were available for determination of prognostic potential. Still images were used for the assessment of variability providing an artificial environment for the grading of embryos. The simplified grading system needs to be externally validated prior to widespread implementation.
Wider implications of the findings: This study demonstrates the prognostic potential and inter- and intra-observer variability of our simplified blastocyst grading system. The grading system is able to effectively predict IVF outcome. Slight variation exists both between and within embryologists but overall the levels of agreement are similar to, if not better than, those associated with more complex grading systems. This, combined with the fact it is quick and easy to use, make it clinically very useful.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s), University of Nottingham, Nurture Fertility.
Trial registration number: NA.
Keywords: blastocyst, grading, prognosis, inter- and intra-observer, variability

P-198 Variations in outdoors pollutants do not affect significantly embryo viability and pregnancy outcome

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Study question: Even though IVF laboratories are equipped with High Efficiency Particulate Air (HEPA) filters, ambient air of in vitro fertilization laboratories could carry small quantities of pollutants. In this regard, the arising question is: Can increased outdoor pollutants circulating affect IVF outcomes?
Summary answer: Episodes of high environmental pollution in Santiago the Chile are not closely related with abnormal fertilization and embryos degeneration, but reduced implantation rate.
What is known already: To obtain optimal results at IVF laboratories, it is important to maintain stable air parameters as pollutants may produce IVF outcome failure. Therefore, our laboratory is equipped with HEPA filters, positive pressure, and all personnel accomplishes with security and hygiene rules to work inside. Despite of these cautions, small quantities of air pollutants can still damage embryo viability especially when Santiago de Chile, the sixth most polluted city in the world, exhibits episodic high contamination levels.
Study design, size, duration: Retrospective cohort study approached with ovum recipients, who asked for an assisted reproduction treatment at IVI – Santiago de Chile during the years 2010–2013. From a total number of 636 cases registered during this term, 562 were taking into consideration for the study.
Participants/materials, setting, methods: High contamination period (HCP) was estimated as the term in which the daily registration of both pollutants NO_2 and CO exceeded steadily the annual average plus one standard deviation. Homogeneity analysis was approached through Student's t-test. The parameters evaluated were: degeneration on day 1, viability on day 2, and embryo quality on day 3. The statistic contrast was performed with chi-squared test and Fisher exact test.
Main results and the role of chance: HCPs were steadily present during autumn and winter from 2010 to 2013. During these periods, embryos degeneration and abnormal fertilization on day 1 exhibit higher but not statistically significant values (**4.5 ± 1.4 vs. 7.5 ± 1.0%**, and **26.0 ± 4.0 vs 37.5 ± 4.8%** respectively) In a similar way, viability on day 3 was slightly affected in HCPs (**18.2 ± 6.2 vs. 15.0 ± 2.1%**); however, embryo quality on this day exhibited similar for cycles accomplished during high and low contamination periods. Pregnancy rate was a little diminished (**53.0 ± 1.5 vs. 47.0 ± 1.8%**); meanwhile implantation rate was significantly reduced (**46.3 ± 0.2 vs 35 ± 0.6%, *p* = 0.0091**) in cycles that took place in HCP but in both All the patients surveyed in this study exhibited similar baseline characteristics as featured from our oocyte donation program.
Limitations, reason for caution: This study does take into account only the daily local registration of NO2, and CO; however, neither these pollutants nor others were measured inside our IVF laboratory facilities. The retrospective design and the potential bias from unknown clinical factors may condition the outcome results presented in this abstract.
Wider implications of the findings: The results of this study reinforce the well known fact of an optimal air quality to obtain the best outcomes in an IVF laboratory. Also, they suggest that outdoors pollution still conditions embryo quality despite controlled laboratory conditions as the low contamination periods may ensure the highest rate of implantation. However, further studies should be designed taking in consideration indoor/outdoor relationship measurements of pollutants.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Clínica IVI – Santiago.
Trial registration number: 0000.
Keywords: IVF, air pollution, pregnancy, implantation rate

P-199 Comparable timings of early developmental events in embryos from fresh and vitrified oocytes

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Study question: Are there differences in early developmental events and early cleavage times between embryos developing from freshly collected oocytes versus those developing from vitrified/warmed oocytes?
Summary answer: There are not significant differences for the median developmental and cleavage times: second polar body extrusion (2nPB), appearance of the two pronuclei (2PN), two pronuclei breakdown (BPN), and cleavage times t2, t3, t4, t5, t6, t7 and t8 between embryos developing from fresh oocytes and embryos developing from vitrified/warmed oocytes.
What is known already: Oocyte vitrification causes a temporary disassembly of the metaphase plate and spindle, which needs recovering after warming. As a result, it is possible that early post fertilization events such as the 2nPB might be altered in timing, with unknown effect on preimplantation development speed, BPN, and cleavage times (t2-t8). Although pregnancy rates are similar when fresh oocytes or vitrified oocytes are used, not much is known about their developmental kinetics.
Study design, size, duration: This is a prospective cohort study carried out in a large private fertility center between February and October 2014; 203 embryos were included. Inclusion criteria were embryos from fresh or vitrified oocytes from women aged 23–29 years, and normozoospermia according to WHO 2010 criteria.
Participants/materials, setting, methods: Fresh or vitrified/warmed oocytes fertilized with either partner or donor sperm. Developmental and cleavage times were collected with Primovision® Analyzer. Statistical analysis was performed by generating Kaplan-Meier curves of each time-lapse marker, and using a Log-rank test to identify differences between the two study groups (fresh vs vitrified/warmed).
Main results and the role of chance: Median developmental times (point at which 50% of the embryos have achieved each developmental stage) did not differ among embryos in the study groups (Log-rank test): **2nPB** = 3:30 vs 3:36 (*p* = 0.72); **2PN** = 7:43 vs 7:48 (*p* = 0.44); **PNB (** = 23:48 vs 23:46 (*p* = 0.64); **t2** = 22:43 vs 27:28 (*p* = 0.40); **t3** = 37:12 vs 39:27 (*p* = 0.25); **t4** = 39:10 vs 40:47 (*p* = 0.39); **t5** = 50:41 vs 50:27 (*p* = 0.79);**t6** = 51:41 vs 51:23 (*p* = 0.54); **t7** = 51:22 vs 53:22 (*p* = 0.14); **t8** = 53:9 vs 50:27 (*p* = 0.33), for fresh and vitrified/warmed oocytes, respectively. Because of the characteristics of oocyte donors, and the inclusion criteria for sperm, the two groups of embryos analyzed were comparable at baseline.
Limitations, reason for caution: Survival times do not follow a normal distribution therefore median times were analyzed. Caution may be exerted when comparing results with other studies where mean times are provided. The study population was selected to include only normospermic samples; results are not generalizable to all patients performing an oocyte donation cycle.
Wider implications of the findings: We conclude that the times of early cleavages and events of embryonic development are not different between embryos originating from fresh and vitrified/warmed oocytes; oocytes vitrification does not affect the preimplantation kinetics of the resulting embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.
Keywords: time-lapse, ICSI, preimplantation development, oocyte donation

P-200 Cumulus cell transcription analysis of in-vitro and in-vivo matured human cumulus cell-oocyte complexes

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Study question: Does the transcriptional profile of selected genes expressed by cumulus cells (CC) vary in in-vivo and in-vitro matured cumulus cell-oocyte complexes (COC)?
Summary answer: In CC, the expression of genes crucially involved in oocyte maturation signalling or cumulus expansion is influenced by in vitro maturation conditions.
What is known already: Many studies suggest that in CC the expression profiles of specific mRNA reflect the functional status of the oocyte and therefore could be adopted as non-invasive biomarkers of oocyte quality. This concept could be extended to COC that are retrieved at the immature germinal vesicle (GV) stage and are matured in vitro in oocyte in vitro maturation (IVM) cycles. However, current evidence on CC gene expression after IVM is very scarce.
Study design, size, duration: The expression profiles of specific genes involved in oocyte maturation were compared between CC obtained from COC matured in vivo or in vitro. In-vivo matured COC were obtained from fully stimulated cycles, while immature COC were retrieved from IVM cycles and cultured for 30 h to achieve oocyte maturation.
Participants/materials, setting, methods: CC were separated from their companion oocytes and RNA was isolated and prepared for expression analysis. RNA was used to produce cDNA libraries whose elements were sequenced, mapped and identified. Relative abundance of transcripts of genes of interest was validated by quantitative RT-PCR and comparatively analyzed.

Main results and the role of chance: Several difference were found in CC gene expression after maturation in vivo or in vitro. The major findings can be summarized as follows: (a) the *FSHR* mRNA was non-detectable in both in-vivo and in-vitro matured sample, while the *LHR* mRNA was four-fold more expressed in in-vitro matured COC; (b) the amphiregulin mRNA was three-fold more represented in in-vivo matured samples, while the mRNA of its cognate receptor (*EGFR*) was only moderately more expressed (1.5 folds) in in-vitro matured COC; (c) transcripts of genes involved in cumulus expansion, such as *PTGS2* and *HAS2,* were much more represented (respectively, three- and ten-folds) under in vivo maturation conditions; (d) the *GJA* gene, encoding for connexin-43, was ten-fold more expressed in IVM COC.
Limitations, reason for caution: Although informative and based on a well-established methodology, the study should be extended to transcripts of other genes known to have a function in oocyte maturation or to reflect oocyte quality.
Wider implications of the findings: The present data indicate that in vitro maturation conditions influence the expression of several genes involved in oocyte maturation signalling networks and cumulus expansion. Interestingly, as shown by collective knowledge on human IVM, such changes do not necessarily preclude the oocyte ability to mature and often give rise to embryos able to establish a viable pregnancy. Therefore, the functional significance of modifications of gene expression during IVM remains to be established.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Biogenesi, Reproductive Medicine Centre – Monza, Italy.
Trial registration number: NA.
Keywords: oocytes, cumulus cells, in vitro maturation, mRNA, human

P-201 Ultrastructural comparison of *in-vitro* and *in-vivo* matured human oocytes

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Study question: Is the ultrastructure of the cytoplasm of human *in-vitro* and *in-vivo* matured oocytes comparable?
Summary answer: Overall, the ultrastructure of human *in-vitro* matured oocytes is comparable to that of *in-vivo* matured controls, although following *in vitro* maturation (IVM) mitochondria-smooth endoplasmic reticulum (M-SER) complexes are partly replaced by mitochondria-vesicles (M-V) aggregates.
What is known already: Immature oocytes retrieved from antral follicles of patients undergoing IVM treatment can achieve meiotic maturation *in vitro*, fertilize and develop into embryos able to implant and give rise to viable pregnancies. However, nothing is known on the ultrastructure of IVM oocytes.
Study design, size, duration: The ultrastructure of *in-vitro* matured oocytes ($n = 7$) was compared with that of *in-vivo* matured oocytes ($n = 10$) by light and transmission electron microscopy (LM, TEM). The study was carried out over a period of 18 months.
Participants/materials, setting, methods: Immature cumulus cell-enclosed oocytes, retrieved from mid-sized antral follicles of women requiring IVM treatment, were matured *in vitro* for 30 h. Mature oocytes were obtained from age-matched women undergoing full ovarian stimulation. *In-vitro* and *in-vivo* matured oocytes were fixed and analysed by LM and TEM.
Main results and the role of chance: *In-vitro* matured oocytes showed general features comparable to *in-vivo* matured controls. All oocytes had normal ooplasm showing uniform distribution of organelles. M-SER aggregates and M-V complexes were commonly found in *in-vivo* matured oocytes. Large M-V complexes partially replaced M-SER aggregates in IVM oocytes. Mitochondria appeared morphologically unaffected by IVM. Cortical granules appeared typically stratified in a single, mostly continuous row just beneath the ooplasm in all oocytes. Microvilli were well preserved after IVM. Vacuoles were only occasionally found in all oocytes and, if present, they were frequently associated with lysosomes. The morphological features of the MII spindle and the first polar body of in-vitro matured oocytes were comparable to those shown by control oocytes.
Limitations, reason for caution: Although informative and based on a well-established methodology, the study should be extended to larger number of oocytes and different maturation conditions.
Wider implications of the findings: Ultrastructural analysis offers an objective approach for the comparison of organelle structure and distribution in in-vitro and *in-vivo* matured oocytes. The present data confirm that following IVM the overall oocyte cytoplasmic architecture is well preserved, although subtle differences in comparison to *in vivo*-matured controls encourage a further refinement of IVM protocols.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Biogenesi, Reproductive Medicine Centre, Monza, Italy.
Trial registration number: NA.
Keywords: oocyte, *in vitro* maturation, cytoplasm, ultrastructure, organelles

P-202 Spindle morphology in human oocytes changes with ageing and causes effect upon developmental competence

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Study question: We analyzed the relationship between biological aging and spindle configuration in human oocytes visualized with a Polscope and evaluated developmental competence. However, in the old group, the blastocyst formation rate of oocytes with a smaller area and grater retardance were higher.
Summary answer: The length and area of oocyte spindles became greater, and spindle density (retardance) became lower, with age. Development to the blastocyst stage was generally reduced with age.
What is known already: Existing reports suggest that human oocytes, with or without birefringent spindles, as imaged with a Polscope, are associated with oocyte quality. However, the relationship between oocyte spindle morphology and patient age and embryo development remains unknown.
Study design, size, duration: This study performed from August 2013 to September 2014. We analyzed 710 matured oocytes from 241 patients following letrozole stimulation.
Participants/materials, setting, methods: Oocyte spindle morphology was analyzed before ICSI using a Polscope. Oocytes were divided into three groups according to patient age: under 36 years (young), between 36 and 41 years (middle), and over 41 years (old). Spindle morphology, fertilization rate, and blastocyst formation rate was compared across the three groups.
Main results and the role of chance: Oocyte spindle area became greater with age (young vs. middle vs. old: 93.8 ± 18.7 nm^2, 101.9 ± 22.6 nm^2, 110.8 ± 26.3 nm^2). Retardance of the old group was lower than either the young or middle group (1.8 ± 0.4 nm, 2.0 ± 0.5 nm, 1.9 ± 0.4 nm). There was no significant difference in fertilization rate among the three groups. Blastocyst formation rate in the oldest group was lower than either the young or middle group (33.6%, 66.7%, 66.3%). However, in the old group, the blastocyst formation rate of oocytes with a smaller area and grater retardance were higher.
Limitations, reason for caution: It is important to examine further whether spindle morphology in human oocytes influences pregnancy and live birth rates.
Wider implications of the findings: This study demonstrates that spindle morphology in human oocytes changes with patient age and influences the developmental competence of embryos. The evaluation of spindle morphology will enable us to select good quality oocytes with high developmental competence.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ochi Yume Clinic, Nagoya.
Trial registration number: NA.
Keywords: oocyte, spindle, polscope, ageing, embryo development

P-203 Positive clinical outcomes when hyaluronan solution replaces polyvinylpyrrolidone in ICSI protocols; a comparative multicentre study

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Study question: Does the replacement of non-biodegradable polyvinylpyrrolidone (PVP) with a commercially available product containing natural hyaluronan (SpermSlow™) lead to superior IVF outcomes?

Summary answer: SpermSlow™ represents a superior alternative to PVP in terms of fertilisation, clinical pregnancy rates and ongoing pregnancy rates.
What is known already: When used as an adjunct during ICSI, PVP can lead to sperm DNA damage and can result in deleterious effects on embryo development (Barak et al., 2001). Hyaluronan (HA) receptors located on the Zona Pellucida are responsible for binding sperm which are mature and have good DNA integrity. Utilising SpermSlow™, which is a viscous HA solution, allows for sperm selection and has the benefits of PVP whilst eliminating the potential deleterious effects.
Study design, size, duration: In this comparative multicentre study, 3023 oocytes injected with HA were retrospectively analysed against 2624 PVP injected oocytes over a 6-month period. Clinical outcomes were measured from 545 transfer cycles. Fisher's exact test was performed using GraphPad™ software where $P < 0.05$ was considered significant.
Participants/materials, setting, methods: Sperm was prepared to a final motile concentration of 1×10^6/mL and added to PVP (Cook®) with ICSI performed in Cleavage Media (Cook®). For the HA group, PVP was replaced with SpermSlow™ (Origio®) and a 10 min incubation included to permit adequate sperm-HA substrate binding.
Main results and the role of chance: Oocytes injected with sperm selected in SpermSlow™ had a significantly higher fertilisation rate when compared to PVP usage (67.8% vs. 64.2%, $p = 0.0048$). An average of 1.3 embryos was transferred in each group. SpermSlow™ resulted in a clinical pregnancy rate of 30.2% ($n = 81$), significantly higher ($p = 0.0077$) than the PVP rate of 20.2% ($n = 56$). SpermSlow™ also had a significantly lower miscarriage rate (20.6% vs. 39.1%, $p = 0.0070$). No statistical difference was observed in biochemical pregnancy rate (38.1% SpermSlow™ vs. 33.2% PVP). There was no difference in the average maternal age, which was 36.3 for SpermSlow™ patients and 36.2 for PVP patients.
Limitations, reason for caution: An inherent property of SpermSlow™ is that it relies on forward progressing sperm and is therefore not suitable for testicular biopsy or severe asthenozoospermic samples.
Wider implications of the findings: SpermSlow™ is a user friendly product that can be easily integrated into ICSI protocols. With a natural HA active ingredient, clinics now have the option of eliminating potentially toxic PVP, selecting mature sperm with high DNA integrity and therefore generating a superior clinical pregnancy rate. Furthermore, a lower miscarriage rate was observed, potentially attributed to better DNA sperm selection at ICSI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – City Fertility Centre.
Trial registration number: NA.
Keywords: hyaluronan, sperm selection, ICSI

P-204 A randomized controlled study comparing two slow-freezing methods for cryopreservation of human cleavage stage embryos

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Study question: To compare two slow-freezing methods for cryopreservation of human cleavage stage embryos.
Summary answer: Embryo slow freezing using Vitrolife® method is superior to the Irvine® method in terms of embryo survival rate, intact embryo rate and clinical pregnancy rate.
What is known already: Slow-freezing protocols have been intensively used for cryopreservation of human embryos. However, over the past few years, vitrification has been shown to be superior to slow freezing by increasing embryo survival rate.
Study design, size, duration: From March to August, 2014, cleavage embryos were randomly cryopreserved with Irvine® or Vitrolife® slow-freezing method. Until November 2014, totally 1581 embryos from 635 cycles were thawed.
Participants/materials, setting, methods: Patient age, oocyte number of Irvine® and Vitrolife® group are 30.49 ± 4.33 and 30.58 ± 4.36; 17.81 ± 7.96 and 17.00 ± 7.05, respectively. Setting: University affiliated IVF center. The embryo survival rate, fully intact embryo rate, clinical pregnancy rate, and implantation rate were compared between the two groups.
Main results and the role of chance: Post warming embryo survival rate was significantly higher in Vitrolife® method compared to Irvine® method (94.3% vs. 84.0%, $p < 0.001$). Fully intact embryo rate was also significantly increased in Vitrolife® method (80.7% vs. 56.5%, $p < 0.001$). Of the 320 thaw cycles using Irvine® method, 9 cycles were cancelled because there were no viable embryos suitable for transfer while none of 315 cycle was cancelled in Vitrolife® method. Significant higher clinical pregnancy rate was observed using Vitrolife® compared to Irvine® method (54.0% vs. 46.0%, $p = 0.046$). Implantation rate was 33.5% and 30.1% for Vitrolife® and Irvine®, respectively.
Limitations, reason for caution: The study was performed at an IVF centre, comparing two commercial slow-freezing reagents.
Wider implications of the findings: For cleavage stage embryos, improved slow-freezing methods can produce comparable results to vitrification.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Women's Hospital, Zhejiang University School of Medicine.
Trial registration number: NA.
Keywords: cleavage embryo, slow-freezing, clinical outcomes

P-205 Comparison of embryo ranking at fixed standard time-points with a decision support tool based on time-lapse data correlated with known implantation

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Study question: This study evaluates how embryologists rank embryos based on static morphological information of embryo development at the ESHRE/ASRM consensus fixed time-points in comparison with a decision support tool (KIDScore) that calculated an implantation potential score for the same embryos using time-lapse information.
Summary answer: Embryo ranking based on strict morphological appearance differ between embryologists. The embryologist's ranking is also different from that of the decision support tool, which makes use of morphokinetic data. The latter could give an immediate benefit to embryologists independent of their working experiences to avoid selecting embryos with a very low chance of implantation, in addition to implementing a more standardized method for embryo ranking.
What is known already: Standard observation of embryo development at fixed time-points could miss irregular cell divisions, inadequate cell cycle length and abnormal developmental pace – events all associated with low implantation rates. A decision support tool based on time-lapse characteristics of thousands of embryos with known implantation has been developed. This determines the relative implantation potential of embryos with a score from 0 (lowest) to 5 (highest). This decision support tool may be useful to support embryologists who apply time-lapse technology in their embryo evaluation.
Study design, size, duration: Embryologists were asked to rank eight embryos based on images taken at seven focal planes at recommended ESHRE/ALPHA consensus fixed time-points (17, 26, 44, 66 h of development). Ranking by embryologists was compared to that calculated by the time-lapse decision support tool (KIDScore Basic D3).
Participants/materials, setting, methods: 80 embryologists (working experience 1–28 years, median 7.0; 44 with and 36 without time-lapse experience) participated. For each embryo the ranking by embryologists was compared to the rank given by KIDScore. Statistics were calculated using Graph Pad Prism (unpaired *t*-test with Welchs correction and linear regression analysis).
Main results and the role of chance: Out of eight embryos, four were ranked by KIDScore with score 1, two with score 3, one with score 4 and one with score 5 (highest implantation potential). From the participants 41.3% gave the top rank to a KIDScore 3 embryo, 27.5% to a score 1, 22.5% to a score 5 and 8.7% to another score 1 embryo. The statistical analysis showed a significant difference in the selection of the score 5 and 1 embryo between time-lapse and non time-lapse users ($P = 0.038$ and 0.005, respectively). Neither the general working experience as embryologist nor the experience in time-lapse did correlate with the decision to select the best embryo (KIDScore 5). In contrast, the score 1 embryo was more likely deselected with increasing time-lapse experience ($P = 0.0006$).
Limitations, reason for caution: This study was limited to eight embryos where still pictures at the different time-points and seven focal planes for each embryo were shown. Embryologists were not able to move embryos as in

normal routine practice. Using a time-lapse based decision tool requires correct and consistent annotation.
Wider implications of the findings: Our findings show, that embryo evaluation at fixed time-points is highly biased by the morphology that is present at the time of observation. Given the different level of experience of the embryologists who participated in this study and the observed differences in ranking of embryos, time-lapse based decision support tools including additional observations from continuous monitoring may help to improve standardization in clinical embryology.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study was conducted at workshops which were organized by FertiliTech (now part of Vitrolife).
Trial registration number: NA.
Keywords: time-lapse imaging, embryo score, morphokinetics, morphology, standardization

P-206 The assessment of the influence of iron, cadmium and lead concentrations on time-lapse parameters in ICSI procedure

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Study question: This research aims to evaluate to what extent the concentrations of iron, cadmium and lead affect the dynamics of embryo development.
Summary answer: It has been shown that the concentrations of cadmium and lead in the follicular fluid affect the dynamics of embryo development from the last observation of both pronuclei (tC) to the four-cell stage embryo (t4). The concentration of iron does not have an impact on the dynamics of embryo development.
What is known already: Heavy metals have a strong capacity to induce oxidative stress in body cells by disintegration of the lipid membrane, and spermatozoa are quite sensitive to oxidative stress. This may be caused by the weakening of cellular-based defensive mechanisms. The oxidant-antioxidant imbalance has an adverse effect on all stages of reproduction. The egg cell is probably damaged by the toxic microenvironment during primordial stage of follicle development and at the time of oocyte maturation.
Study design, size, duration: The growth of all the embryos from each patient was monitored continuously by obtaining images at 10 min intervals. The Pb, Cd, Fe measurements were performed by the electrothermal-atomic absorption spectrometry method.
Participants/materials, setting, methods: The present study was conducted in 2013 and 2014 in the 'Ovum Reproduction and Andrology' Non-Public Health Care Unit in Lublin (Poland), and involved 219 women aged 25–35 years undergoing fresh IVF. The study has been approved by the ethics committee of the Institute of Rural Health in Lublin.
Main results and the role of chance: We have shown that there is a positive correlation between the concentration of lead and the following times: tC(r = ,321,p = ,000), t1(r = 0,311, p = 0,000), t2(r = 0,359, p = 0,000), t3(r = 0,305, p = 0,000), t4(r = 0,235, p = 0,000), as well as between cadmium and times: tC(r = 0,388, p = 0,000), t1(r = 0,357, p = 0,000), t2(r = 0,395, p = 0,000), t3(r = 0,282, p = 0,000), t4(r = 0,296, p = 0,000). Considering other correlations, no statistically significant correlations have been found between iron, cadmium and lead.
Limitations, reason for caution: None
Wider implications of the findings: Overexposition of an organism to cadmium and lead adversely affects the dynamics of embryo development up to the four-cell embryo stage. Exposition to iron does not affect the dynamics of embryo development.
Study funding/competing interest(s): Funding by national/international organization(s) and International Scientific Association for the Support and Development of Medical Technologies.
Trial registration number: Local institutional registration
Keywords: iron, cadmium, lead, time lapse

P-207 Binding of FGF2 to FGFR2 in an autocrine manner in trophectoderm cells is indispensable for expanded blastocyst formation through activation of the PKC-p38 signalling pathway

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Study question: Fibroblast growth factors (FGF1, FGF2 and FGF4) and fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3 and FGFR4) have been reported to be expressed in preimplantation embryos and be required for their development. However, the functions of these molecules in trophectoderm cells (TEs) that lead to the formation of the blastocyst as well as the underlying mechanism have not been elucidated.
Summary answer: The present study has demonstrated for the first time that endogenous FGF2 secreted by TEs can regulate protein expression and distribution in TEs via the FGFR2-mediated activation of PKC and p38, which are important for the development of expanded blastocysts.
What is known already: Fibroblast growth factors (FGF1, FGF2 and FGF4) and fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3 and FGFR4) have been reported to be expressed in preimplantation embryos and be required for their development.
Study design, size, duration: Total number of subjects were involved. About one thousand of embryos were involved. It took 1 year for this experiment.
Participants/materials, setting, methods: We collected ICR mice 4–8 cells embryos to study the formation of expanded Blastocysts. SiRNA, immunoflurence technology, kinase inhibitors co-culture and Real-time PCR was used.
Main results and the role of chance: Endogenous FGF2 secreted by TEs can regulate protein expression and distribution in TEs via the FGFR2-mediated activation of PKC and p38, which are important for the development of expanded blastocysts.
Limitations, reason for caution: It is difficult to obtain a certain amount of embryos to test from protein quantitative level.
Wider implications of the findings: This finding provides the first explanation for the long-observed phenomenon that only high concentrations of exogenous FGFs have effects on embryonic development, but *in vivo* the amount of endogenous FGFs are trace. Besides, the present results suggest that FGF2/FGFR2 may act in an autocrine fashion and activate the downstream PKC/p38 pathway in TEs during expanded blastocyst formation.
Study funding/competing interest(s): Funding by University(ies) – (1) International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China. (2) The Key Laboratory of Reproductive Genetics, Ministry of Education (Zhejiang University), Hangzhou, Zhejiang 310006, China.
Trial registration number: No clinical.
Keywords: expanded blastocyst formation, fibroblast growth factor 2, fibroblast growth factor receptor 2, p38 MAP

P-208 Outgrowth embryo-derived extracellular vesicles improve the developmental competence of mouse pre- and peri-implantation embryos

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Study question: Does outgrowth embryos secrete extracellular vesicles and Does it improve the pre- and peri-implantation embryonic development?
Summary answer: Outgrowth embryos secrete extracellular vesicles and it improves mouse embryonic development on blastulation rate, blastocyst cell number and outgrowth rate as well as mean of outgrowth areas and morphological scores.
What is known already: A large variety of extracellular vesicles produced within the mammalian embryos and secreted into the surrounding environment of uterus have been called exosomes, shedding vesicles, and extracellular vesicles (EVs). Embryos alter the microenvironment through secretions of those

vesicles and it has physiological roles during embryonic development and gene expressions.
Study design, size, duration: Two-cell embryos were collected from super-ovulated ICR mice (day-2) and cultured in basal medium, Quinn's advantage blastocyst medium (QABM), up to blastocyst (day-5). In vitro cultured blastocysts were transferred to be outgrowth on fibronectin-coated dish without serum supplementation for 3 days (day-8). Then, EVs were prepared from conditioned media of outgrowth embryos (EVs-QABM) on day-8. Two-cell embryos were cultured in basal medium without or with outgrowth embryo derived-EVs. Pre-implantation embryonic development was assessed by rates of morula, blastocyst and hatching and TUNEL assay and peri-implantation embryonic development was assessed by outgrowth rates, spreading area and morphological scoring.
Participants/materials, setting, methods: Conditioned media of outgrowth embryos was examined by using TEM to observe the morphology and size of EVs. Mouse 2-cell embryos were treated with 0–50% of EVs-QABM and 10% serum protein substitute (SPS) for the positive control (PC). Blastocysts were (i) stained for cell number, (ii) for TUNEL assay, (iii) analyzed quantitatively the expression of survival-related genes and (iv) transferred to wells coated with fibronectin for peri-implantation outgrowth. Outgrowth embryos were (iv) measured trophoblastic spreading area and ranked against the morphological scoring of trophoblast cells and inner cell mass (ICM).
Main results and the role of chance: The EVs had conventional membrane structures and the sizes were 50–200 nm. Significant differences in pre-implantation embryonic development, as well as the cell number of blastocyst (0% vs. 12.5%, $p < 0.05$), apoptotic index (0% vs. 12.5%, PC% vs. 12.5%, $p < 0.01$) and rates of morula (0% vs. 25%, $p < 0.01$) and blastocyst (0% vs. 12.5%, 0% vs. 25%, $p < 0.01$), were found when the embryos were treated with EVs-QABM. And survival-related genes such as Bcl-2, Survivn and LIF were up-regulated in 12.5% and 25% of EVs-QABM treated group ($p < 0.01$) compared to negative control while pro-apoptosis gene, Bax, was down-regulated in EVs-QABM group ($p < 0.05$). Also EVs-QABM affected the peri-implantation embryonic development by improving outgrowth rates (0% vs. 12.5%, $p < 0.01$), trophoblastic spreading area (0% vs. 12.5%, PC vs. 12.5%, $p < 0.01$) and morphological scoring of trophoblastic outgrowth (0% vs. 12.5%, PC vs. 12.5%, $p < 0.01$) and ICM (0% vs. 12.5%, $p < 0.01$).
Limitations, reason for caution: Findings in the mouse embryos were assessed only in vitro, not in vivo. Due to the nature of this work, this study was not fully transferable to humans.
Wider implications of the findings: Our study showed the extraction of EVs from outgrowth embryos was successful, and our study is reassuring in what kind of factors exists in EVs-QAMB and investigating the biological mechanisms of EVs.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare (A120043).
Trial registration number: EUIACUC 13-18.
Keywords: pre-implantation embryo, peri-implantation embryo, extracellular vesicles, outgrowth embryo, developmental competence

P-209 A historical cohort study to investigate the relation between blastomere survival and mitosis resumption in frozen-thawed embryo's and ongoing pregnancy

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Study question: What is the association between frozen-thawed embryo morphological characteristics (FEMC: blastomere survival and mitosis resumption) and ongoing pregnancy (OP), when adjusted for potential confounders belonging to embryo morphological characteristics before cryopreservation, maternal characteristics and cycle influences. In addition we investigated whether FEMC are also predictors of OP.
Summary answer: Mitosis resumption is positively associated with OP. After adjustment of mitosis resumption for embryo morphologic characteristics before cryopreservation, the association with OP no longer existed. Therefore mitosis resumption is not an independent predictor of OP. Adjusting for the other confounders did not alter the association between FEMC and OP.
What is known already: Frozen-thawed embryo transfers (FETs) have a reduced pregnancy rate compared to fresh embryo transfers. Next to FEMC, embryo characteristics before cryopreservation (number of blastomeres and embryo quality), maternal characteristics (age, cause of infertility and pregnancy history) and cycle influences (type of stimulation, number of attempts, number of oocytes and embryo availability) influence the chance on OP. Adjusting for these confounders to assess whether FEMC are true predictors of OP has not been performed before.
Study design, size, duration: In this historical cohort study, 1242 first unique single FETs in our database between 2001 and 2012, consisting of 1242 patients who underwent in vitro fertilization treatment, were analyzed. This approach was used to prevent overestimation of the results by individuals appearing more than once in the dataset.
Participants/materials, setting, methods: Patients at Leiden University Medical Center, whose embryos were cryopreserved 72 h after oocyte retrieval and cultured overnight prior to FET were included. To assess the association between FEMC with OP an association model was built. FEMC were also evaluated as OP predictors using univariable and multivariable logistic regression analysis.
Main results and the role of chance: There was a significant ($P < 0.001$) positive association (OR 2.15, 95% CI 1.5–2.97) of mitosis resumption and OP in the univariable logistic regression analysis. Blastomere survival was not associated ($P = 0.26$) with OP. After adjustment of mitosis resumption for number of blastomeres after overnight culture, the association ($P = 0.86$) with OP no longer existed. This study shows that the number of blastomeres after overnight culture is the only embryo characteristic that serves as an independent predictor of OP. As a result of mitosis resumption, which results in an increased number of blastomeres, the latter becomes an independent predictor.
Limitations, reason for caution: In contrast to other studies we were unable to identify a significant association between blastomere survival and OP. In our database blastomere survival was divided into 4 groups: 0%, >0%–<50%, >50%–<100% and 100%. Splitting those groups in subgroups might give more accurate results. We adapted our database for future studies.
Wider implications of the findings: Our findings may contribute to cost-effectiveness studies to increase the efficiency of FETs. E.g., cancelation of transfers with <8 blastomeres, would almost halve ($N = 615$) the number of transfers while losing 25% ($N = 44$) OPs. A cut off point for the number of blastomeres at time of FET might be determined. Thawing another embryo if available may reduce the time to pregnancy in such cases.
Study funding/competing interest(s): Funding by University(ies) – Leiden University Medical Center.
Trial registration number: No Trial registration number.
Keywords: cryopreservation, blastomere survival, mitosis resumption

P-210 Intracytoplasmic sperm injection (ICSI) outcome versus intracytoplasmic morphologically selected sperm injection (IMSI) outcome according to male age: a retrospective trial

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Study question: Is there any difference between IMSI and ICSI in terms of IVF outcomes in selected infertile patient population in according to male ages?
Summary answer: A trend towards improvement in IVF outcomes was detected in all male age subgroups when IMSI was applied. The subgroup analyses according to male age did not show a statistical difference between ICSI and IMSI. Our results show that the IMSI technique slightly but not significantly improves IVF outcomes in a selected infertile population
What is known already: It was known that selection of vacuole-free spermatozoa by IMSI under high magnification (X6400) may have a positive impact on IVF outcome in severe male infertility factor or repeated pregnancy failure cases. In some studies reported that there is no significant clinical improvements with IMSI in an unselected patient population (with various aetiologies of infertility and with and without previous ICSI failures).
Study design, size, duration: Retrospectively 4022 cycles (1399 ICSI and 2623 IMSI) were included from July 2011 to July 2014. The study was approved by the local Institute Review Board.
Participants/materials, setting, methods: Patients with any of leucospermia, orchiopexy/undescended testis, genetically problems and chemotherapy/radiotherapy and for women age >40, BMI >30, genetically problems and chemotherapy/radiotherapy were excluded from the study. Patients separated four group as <30, 30–35, 36–39 and >40-years old according to male ages.

Main results and the role of chance: ICSI vs IMSI groups were comparable when considering (+) βhcG (ICSI: 49.7%; IMSI: 56.2%, $p < 0.0001$), clinical pregnancy rate (ICSI: 45.5%; IMSI: 51.1%, $p < 0.0001$), ongoing pregnancy rate (ICSI: 38.5%; IMSI: 45.0%, $p < 0.0001$) and miscarriage rate (ICSI: 13.3; IMSI: 12.0%, $p = 0.82$) in overall. However, in the subgroup analyses there were an increase in the mean of (+)βhcG, clinical pregnancy rate, ongoing pregnancy rate, but slightly decrease in miscarriages in IMSI groups compared to ICSI groups, but not statistically significant. A trend towards improvement in IVF outcomes was detected in all male age subgroups when IMSI was applied.
Limitations, reason for caution: Retrospective study. Take home baby rate should be giving.
Wider implications of the findings: Our results show that a nonsignificant trend towards higher pregnancy and lower misscarriages rates in the IMSI group than in the ICSI group in according to male age. IMSI seems to be an expensive and time-consuming procedure, depending on the degree of sperm morphology impairment and the number of oocytes to be injected, so it is should be better to apply only selected patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital IVF Clinics.
Trial registration number: NA.
Keywords: ICSI, IMSI, ART, male Age

P-211 Differences in morphokinetic parameters and clinical outcomes between IMSI and ICSI-derived embryos by time-lapse imaging

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Study question: The aim of this study was to evaluate the efficiency of the intracytoplasmic morphologically selected sperm injection (IMSI) technique compared with conventional ICSI.
Summary answer: No correlation has been found between morphokinetic variables. However, Compared to conventional ICSI, this study found that IMSI increased the IVF-ET success rates in patients with oligoasthenoteratozoospermia (OAT).
What is known already: Many studies have reported that the presence of large nuclear vacuoles in the spermatozoa is associated with reduced fertilization, pregnancy rates, and with an increased risk of miscarriage. IMSI treatment results in higher success rates compared to conventional ICSI treatment. However, it is unknown whether this difference is linked to embryo morphokinetic.
Study design, size, duration: Retrospective analysis of fertilized embryos ($n = 506$, IMSI: 236 vs. ICSI: 270) were cultured in time-lapse incubator from June 2013 to August 2014. This study was performed in 101 cycles that had undergone ICSI treatment in the previous cycle for OAT infertility.
Participants/materials, setting, methods: The motile spermatozoa were analyzed under high magnification (6,600x) microscopy incorporating with modulation contrast illumination. Conventional ICSI was performed under an optical magnification of approximately 200 or 400×. All embryos were cultured after IMSI/ICSI assessed a time-lapse incubator (EmbryoScope, Unisense Fertilitech, Denmark) and annotated for pattern time of cleavage.
Main results and the role of chance: No significant differences were observed between groups for age, fertilization rate, good quality embryo rate and number of embryos transferred. There was not and significant differences were shown in embryo morphokinetics. The mean time-points for IMSI and conventional ICSI respectively were; (tPB2) 3.1 vs. 3.2, (tPNf) 24.1 vs. 24.6, (t2) 27.5 vs. 27.2, (t3) 36.8 vs. 36.5, (t4) 39.9 vs. 39.5, (t5) 49.9 vs. 49.2, (cc2) 8.4 vs. 9.0, (s2) 2.7 vs. 2.6. The pregnancy and implantation rates with IMSI were significantly higher than those of the ICSI cycles (36.5% vs. 15.6% and 16.3% vs. 7.8%, respectively; $p < 0.05$). The miscarriage rate among pregnant patients (12.8% vs. 28.1%) showed statistically significant difference between groups ($p < 0.05$).
Limitations, reason for caution: This retrospective study was conducted in a limited cohort of patients. Data set size is a limiting factor, making reliable statistical analyses difficult.
Wider implications of the findings: IMSI performed at high magnification such as 6,600x has the advantage of enabling the sorting of sperm with vacuoles in their heads or other defects that cannot be observed during conventional ICSI. The overall embryological parameters remain similar regardless of the between the two groups. However, clinical IMSI is an effective technique for IVF-ET with positive clinical outcomes such as better pregnancy and implantation rates than conventional ICSI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maria Fertility Hospital.
Trial registration number: We don't need to Trial registration number.
Keywords: IMSI, morphokinetic, time-lapse

P-212 The impact of embryo fragmentation and morphokinetic events within the first 48 h of culture on the rate of blastocyst formation

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Study question: Does the degree of embryo fragmentation affect the rate of blastocyst formation?
Summary answer: The degree of fragmentation affects the rate of blastocyst formation. Fragmentation in the 4 cell stage embryo was the most important predictor of blastocyst formation.
What is known already: Findings of previous studies suggest that dynamic markers of viability improve blastocyst formation rates more than morphological grading. The results of studies on the affect of fragmentation on blastocycst formation are somewhat ambigious, as several reports have stated that fragments are resorbed into the blastomere.
Study design, size, duration: During the 1st quarter of 2013, 262 embryos were obtained by IVF from 41 cycles and cultured to day 7 without medium change in a Time-lapse Imaging system (EmbryoScope, Vitrolife).
Participants/materials, setting, methods: Outcomes were assessed by multiple logistic regression analyses with repeated measures. Logistic regression models were built to predict blastocyst formation as determined by fragmentation degree and time-lapse derived morphokinetic parameters. Independent predictors included degree of fragmentation at 2- and 4-cell stage, and multiple embryo events within the first 48 h of development. Statistically significant effects were determined at a $P < 0.05$ level.
Main results and the role of chance: The time-lapse variables as potential predictors of blastocyst development were degree of fragmentation at 4 cell-stage (OR; 2.928 95% CI; 01.794–4.781), duration of 3-cell stage (OR; 1.092 95% CI; 1.008–1.183) and time point of division to 2-cell (OR; 1.234 95% CI; 1.102–1.383).
Limitations, reason for caution: The study was a retrospective study.
Wider implications of the findings: These results suggest that the degree of fragmentation at the 4 cell stage is a relevant predictor of blastocyst formation and should be routinely measured.
Study funding/competing interest(s): Funding by hospital/clinic(s), None.
Trial registration number: NA.
Keywords: fragmentation, development, morphokinetic

P-213 Gender Specific Selection of Individual spermatozoa by ICSI

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Study question: Attempts to select spermatozoa of a specific chromosomal character for use in IUI and/or IVF may fail to produce the desired gender due to disparity in motility and fertilizing capacity. We questioned whether it is possible to pick up individual spermatozoa during ICSI to maintain the gender-specific specimen selection.
Summary answer: Gradient self-selection of spermatozoa is capable of providing X-bearing spermatozoon at 85.2% Y-bearing at 79.6%. Individual selection of spermatozoa post-enrichment maintained the gender proportion, as confirmed by in situ hybridization, and provides improved chances of generating the conceptus of the wished sex.

What is known already: Several techniques have been proposed to successfully select X- or Y-bearing spermatozoa. These include centrifugation methods and layering techniques, as well as the use of electrophoretic devices. The most effective method to date uses flow cytometry, capable of achieving selection of 90% for X- and 80% for Y-bearing spermatozoa. This method, however, is expensive and requires exposure of spermatozoa to a fluorescent dye.
Study design, size, duration: In a 19-month period, we processed ejaculates by gradient self-selection (GSS) and gradient self-selection plus (GSS+) prior to cytogenetic analysis. Single spermatozoa were aspirated by microtool from the selected and unselected aliquots and dispensed on a glass slide for cytogenetics.
Participants/materials, setting, methods: From six formulations of gradients, GSS and GSS+ carried out at 4°C were the most successful. GSS+, however, yielded lower spermatozoon concentrations. For consistency, we assessed chromosomes X, Y, 15, 16, 17 and 18 on at least 1000 cells and on individually isolated sperm cells.
Main results and the role of chance: In 15 samples with a concentration of $47.9 \pm 15 \times 10^6$/ml, 49.4 ± 5% motility and normal morphology, GSS method yielded 78.9% for X- and 75.0% for Y-bearing spermatozoa. When GSS+ was used, 85.2% X- and 79.6% Y-bearing fraction were obtained, albeit at concentrations under 1×10^6. For the single cell experiment, an ejaculate was processed for routine semen analysis and yielded 53.8% X- and 46.2% Y-bearing spermatozoa. After retrieving 10 individual spermatozoa from this original specimen, 6(60.0%) were found to be female and 4(40.0%) to be male. An aliquot of the specimen was subjected to GSS, obtaining 78.9% X- and 75.0% Y-bearing spermatozoa at 5- and 10×10^6 concentration, respectively. Finally, we individually retrieved 10 spermatozoa from each gender-specific fraction, resulting in 7(70.0%) for X- and 7(70.0%) for Y-cells.
Limitations, reason for caution: These observations aim at limiting interference of cell motility, natural competition, and functional cell integrity in a spermatozoa population purposely skewed in favor of the desired gender. Individual sperm selection may or may not maintain the gender-enriched proportion, unless gender-specific morphometric characteristics are integrated during selection of the cell.
Wider implications of the findings: Multi-gradient methods of spermatozoa self-selection offer the ability to offset the proportion of spermatozoa of the desired gender in an economical and safe manner. Individual retrieval of spermatozoa during an ICSI procedure is able to maintain the same proportion of gender-specific cells achieved by GSS. Single sperm sex selection may be enhanced by the identification of eventual morphometric characteristics distinctive of a sex-specific spermatozoon.
Study funding/competing interest(s): Funding by University(ies) – Reproductive Medicine, Weill Cornell Medical College.
Trial registration number: NA.
Keywords: pre-gender selection, Y-bearing sperm, X-bearing sperm, gradient selection, ICSI

P-214 EGF-like growth factors improve in vitro maturation and embryo development by enhanced normal distribution of cortical granules and global DNA methylation

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Study question: The objectives of this study were (1) to examine whether epidermal growth factor (EGF)-like growth factors should be influence maturation rates and developmental competences and (2) to investigate the effects of EGF-like growth factor on distribution of cortical granules (CGs) and the DNA methylation changes at metaphase II stage.
Summary answer: (1) The maturation rate of mouse germinal vesicle (GV) oocytes was increased by EGF-like growth factors (amphiregulin: AR and epiregulin: EPI). (2) AR or EPI treatment increased the proportion of oocytes with normal CGs distribution and the global DNA methylation.
What is known already: It was reported that AR and EPI affect both in vitro maturation (IVM) of mouse immature oocytes and embryo development. However, little is known about how the AR and EPI relate to oocyte maturation and developmental competence. It has been demonstrated that distribution of CGs has been used as a criterion for the assessment of cytoplasmic maturation. Recently, a reduction in global DNA methylation of MII oocytes result in compromised in vitro developmental potential in mouse embryos (Liang et al., 2014).
Study design, size, duration: In this prospective experimental study, GV oocytes were randomly divided into three groups; IVM medium only (Control), IVM medium with AR, and IVM medium with EPI.
Participants/materials, setting, methods: (1) GV oocytes were matured in maturation medium with/without AR or EPI. Maturation, fertilization rate and embryo development were monitored. (2) CGs distribution in MII oocytes after IVM was stained with Lens culinaris agglutinin probe. The level of DNA methylation was examined by immunofluorescence staining using an anti-5-methylcytosine antibody.
Main results and the role of chance: (1) A total of 776 immature oocytes were cultured in vitro (control: 268; AR: 253; EPI: 255). The maturation rates were statistically higher in AR and EPI-treated groups than in control group (63.2%, 61.6% and 46.6%, respectively) ($P < 0.05$). There were no statistically differences in fertilization rates among groups. Only EPI groups (87.0%) were increased the blastocyst rates rather than control group (77.2%, $P < 0.05$). (2) The percentage of matured MII oocytes with normal distribution of CGs (appear as small granules linearly arranged under the oolemma) was high in the AR and EPI groups (78.8% and 75.5%, $P < 0.05$) as compare to the control group (61.8%). The density of global DNA methylation was significantly increased in AR and EPI-treated group ($p < 0.001$).
Limitations, reason for caution: The effect of AR and EPI can be tested reliably with mouse oocytes, yet the findings should be extended to the human oocytes with caution.
Wider implications of the findings: Our data suggested that AR and EPI are involved in oocyte maturation process. This finding may be applied to human IVM protocol, which is still a low-success rate in the clinical practice. The addition of AR and EPI to human IVM media may improve the outcome of human IVM cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maria Fertility Hospital.
Trial registration number: NA.
Keywords: EGF-like growth factor, cortical granule, DNA methylation

P-215 Effects of sperm insemination on final meiotic maturation of mouse oocytes arrested at metaphase I after *in vitro* maturation

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Study question: The aims of this study were (1) to investigate whether fertilization could induce the resumption of meiosis in mouse oocytes that are arrested metaphase I (MI) after *in vitro* maturation (IVM) and (2) to investigate the effect of calcium oscillation by fertilization on MI to MII transition.
Summary answer: (1) Higher proportions of arrested MI oocytes progressed MII by fertilization. Although the fertilization rates were similar, the blastocyst rates and live birth were significantly higher in MII oocytes than in MI oocytes. (2) Fertilization may induce MI oocyte maturation by calcium oscillation.
What is known already: During IVM, some oocytes arrested spontaneously at MI that unable to achieve MII even after prolonged culture. There were some reports that these arrested MI oocytes have similar sperm-induced calcium oscillation pattern with MII oocytes. Moreover, insemination of immature oocytes enhanced maturation rates in vitro. However, it is unknown whether (1) fertilization could induce the resumption of meiosis in arrested MI oocytes and these oocytes can lead to live birth (2) calcium oscillation may have influence on overcome the MI arrest.
Study design, size, duration: (1) MII (MII group) and arrested MI (MI group) mouse oocytes after IVM were fertilized with spermatozoa, and then assessed their maturity and developmental potency. (2) Arrested MI oocytes after IVM were treated with calcium chelator to investigate the effect of calcium oscillation driven by fertilization on MI to MII transition.
Participants/materials, setting, methods: (1) The embryonic development was monitored and cell numbers were counted. Blastocysts from each group were transferred into 2.5 dpc pseudo-pregnancy ICR mice using non-surgical embryo-transfer device. (2) Arrested MI oocytes were treated with calcium chelator and fertilized with sperm, then evaluated their maturity by monitoring of first polar body.

Main results and the role of chance: (1) As insemination time increased, the oocytes reached to MII stage in MI group also increased (5.5% at 2 h, 72.9% at 3 h, and 94.8% at 4 h, respectively). However, the spontaneous maturation rate of oocytes at the MI stage cultured for up to 4 h without sperm was 0.7%. There was no difference in fertilization rate between two groups. However, the blastocyst rates and the total cell numbers in MII group were significantly higher than those in MI group ($P < 0.05$). Ninety-six blastocysts of each group were transferred in 12 recipients. No pregnancy was obtained from MI group. However, 10 pregnancies were achieved (10/12, 83.3%) in MII group and the rate of live pups per transferred embryos was 32.3% (31/96). (2) The proportion of MI maturing to MII oocytes after fertilization was significantly higher in non-treated group (93.2%) compared with the 1 μM (3.0%) or 10 μM (1.2%) calcium chelator treated groups ($P < 0.01$).
Limitations, reason for caution: According to the previous report, arrested human MI oocyte was fertilized and developed to a healthy neonate who had a normal karyotype. However, in this study, no pregnancy was obtained from arrested mouse MI oocytes. Further investigations are needed to confirm this difference.
Wider implications of the findings: Although the oocytes in the MI group was fertilized and developed to the blastocyst normally, no pregnancy was obtained from MI group. It suggested that their developmental competence was lower than the MII group. Higher proportions of arrested MI oocytes progressed MII by fertilization. MI to MII transition was blocked by calcium chelator treatments before fertilization. This result indicated that MI oocytes maturing to MII after fertilization were related to the intracellular calcium oscillation driven by fertilization.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maria Fertility Hospital.
Trial registration number: NA.
Keywords: arrested metaphase I oocyte, fertilization, calcium oscillation

P-216 Sperm telomere length is positively associated with the quality of early embryonic development

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Study question: What is the relationship between telomere length in sperm and early embryonic development in *in vitro* fertilization (IVF)?
Summary answer: We provide the first report that sperm telomere length (STL) is positively associated with embryo quality in IVF. This suggests that STL plays an essential role in early embryonic development and that selecting sperm with longer telomeres might help us obtain good-quality embryos in assisted reproductive technology (ART).
What is known already: Previous studies have shown that sperm telomere STL differs among human males. However, little is known about whether STL is related to embryo quality and whether it is relevant in clinical pregnancy for IVF in ART.
Study design, size, duration: Patients undergoing IVF treatment from August 2013 to August 2014, we analyzed the associations between STL, fertilization laboratory parameters and clinical pregnancy using Pearson's correlation, partial Pearson's correlation or multiple linear regression, as appropriate.
Participants/materials, setting, methods: We used quantitative PCR technique to detect the mean STL in 418 couples undergoing IVF treatment.
Main results and the role of chance: The mean STL was positively correlated with age of patient and total sperm count/ejaculate Analysis of the age-adjusted mean STL in relation to the male patient's paternal and maternal ages at the time of conception showed a significant positive relationship between STL and both paternal and maternal age. But, the relative contribution of paternal age at conception to the patient's STL was great than that of maternal age analyzed by multivariable linear regression. In addition, significant correlations were found between STL and good quality embryo and transplantable embryorates, but clinical pregnancy rate was not affected.
Limitations, reason for caution: This is the first study report that that STL is positively associated with embryo quality in IVF. Additional studies are needed to confirm these observations, and the methods selecting sperm with longer telomere effectively need to be exploited in the future.
Wider implications of the findings: Sperm telomere length may be as a indicator of early embryonic development.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Science Foundation of China, and the National Science Foundation for Young Scientists of China.
Trial registration number: Grants 31271605 and 31471404, Grant 31401274.
Keywords: telomere length, infertility, embyro, *in vitro* fertilization

P-217 Morphokinetic differences between bovine blastocysts cultured in media supplemented with human serum albumin or recombinant human albumin

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Study question: A bovine model was used to compare the morphokinetics between matured oocytes fertilised/cultured in conventional commercial media supplemented with human serum albumin (HSA) or recombinant human albumin (rHA).
Summary answer: The HSA group took longer to reach the 9-cell stage from insemination, but reached the compact morula stage from the 9-cell stage and the start of blastulation from the compact morula stage faster than the rHA group. The blastocyst yield and time from insemination to blastocysts were similar between groups.
What is known already: HSA, a common protein source in ART, can contribute to biological variation and disease transmission. Using rHA can reduce these risks, but the extant literature is limited. We previously reported that replacement of HSA supplemented during culture with lower amounts of rHA produced good-quality bovine blastocysts (ESHRE 2014). On the other hand, studies have shown that the presence of whole serum during culture causes altered morphokinetics of mammalian embryos (e.g., impaired compaction and premature blastulation).
Study design, size, duration: Bovine oocytes were collected from abattoir ovaries and matured in defined media. Matured oocytes were randomly divided into 2 groups and fertilised/cultured in media containing HSA or rHA. Morphokinetics were evaluated from the start of insemination to the blastocyst stage (day 8) by time-lapse microscopy (Vitrolife) ($n = 416$, 13 trials).
Participants/materials, setting, methods: Oocytes underwent conventional IVF (with sperm from a bull) in G-IVF medium containing 10 μg/mL HSA or 1 μg/mL rHA, and were cultured in G1/G2 media containing 5 μg/mL HSA or 0.5 μg/mL rHA. Morphokinetics of good-quality blastocysts (expanded [diameter >160 mm], <50% fragmentation at the morula stage) were analysed.
Main results and the role of chance: Good-quality blastocyst rates were similar for the HSA (31/208, 14.9 ± 3.2%) and rHA (40/208, 19.2 ± 2.5%) groups. The HSA group took longer ($P < 0.01$) to reach the 9-cell stage from insemination than the rHA group (98.9 ± 1.2 h vs. 92.9 ± 1.3 h). Conversely, the HSA group showed shorter ($P < 0.0001$) times from the 9-cell stage to compact morula (26.5 ± 0.9 h vs. 32.2 ± 0.8 h) and from the compact morula to the start of blastulation (20.6 ± 1.0 h vs. 29.2 ± 0.9 h). Typically, the compact morula was tighter in the rHA group. Overall, the times to reach full blastocysts (blastocoel completely filling the embryo) from insemination were similar for the HSA (160.2 ± 1.9 h) and rHA (164.0 ± 1.9 h) groups, which were slightly shorter than the estimated time for in vivo-derived bovine blastocysts (7–8 days).
Limitations, reason for caution: HSA supplementation during culture may retard the precompaction development of bovine embryos, affect compaction, and trigger premature blastulation. However, to confirm this possibility, further studies, including comparisons of morphokinetics and gene expression between the in vivo-derived embryos and in vitro-produced embryos in the presence of HSA or rHA, are warranted.
Wider implications of the findings: To our knowledge, this is the first study to illustrate clear differences in morphokinetics between mammalian blastocysts cultured in conventional (HSA) and defined (rHA) media. Based on our results, we are currently investigating the yield, morphokinetics, and pregnancy rate after ET of human blastocysts cultured with HSA or rHA in clinical settings. These investigations will aid in the development of standardised and defined ART systems to eliminate the variation and risks associated with blood-derived products.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kuramoto Women's Clinic.
Trial registration number: NA.
Keywords: bovine, blastocysts, morphokinetics, recombinant human albumin, defined media

P-218 Day 3 embryos release intact mitochondrial DNA into the surrounding culture medium

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Study question: Do day 3 embryos release intact mitochondrial DNA (mtDNA) into the surrounding culture medium?
Summary answer: 60 percent of day 3 embryos release detectable levels of intact mtDNA into the surrounding culture medium.
What is known already: Recent evidence suggests that human embryos release mtDNA into the surrounding culture medium, and that this process may be modulated by embryo quality. Previous studies have confirmed the presence of short mtDNA fragments in the embryo culture medium, but no study has assessed whether the mtDNA released by day 3 embryos is intact or is largely fragmented or deleted.
Study design, size, duration: 48 individually cultured embryos from 12 patients were analysed for the presence of intact mtDNA in day 3 culture medium droplets (20 ml), along with controls. Embryos were cultured to day 5 using a sequential culture medium protocol and day 3 medium was analysed for the presence of intact mtDNA.
Participants/materials, setting, methods: To confirm the presence of the entire mtDNA genome, a long range PCR protocol was developed for the detection of low template mtDNA, and targeted to a 15 kb fragment encompassing the entire mitochondrial genome and a 2 kb fragment encompassing the ND2 and COX1 gene in purified embryo culture medium.
Main results and the role of chance: The 2 kb mtDNA fragment was detected in 85% of day 3 embryo culture medium samples. The entire mtDNA genome was detected in 60% of day 3 embryo culture medium samples, and was absent in culture medium control droplets, suggesting that the mtDNA was released from the embryo rather than originating from contamination during the culture period. mtDNA deletions were detected in only 2% of embryo culture medium samples, suggesting that the embryo may not preferentially release damaged mtDNA. The high percentage of intact mtDNA detected in culture medium samples suggests that the release of the entire mitochondrial genome may be a normal phenomenon occurring in day 3 embryos.
Limitations, reason for caution: Data is from a preliminary study that is limited by a small sample size.
Wider implications of the findings: Results presented in this study support previous reports that day 3 embryos release mtDNA into the surrounding culture medium. For the first time, the current study shows that embryos release the entire mitochondrial genome into the surrounding culture medium. It is possible that the presence of intact mtDNA in the embryo culture medium may relate to embryo quality on day 3 or be predictive of blastocyst quality, where this should be elucidated in a larger cohort.
Study funding/competing interest(s): Funding by national/international organization(s) – Auckland Medical Research foundation.
Trial registration number: NA.
Keywords: embryo, mitochondrial DNA, deletions, cell-free DNA

P-219 Peroxiredoxin 4, a new oxidative stress marker in follicular fluid may predict IVF outcomes

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Study question: For better predicting *in vitro* fertilization (IVF) outcomes, it is necessary to identify some non-invasive and sensitive markers.
Summary answer: Our results provide evidence that the upregulated expression of antioxidants in IVF patients follicular fluid (FF), such as peroxiredoxin4 (Prdx4), tend to increase the potential pregnancy via oocyte quality mechanism.
What is known already: Studies indicated that oxidative stress status in patients was closely associated with IVF outcomes, while the results are still controversial. Prdx4 as one member in Prdx family, can catalyze the reduction of reactive oxygen species. While little data on the relationship of Prdx4 and female reproduction were demonstrated.
Study design, size, duration: Our study is a prospective clinical study. All participants were recruited in the center of clinical reproductive medicine from September 2013 to December 2014. Infertile women with either tubal factor or male factor (n = 85) and polycystic ovary syndrome (PCOS) patients (n = 85) undergoing controlled ovarian hyperstimulation and IVF were recruited in our study.
Participants/materials, setting, methods: FF samples from patients were collected on the day of oocyte collection and then centrifuged and frozen up for analysis. Prdx4 concentration in FF was measured in each participant. Furthermore, the correlation between Prdx4 level and IVF outcomes, such as clinical pregnancy rate and oocyte quality was analyzed.
Main results and the role of chance: In both control and PCOS groups, pregnant women had higher levels of Prdx4 in FF than non-pregnant women. Prdx4 was also positively correlated with oocyte fertilization rates (r = 0.326; p = 0.013) and good quality embryo rates (r = 0.334; p = 0.011).
Limitations, reason for caution: A larger study should be conducted in order to investigate whether follicular fluid Prdx4 levels are correlated with good quality embryo rates and pregnancy outcomes. Moreover, the role of Prdx4 on oocyte and embryo quality should be studied to determine whether low Prdx4 concentration in FF is only a consequence or also a cause of follicular dysfunction.
Wider implications of the findings: Prdx4 evaluation in individual FF samples might represent an innovative biomarker of embryo quality to use as a supplemental tool to predict oocyte and embryo quality during IVF.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s) – The present work was supported by grants from China 973 Program (2012CB944703, 2012CB944702), the National Science Foundation of China (81200439) and the Graduate Students Scientific Research Innovation Plan of Jiangsu Higher Education Institutions.
Trial registration number: Not an RCT.
Keywords: antioxidant, follicular fluid, ovarian stimulation, oxidative stress, peroxiredoxin 4

P-220 Cumulative live birth rate in conditions where according to the restrictive law only up to 3 oocytes could be inseminated per cycle

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Study question: The purpose of this study was to examine the cumulative live birth rates of infertile couples, where according to the restrictive legal conditions only a limited number of oocytes (up to 3) could be inseminated per cycle.
Summary answer: Our results indicate that acceptable cumulative live birth rates of infertile couples can be obtained in conditions where according to the restrictive law only a limited number of oocytes could be inseminated per cycle.
What is known already: Cryopreservation allows expanding the options available to infertile couples. Oocyte cryopreservation has become increasingly available as an option for fertility preservation. During the last few years oocyte cryopreservation has become more successful contributing to the cumulative live birth rates. The transfer of cryopreserved embryos and oocytes increases the cumulative success rates after a single IVF stimulation.
Study design, size, duration: This retrospective cohort study included 148 stimulated cycles performed between May 2010 and December 2011. The subsequent 82 cycles with warmed oocytes and 20 frozen embryo transfers were done till March 2014.
Participants/materials, setting, methods: Metaphase II oocytes and embryos were vitrified according to Cryotop protocol. If pregnancy was not obtained during stimulated cycle a subsequent ICSI cycle was done with warmed oocytes. Also, if embryo transfer was not performed during stimulated cycle a frozen embryo transfer was done in subsequent natural cycle.
Main results and the role of chance: The mean numbers of aspirated oocytes and embryos transferred per patient during stimulated cycles were 9.12 ± 3.06 and 2.11 ± 0.62, respectively. In the fresh cycles, the clinical pregnancy and live birth rates obtained were 32.5% and 23.6%, respectively. The mean numbers of warmed oocytes and embryos transferred per patient were 3.04 ± 1.14 and 1.68 ± 0.71, respectively. The clinical pregnancy and live birth rates per embryo transfer obtained with vitrified oocytes were 16% and 10%, respectively. The mean number of frozen embryos transferred was 1.75 ± 0.55. The clinical pregnancy and live birth rates obtained with frozen embryo transfer were 60% and 50%, respectively. The cumulative pregnancy rate and live birth rate observed per started stimulated cycle was 42.5% and 31%, respectively.

Limitations, reason for caution: Results are specific and limited due to our restrictive law in that period - maximum of 3 oocytes per infertile couple were fertilized. Also, if embryo transfer was not performed during stimulated cycle in cases of ovarian hyperstimulation syndrome, endometrial polyp or hydrosalpinx a frozen embryo transfer was done in subsequent natural cycle.
Wider implications of the findings: Our results indicate that contribution of oocyte vitrification procedure followed by ICSI to cumulative live birth rate is inferior to fresh insemination procedure and frozen embryo transfer. Cumulative live birth rates from oocyte collections may provide the most relevant index of success.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University Hospital Centre Zagreb, Croatia.
Trial registration number: NA.
Keywords: cumulative live birth rate, restrictive law, oocyte cryopreservation, embryo cryopreservation

P-221 Comparison of the clinical outcomes following the transfer of blastocysts vitrified at day 5 and day 6

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Study question: The aim of the present study was to compare the clinical outcomes following the transfer according to the day (day 5 versus day 6) of vitrification.
Summary answer: In both blastocysts transfer of vitrified at day 5 and day 6 groups, the implantation and ongoing pregnancy rates were not statistically significant differences. The surplus day 6 blastocysts available for vitrification compromise to enhance the chance of pregnancy per oocytes retrieval and improve the cumulative pregnancy rate.
What is known already: The day 5 blastocysts include early blastocysts tends to result in higher pregnancy rates than day 6 blastocysts in the fresh cycle. However, in the thawed cycle, the blastocysts vitrified on day 5 and day 6 had the same morphological quality at the time of freezing showed no difference in clinical and ongoing pregnancy rates. The vitrification of the delayed blastocysts on day 6 reduces the embryo wastage and increases the cumulative pregnancy rate.
Study design, size, duration: The study was retrospectively 541 cycles (day 5 group: 334 and day 6 group: 207) analyzed from blastocyst transfers between January 2009 and April 2012. A total of 1292 embryos were vitrified-thawed and 1076 embryos were transferred.
Participants/materials, setting, methods: The surplus day 5 and 6 embryos were vitrified by EM-grid following artificial shrinkage. The equilibrium (EG20) and vitrification (EFS40) solutions were prepared, and embryo thawing was performed by a two-step dilution method. The thawed embryos were cultured overnight and survived embryos were transferred into the patient's uterine cavity.
Main results and the role of chance: The implantation and clinical pregnancy rates per transfer did not differ in the day 5 group (29.6% and 35.0%, retrospectively) compared with the day 6 group (24.2% and 28.0%). The corresponding ongoing pregnancy rate was 53.0% (62/117) in the day 5 group, and 51.7% (30/58) in the day 6 group, retrospectively ($p = 0.874$). The percentage of multiple pregnancy rate was not different between the two groups (33.9% versus 20%, $p = 0.171$). The number of transferred embryos in the vitrified blastocysts in the day 5 and the day 6 was 1.97 and 2.01 and the average of female age was 32.72 and 32.97, retrospectively, there were no significant differences.
Limitations, reason for caution: This is a relatively small study and the results are required large study population. The study of comparison was limited by the differences sample size in two groups.
Wider implications of the findings: The results showed the vitrified blastocysts on day 5 and day 6 lead to similar clinical outcomes when replaced in thawed embryo transfer cycles. The delayed day 6 blastocyst development does not influence the implantation and ongoing rates of vitrified-thawed transfer. Consequently, the vitrified-thawed day 6 blastocysts would be transferred in subsequent cycles to contribute to increasing cumulative pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maria Fertility hospital. No study funding or competing interest.
Trial registration number: NA.
Keywords: vitrification, blastocyst transfer, implantation, clinical pregnancy, ongoing pregnancy

P-222 Comparative time lapse imaging of embryos obtained after oocyte in vitro maturation (IVM) in two different IVM media

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Study question: Are there any differences in efficiency of oocyte maturation between two IVM media and is the timing of embryo cleavage divisions after oocyte IVM altered when comparing two different IVM media with standard ICSI (control).
Summary answer: Overall, both IVM media were comparable in terms of oocyte maturation rate, fertilization rate and embryo utilization rate. Top quality embryos were obtained in both media. Embryo kinetics after IVM in two different media were similar, but cleavage after the 4-cell stage was delayed when compared with standard ICSI.
What is known already: Non-hCG triggered oocyte IVM is particularly useful in women diagnosed with PCO(S) in order to avoid the risk of OHSS. Recently high maturation rates after oocyte IVM (69.7%; Junk et al., 2012) were reported using blastocyst medium for IVM with subsequently good embryo development. The availability of time lapse (TL) monitoring offers new insights in embryological development, although the predictive power of implantation potential by using TL monitoring is still under debate.
Study design, size, duration: Between January and April 2013, 27 PCO(S) patients were recruited for 29 IVM cycles. 473 sibling COCs were incubated in one of two IVM media. Retrospective TL analysis (Embryoscope; Fertilitech) was performed on 45 'Medicult-IVM' and 38 'BlastoCook-IVM' embryos and compared with 54 standard ICSI embryos.
Participants/materials, setting, methods: IVM media were: 'Medicult-IVM' containing Medicult IVM system (Origio) plus HSA, FSH, hCG and 'BlastoCook-IVM' containing Blastocyst medium (Cook) plus protein supplement, FSH, hCG. After 28 h, mature oocytes were inseminated and cultured 3 days in Quinn's Advantage Cleavage medium (Sage) in the Embryoscope. Statistics were performed using Wilcoxon and ANOVA + Tukey.
Main results and the role of chance: In 'Medicult-IVM' and BlastoCook-IVM' maturation rates were 37.3% and 35.2% (NS), fertilisation rates 57.4% and 59.7% (NS), utilisation rates of day 3 embryos 50.0% and 48.7% (NS) and top quality rates 18.1% vs. 34.9% ($p = 0.06$; Wilcoxon signed rank test). Normally fertilised embryos were analysed for cleavage timings. No differences were recorded after IVM in the two IVM media. Compared to control embryos, extrusion of the second polar body was slower in embryos generated with 'Medicult-IVM' (4.2 h vs. 3.4 h in control, $p = 0.03$), division to 5-cell was slower in embryos generated with 'BlastoCook-IVM' (53.1 h vs. 47.3 h; $p = 0.005$) and division to 6-cell appeared slower in embryos from both IVM media (53.1 h in 'Medicult-IVM' and 54.4 h in 'BlastoCook-IVM vs. 48.8 h in control $p = 0.001$; ANOVA + Tukey).
Limitations, reason for caution: The number of embryos for TL analysis in this study is too limited to analyse pregnancy rates. The optimal control group for TL would be embryos generated in standard ICSI cycles of PCO(S) women, as they show a delay in timing of embryonic development compared to non-PCO(S) women.
Wider implications of the findings: Although the use of blastocyst medium for IVM has been reported to generate high rates of mature oocytes, this value was not confirmed by our data. Not the same blastocyst medium has been used in our study and patient serum was averted due to its undefined nature. Oocyte IVM delays embryonic cleavage divisions and possibly contributes to the currently lower live birth rates obtained after IVM compared to standard ICSI.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Free University Brussels (Vrije Universiteit Brussel – VUB), Universitair Ziekenhuis Brussel.
Trial registration number: NA.
Keywords: IVM, time lapse imaging, culture media

P-223 Blastocyst versus day 2–3 transfer in ICSI cycles for male factor infertility: a multicenter randomized controlled trial

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Study question: What is the effect of extended embryo culture till day 5 in cases suffering from male factor infertility on clinical pregnancy rate?
Summary answer: Blastocyst transfer in cases suffering from male factor infertility resulted in significant increase in clinical pregnancy rate and insignificant decrease in abortion rate.
What is known already: A recent meta-analysis concluded that implantation and pregnancy rates are improved and abortion rates are decreased following blastocyst transfer as compared to cleavage stage embryos. However, in male factor infertility the evidence is still insufficient to recommend blastocyst transfer in these cases.
Study design, size, duration: Single blinded prospective multicenteric randomized controlled trial. 326 participates were randomized for day 2/3 or day 5 transfer. Randomization was done by computer permuted blocks size of 4, allocation by closed envelops, parallel technique. The study was conducted between June 2013 and March 2014.
Participants/materials, setting, methods: 400 couples suffering of male factor agreed to participate in the study of which 326 were eligible. Long agonist protocol was used and ICSI was done. 126 cases had day 2/3 transfer and 126 had blastocyst transfer. Primary outcome was clinical pregnancy rate. Secondary outcome was abortion rate.
Main results and the role of chance: There were insignificant difference between both groups regarding the number of retrieved oocytes, fertilization and cleavage rates and day 2 class A embryos. There was significant increase in the clinical pregnancy rates in the blastocyst group as compared to the cleavage stage group (60% vs. 35%) $p < 0.01$. The abortion rate was insignificant lower in the blastocyst group as compared to day 2/3 group (16% vs. 27%) $p = 0.237$
Limitations, reason for caution: The study only included fresh transfers and adding the data of cryopreserved embryos may affect the cumulative pregnancy rates in both groups. In addition the study was not extended to detect live birth rates as literature proved improvement in live birth rate after blastocyst transfer.
Wider implications of the findings: Infertility etiology as male factor is an important variable that should be considered when deciding the timing of transfer. Individualized policy for embryo transfer regarding timing, number of embryos and fresh or frozen cycles is mandatory to improve implantation and pregnancy rates. Blastocyst transfer should be the first choice in fresh cycles and further studies are needed to compare cumulative pregnancy rates as in cleavage stage embryos usually there are more embryos available for cryopreservation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Alexandria ICSI – IVF Center, Egypt, Misrata National Center for Infertility, Libya.
Trial registration number: PACTR201308000581376.
Keywords: blastocyst, ICSI, male factor

P-224 Ultrastructural assessment of human metaphase II oocytes after cryopreservation with media containing different macromolecular supplements

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Study question: To verify whether type and concentration of protein supplement included in freezing solutions affect the ultrastructure of human metaphase II (MII) oocytes cryopreserved by slow cooling and therefore optimize cryopreservation conditions.
Summary answer: This study confirms: (1) slow freezing ensures good preservation of the oocyte; (2) premature cortical granules (CG) exocytosis and vacuolization are both markers of cryodamage; (3) prolonged culture before cryopreservation may cause enlargement of mitochondria-vesicle (MV) complexes. Furthermore serum supplementation induces good preservation of the ooplasm avoiding extensive vacuolization.
What is known already: Cryoprotective agents (CPAs) are essential components in freezing solutions, but may also disrupt the meiotic spindle and organelles. Together with conventional CPAs, protein supplement is known to preserve cell structure. CG exocytosis requires a healthy plasma membrane and cytoskeleton. This process may be affected by cryopreservation as a result of shrinkage during CPA addition leading to subjacent localization of cortical granules and resulting in release of their contents because of plasma membrane fusion after rewarming.
Study design, size, duration: Forty supernumerary MII oocytes were donated by consenting patients (aged 28–36) enrolled in an IVF program. Thirty-four oocytes were cryopreserved using slow freezing with 0.2M sucrose, 1.5M 1–2 propanediol (PROH) and either serum or Plasma Protein Solution (PPS) in the freezing mixture. Six oocytes were used as fresh controls.
Participants/materials, setting, methods: Oocytes were cryopreserved with 20% ($n = 12$) and 10% ($n = 10$) serum or 10% PPS ($n = 12$). Samples were fixed by 2 h after thawing and prepared for light and transmission electron microscopy (LM and TEM) for ultrastructural analysis of CG, mitochondria-smooth endoplasmic reticulum aggregates (M-SER) and vacuolization.
Main results and the role of chance: By LM, both control and cryopreserved oocytes appeared rounded and with uniform distribution of organelles. By TEM, M-SER and small (MV) complexes were the most numerous structures found in all oocytes. Only in a few cryopreserved oocytes, irrespective of macromolecular supplement, numerous large MV complexes were found, probably due prolonged culture (3–4 h) before cryopreservation. Amount and density of CG appeared abnormally reduced in all samples. Different degrees of vacuolization were present in the ooplasm of cryopreserved, but not fresh oocytes. Extensive vacuolization was present only in a minority of oocytes cryopreserved with serum (16.6% of the oocytes supplemented with 20% serum and 20% of the oocytes supplemented with 10% serum), whereas a higher number (66.6%) of oocytes supplemented with 10% PPS were largely vacuolized.
Limitations, reason for caution: Although the interest raised by the investigation of which macromolecular supplement gives best results in terms of ultrastructural preservation of oocyte quality and integrity, this study should be extended in order to verify this finding in clinical routine.
Wider implications of the findings: This approach can be considered of interest for different cryopreservation methods extensively adopted in assisted reproductive treatments. In fact, the matter of protein supplement in different formulations and concentrations is still debated both for culture media supplementation and vitrification solutions.
Study funding/competing interest(s): Funding by hospital/clinic(s) – San Raffaele Scientific Institute, Milan, Italy.
Trial registration number: NA.
Keywords: oocyte cryopreservation, protein supplementation, cortical granules, transmission electron microscopy, ultrastructure

P-225 Day 3 low quality embryos can develop to blastocysts and produce pregnancies: A retrospective study

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Study question: Can day three embryos of low quality, which would usually be discarded, develop into blastocysts and produce a pregnancy?
Summary answer: The culture of these low quality embryos to blastocyst stage allows us to recover and freeze some embryos that would have otherwise been discarded. When transferred in a posterior frozen cycle, these embryos can produce a pregnancy.
What is known already: There is not a consensus to decide the fate of the poor quality supernumerary embryos on day 3. Vitrification of these embryos is highly inefficient due to their low implantation rates. However, by discarding the embryos we risk losing embryos that might implant. Their culture to blastocyst stage is an additional opportunity to assess whether it is worth it to cryopreserve them.

Study design, size, duration: This retrospective study includes 743 IVF cycles performed between January 2013 and September 2014.
Participants/materials, setting, methods: 743 patients were recruited, with ages between 22 and 45. They had fresh embryo transfers in day 3 and low quality supernumerary embryos that were not selected for transfer nor vitrification. Those embryos remained in culture up to day 5–6, and were re-assessed and cryopreserved if they had developed into blastocysts.
Main results and the role of chance: Of 1574 supernumerary embryos of low quality in day 3 that were left in culture until day 5–6, 80 (5.1%) developed into blastocysts (Grade 3CC or superior). 11 couples underwent a transfer of the study embryos, obtaining 6 pregnancies (54.5%), 5 of them being confirmed as clinical pregnancy rates (45.4%), resulting in 3 deliveries (27.3%).
Limitations, reason for caution: This study included patients of different characteristics, so we cannot determine if this policy is useful for all kind of couples.
Wider implications of the findings: Extended culture to day 5–6 of low quality supernumerary embryos on day 3 is an efficient strategy to cryopreserve embryos with low implantation potential on day 3. This strategy allows re-assessing embryos in their blastocyst stage, which is more indicative of their potential to produce a pregnancy. It its time and resource saving and provides a second opportunity to embryos that would otherwise be discarded.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ginemed Clinic.
Trial registration number: NA.
Keywords: human reproduction, embryo culture, blastocyst transfer, low quality embryos

P-226 Global single step medium for uninterrupted culture to blastocyst in the EmbryoScope: examination of clinical outcome and live birth data from blastocyst transfer cycles

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Study question: Is medium refreshment beneficial in maximizing blastocyst formation, clinical pregnancy and live birth rate when culturing to day 5 in a single step medium?
Summary answer: Global single step medium could be successfully used for continuous uninterrupted culture to blastocyst in the EmbryoScope. Blastocyst formation, overall embryo utilization, implantation rate, on-going pregnancy and live birth rates were not enhanced by medium refreshment on day 3. Mean birth weight was however higher with uninterruped culture.
What is known already: Sequential two-step culture media for blastocyst formation present embryos with different nutrients pre and post genomic activation. In contrast, single step media allow the embryo to select the nutrients it needs at the appropriate time. With the introduction of time-lapse imaging systems, transition to a single step media that allows uninterrupted culture to blastocyst is highly desirable. However, depletion of nutrients or build-up of deleterious embryonic metabolites in microdrop culture may negatively influence outcomes.
Study design, size, duration: Data from three consecutive years was retrospectively analyzed for patients having a blastocyst stage transfer. The study group included patients <40 with ≥6 zygotes cultured in the Embryoscope time-lapse incubator. Blastocyst development, implantation, pregnancy and neonatal outcomes were contrasted after continuous culture with or without medium refreshment.
Participants/materials, setting, methods: Zygotes were cultured (25 µl) in GB medium supplemented with 10% SPS (Synthetic Protein Substitute) in the EmbryoScope at 37°C, 6% CO_2, 6% O_2. Group A embryos (medium refreshed on day 3) were contrasted to Group B embryos (uninterrupted culture). Blastocysts were transferred on day 5 and surplus embryos frozen.
Main results and the role of chance: With exception of mean birth weight, blastocyst development and clinical outcome parameters did not differ between treatments. Embryonic metabolite accumulation in microdrops did not appear to be deleterious. Overall embryo utilization i.e. percentage of blastocysts available for either transfer or freezing was not enhanced by day 3 medium exchange.

	Medium refresh	Uninterrupted culture
Transfer Cycles	59	220
Patient Age	33.9 ± 3.4	3.4 ± 3.3
Mature Oocytes	12.7 ± 4.0	12.0 ± 4.0
Fertilization rate	66%	61%
Total Zygotes	718	2332
Embryos transferred	1.9 ± 0.5	1.9 ± 0.4
Positive hCG	80% (47/59)	86% (176/220)
Clinical Pregnancy	69% (41/59)	74% (163/220)
Implantation Rate	53%	59%
Blastulation Rate	64%	65%
Blastocysts Frozen	74%	70%
Embryo Utilization	65%	63%
Ongoing pregnancies	–	97
Pregnancy losses (to date)	1/41 (2.4%)	7/163 (4.3%)
Delivered pregnancies	40	59
Singleton	21	37
Twins/Triplet	19/0	21 /1
Birth weight (grams)	2531.0 ± 1.3	2748.8 ± 1.5*

*p-value <0.05 considered to be significant.

Limitations, reason for caution: This was a retrospective analysis. Although no differences were observed to the point of establishing a clinical pregnancy, detailed comparison of neonatal data is still pending as not all pregnancies have delivered. Confounding factors such as patient age, diagnosis and cycle characteristics were not controlled for.
Wider implications of the findings: With new time-lapse culture systems, it is highly desirable to perform 'hands- off' uninterrupted culture of zygotes to the blastocyst stage. These preliminary data indicate that nutrient depletion and metabolic waste buildup in microdrops is not a significant problem when using one-step Global medium. Moreover, the environmental conditions for blastocyst culture provided with this medium and the EmbryoScope incubation chamber translated in to high implantation, pregnancy and live birth rates with day 5 embryo transfers.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Cleveland Clinic.
Trial registration number: NA.
Keywords: time-lapse, blastocyst, EmbryoScope, implantation, culture media

P-227 Morphokinetic range reduces from embryos that implant, to embryos that blastulate but cannot implant, to embryos that cannot blastulate: Evidence in favour of time-lapse

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Study question: Can we narrow down the choice of embryos for transfer through determining minimum and maximum time-points of embryonic developmental stages in cleaving, blastulating and implanting embryos?
Summary answer: There is a narrower time-range at all developmental stages for embryos that go on to form an on-going clinical pregnancy compared to those that do not. These maximum and minimum values can be used to de-select non-viable embryos from treatment to assist in counselling patients on their chances of pregnancy.
What is known already: The efficacy of time-lapse technology is dependent on understanding the relationship between morphokinetics and implantation. Originally, models were designed around blastulation rather than implantation and consequently some models are not accurate predictors of implantation. Models have also been demonstrated to require local validations to identify optimal morphokinetic limits for each clinic. Consequently, there has been considerable debate as to the clinical use of time-lapse technology over conventional static observations in predicting embryo viability.
Study design, size, duration: Data were collected from 3201 cleaved 2PN embryos with known implantation data (KID) cultured in Embryoscope incubators from September 2013 to November 2014 and annotated using the Academic Reproductive Partnership annotation protocols from insemination to transfer, vitrification or discard.
Participants/materials, setting, methods: Embryos were divided into three categories: cleaved but not blastulated (C, n = 2027); blastulated but not implanted(B, n = 1111); implanted(I, n = 63). Minimum, maximum, ranges for morphokinetic parameters for each category (C, B, I) were compared using relevant statistical tests. t2 embryos were categorised between <22, 22–25, 25–28, 28–32 and ≥32 h post insemination (hpi).
Main results and the role of chance: With all morphokinetic parameters, the range was largest for cleaved, followed by blastulated and smallest for

implanted embryos(paired *t*-test, $p < 0.001$). For C,B and I, range increased as the embryo progressed in development from tPB2-tsB (correlation, $p < 0.001$), demonstrating that absolute time points become more variable at later stages of development. No embryos implanted outside the I ranges[(minimum-maximum): tPNf(19.21–28.61), t2(21.72–31.78), t3(28.65–44.56), t4(30.15–44.56), t5(33.99–63.87), t6(37.49–63.87), t7(37.82–64.84), t8(40.83–78.29), tSC(52.04–98.31), tM(70.88–108.22), tSB(81.02–114.03), tB(88.69–126.33), tEB(96.39–135.51), tHB(96.89–119.38), relative cc2(1.00–6.67), cc2(1.00–6.67), s2(0.00–4.80)]. The range in morphokinetics for implanting embryos as a proportion of the range by blastulating embryos were: tPB2(63%), tPNa(43%); tPNf(28%); t2(30%); t3(35%); t4(26%); t5(51%); t6(35%); t7(35%); t8(49%); tsC(50%); tM(41%); tSB(41%); tB(48%); tEB(47%); tHB(29%); cc2(77%); s2(19%); relative cc2(5%). Therefore, de-selection of a considerable proportion of blastocysts is possible and is most effective at earlier stages of embryo development(tPNf-t4) compared to later stages (t8-teB). In 1583 embryos, frequencies of embryos cleaving <22, 22–25, 25–28, 28–32 and ≥32 hpi were 3%, 21%, 27%, 25%, 24% and KID ratios were 3%, 14%, 9%, 4%, 0% ($p < 0.001$). Therefore, despite 24% of embryos dividing post 32 hpi, none implanted.

Limitations, reason for caution: Data were collected from one laboratory using a specific culture system and protocols. Since multiple environmental factors affect embryo development, these data should not be assumed to be applicable to all IVF laboratories. It is therefore important that each IVF clinic validates their own embryo selection models.

Wider implications of the findings: Ability to predict implantation potential will not only aid embryo selection (with possible increases in success rates), but also aid management of patients' expectations of pregnancy prior to transfer. A considerable proportion of blastocysts cannot lead to implantation, some of which can be predicted and deselected using time-lapse technology.

Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Hammersmith, Boston Place Clinic.

Trial registration number: NA.

Keywords: time-lapse, morphokinetics, embryology, embryo development, embryo selection

P-228 The benefit of prolonged culture with primary aseptic vitrification embryo transfer (aVET) in cases of slow blastulating embryos

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Study question: Aim of this study was to evaluate whether IVF/ IMSI success rates of slow blastulating embryo (SBEs) can be improved with prolonged embryo culture and aVET. We compared clinical outcome of fresh day 5 embryo transfer (ET) of SBEs, with aVET of SBEs after prolonged culture.

Summary answer: We observed very low pregnancy- (PR) and birth rates (BR) after fresh ET of SBEs on day 5. Vitrification of SBEs and prolonged culture, either before or after cryopreservation remarkably improved clinical outcomes. Better synchronization between embryo development and endometrium are suggested to be mainly responsible for this finding.

What is known already: IVF-embryos should present as fully expanded or hatching blastocysts on day 5 (Istanbul Consensus, 2011). These embryos show the highest chance to establish a pregnancy. However, in some cases often related with patient's age and medical histories, it is observed that a embryos undergo a slower development with delayed blastulation (SBE). It is well recognized, that such embryos, when transferred on day 5 show strongly reduced implantation rates.

Study design, size, duration: A retrospective study during 2007–2013 was conducted comparing clinical outcome after fresh day 5 ET of SBEs, and after aVET following prolonged embryo culture in 2 protocols: (i) SBEs vitrified day 5 and warmed 24 h before aVET, (ii) SBEs vitrified day 6 and warmed 3 h prior to aVET.

Participants/materials, setting, methods: Outcomes of 488 fresh day 5 ETs with SBEs were included. Data were compared with clinical outcomes of (i) 82 aVETs with SBEs vitrified day 5 and warmed 24 h before ET and (ii) 58 aVETs with SBEs vitrified day 6. All aVETs were transferred in a day 5 receptive endometrium.

Main results and the role of chance: PR after day 5 fresh single embryo transfer (SET) with top early blastocysts (mean female age 37.5 years) was 36.4%, with non-top early blastocysts (mean age 37.1 years) PR was 13.7%. BR was 26.1% and 6.5%, respectively. In 181 SETs and 95 DETs with morulae or compacting embryos on day 5, PR was 4.4% and 7.4%, BR 3.9% and 3.2%, respectively. In aVET cycles (mean age 38.2 years) of (i) SBEs vitrified day 5 and warmed 24hrs prior to ET, PR rate was 35.4%, BR 18.3%; after aVET of (ii) embryos vitrified day 6, PR and BR with top-grading blastocysts derived from SBEs was 57.1% and 42.9% respectively. Non-top grading blastocysts vitrified on day 6 showed PR of 26.7% and BR 18.3%.

Limitations, reason for caution: Although results are promising and no increase in the rate of congenital malformations was observed in our data set, further studies need to verify that there is no health risk for the offspring derived from SBEs.

Wider implications of the findings: This study demonstrates that prolonged embryo culture and aVET into a day 5 receptive endometrium can improve clinical outcome of SBEs. This strategy could be an important improvement for patients with recurrent slow embryo development in IVF-culture. We show that prolonged culture and a primary aVET should be proposed over a fresh day 5 ET with SBEs. We hypothesize that a better synchronization between embryo development and endometrium might be responsible for this finding.

Study funding/competing interest(s): Funding by hospital/clinic(s) – This study was not externally funded.

Trial registration number: All research was conducted in accordance with the Helsinki Declaration.

Keywords: implantation rate, slow embryo development, blastocyst, birth outcome

P-229 Clinical outcome of multinucleated embryos: is it worthwhile vitrifying them?

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Study question: What is the clinical outcome in single embryo transfers (SET) of vitrified-warmed day 3 embryos and day 5/6 blastocysts that showed multinucleation in the early-cleavage stage. Is it worthwhile to vitrify multinucleated embryos?

Summary answer: Multinucleated embryos may have a good implantation potential, especially when cultured up to day 5. For embryos cultured up to day 3, a higher implantation rate was found when the multinucleation was seen on day 1, instead of day 2 or day 3.

What is known already: Since multinucleated embryos are associated with an increased risk for aneuploidy and lower implantation rates, they are preferentially not selected for fresh embryo transfer. However, some multinucleated embryos are cryopreserved when they develop into morphologically good quality embryos. Therefore it is important to assess the outcome of warming cycles in which multinucleated embryos have been transferred.

Study design, size, duration: Retrospective analysis of 189 SET warming cycles performed between 2010 and 2012, in which day 3 ($n = 99$) or day 5/6 ($n = 90$) embryos showing multinucleation on day 1 ($n = 38$), 2 ($n = 104$) or 3 ($n = 47$) of development were warmed. The main outcome parameters were clinical pregnancy rate (CPR) and implantation rate (IR, with FHB).

Participants/materials, setting, methods: Multinucleation was typed as either K2 (2 nuclei in ≤ 50% of blastomeres, $n = 70$), K3 (>2 nuclei in ≤50% of blastomeres, $n = 97$) or K4 (≥2 nuclei in >50% of blastomeres, $n = 22$). The outcome was compared according to the type and day (1, 2 or 3) of multinucleation.

Main results and the role of chance: No significant differences in CPR and in IR were observed between multinucleation type K2 (24.3% and 18.6%), K3 (24.7% and 18.6%) and K4 (36.4% and 31.8%). CPR and IR were similar for multinucleation detected on day 1 (36.8% and 31.6%), day 2 (22.1% and 16.4%) and day 3 (25.5% and 19.2%). CPR and IR were significantly higher after day 5/6 vitrification (38.9% and 30.0%) compared to day 3 (14.1% and 11.1%, $p < 0.01$). Also a subgroup analysis according to the day of vitrification was performed. While day 3 embryos had a significantly higher CPR for multinucleation on day 1 (35.7%) compared to day 2 and day 3 (10.5%, $p = 0.018$ and 10.7%, $p = 0.049$, respectively), this difference was not observed for embryos vitrified on day 5.

Limitations, reason for caution: The results are based on a retrospective analysis with a relatively small sample size.

Wider implications of the findings: Our current findings indicate that transfer of multinucleated embryos may result in good IR. However, it seems preferable to culture multinucleated embryos up to day 5/6 and selectively vitrify

those that develop into blastocysts. When cryopreservation is performed on day 3, selection criteria should take into account which day multinucleation was detected, since day 1 multinucleated embryos show the highest implantation potential. Furthermore, multinucleated embryos may be used as second choice embryos in fresh transfers.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Universitair ziekenhuis Brussel.

Trial registration number: NA, no RCT.

Keywords: multinucleation, vitrification, IVF/ICSI, embryo selection

P-230 Prospective multicentre study to evaluate the influence of morphological embryo categories and day-3 embryokinetic markers on implantation rates

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Study question: Is there a marker of embryo morphokinetics that improves the prognostic capability of morphological embryo categories in embryo implantation?

Summary answer: Pronuclear fading was the only morphokinetic parameter found to improve the prognostic capability of morphological embryo categories in embryo implantation.

What is known already: To date, most studies of morphokinetics and embryo implantation have proposed contradictory prognostic models, based solely on morphokinetic criteria. At present, there is no consensus as to which is the best morphokinetic parameter for predicting embryo implantation. One strategy for improving the performance of morphokinetic parameters is to incorporate in predictive models the conventional static morphological parameters used before the onset of time-lapse technology.

Study design, size, duration: A multicentre prospective study was conducted from January to November 2014. Fourteen centres participated in this study, which included 347 ICSI cycles with day-3 transfers, resulting in 85 implanted embryos, 206 non-implanted embryos and 118 embryos in which the implantation result was unknown.

Participants/materials, setting, methods: The embryos were analysed with a time-lapse system (Primo Vision, Vitrolife, Sweden). All the embryos were classified into four categories (A, B, C, D), following the Spanish Association of Reproduction Biology Studies recommendations (ASEBIR categories), according to which category A gave the best and category D the worst prognosis for a combination of various morphological parameters. Confounding factors such as diagnosis, patient age, years of sterility, origin of oocytes, stimulation and lab protocols were controlled.

Main results and the role of chance: In the univariate analysis, ASEBIR categories, pronuclear fading, CC1, t5-t4 and years of sterility were found to be related with embryo implantation. Logistic regression showed that ASEBIR categories and pronuclear fading were significant predictors of implantation. The implantation rate and pronuclear fading in the ASEBIR categories were statistically different (Implantation rate: A = 48.2%; B = 26.5%; C = 20.4%; D = 6.0%; Pronuclear fading: A = 22.8 ± 2.8; B = 24.0 ± 5.1; C = 24.3 ± 6.3; D = 25.8 ± 5.0). The Nagerlkerke-corrected R^2 value showed that the model with both variables produced a better model of implantation (0.227) than when pronuclear fading (0.071) or ASEBIR categories (0.172) were analysed independently. The area under the curve obtained for embryo implantation was similar for pronuclear fading (0.619 [0.555–0.683]) and for ASEBIR categories (0.701 [0.642–0.760]).

Limitations, reason for caution: This model was developed using embryos obtained by ICSI alone, and so requires further validation for embryos obtained by IVF. The sample size must be increased in order to improve the prognostic value of the model obtained.

Wider implications of the findings: The inclusion of kinetic parameters within morphological embryo categories improves embryo selection criteria and can predict implantation. The study findings support the utility of time lapse and kinetic markers to assess embryo competence. In contrast to retrospective single-centre studies, in our multicentre study, the implanted and non-implanted embryos differ mainly in the existence of an early morphokinetic marker. Later morphokinetic markers could be affected by individual laboratory conditions (workflow, number of incubator, air quality, etc.) and thus have poorer prognostic value for embryo implantation between laboratory.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by Hospital Universitario Virgen de las Nieves (Granada) and Centro MasVida Reproducción (Sevilla). Internal funding.

Trial registration number: NA.

Keywords: time-lapse, implantation, morphokinetic.

P-231 Modified, successful method of cryoprotectant dilution after oocyte slow-freezing

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Study question: Does it exist any difference in survival rate of slow-frozen oocytes depending on cryoprotectant dilution approach, reflecting on clinical effects?

Summary answer: Different from traditional, sucrose mediated propanediol (PrOH) dilution leads to significantly higher survival rate of slow-frozen oocytes and may have positive effect on implantation and pregnancy rates.

What is known already: Slow-frozen human oocytes usually survive thawing with lower success rate than their vitrified-warmed counterparts. However, very recently it was shown that an improvement of survival and activation rates of slow-frozen oocytes may be achieved using an alternative, warming-like thawing system (Parmegiani et al., *Reprod Biomed Online* 2014;28:614–623).

Study design, size, duration: Retrospective analysis of thawing effects of 291 slow-frozen oocytes, obtained in 56 thawing cycles, performed in two consecutive periods: March 2007 – February 2012 and March 2012 – June 2014. Oocyte survival rate in both periods was compared, following with analysis of clinical data after fertilization and embryo transfer.

Participants/materials, setting, methods: Oocytes were subjected to thawing and traditional, 4 step propanediol dilution method or to modified, sucrose mediated 3 step dilution. Two concentrations of sucrose solution (0.5 and 0.1M) were applied for this purpose. After 1 and 2 min of treatment, respectively, oocytes were moved to IVF medium and fertilized.

Main results and the role of chance: Twenty two and 34 thawing cycles were performed in the first and second period, respectively. Survival rate of oocytes subjected to the traditional dilution was 65.1% whereas 92.9% of oocytes survived thawing procedure if followed with the modified dilution method ($P \leq 0.0001$, the two-sided P value, Fisher's exact test). Clinical pregnancy (CPR) and implantation rates (IR) tended to be higher after transfer of embryos developed in the modified dilution group (33.3% and 27.8% vs. 23.5% and 14.8%, respectively). After transfer of embryos developed from oocytes subjected to the second, modified dilution method 8 births resulting in 9 healthy and one preterm baby have occurred by now. Two twin pregnancies are still ongoing.

Limitations, reason for caution: Study presented here bases on retrospective data, collected during 2 subsequent periods. Number of thawing cycles and number of oocytes thawed were rather limited. It was feasible to confirm

statistical difference in survival rate of oocytes between analyzed groups, but differences in CPR and IR did not reach statistical significance.
Wider implications of the findings: The high oocyte survival, CPR and IR obtained using modified, sucrose mediated dilution method, resembling data obtained using vitrified/warmed oocytes, suggest a new, simple way to obtain a satisfactory yield from slow-frozen oocytes. Healthy babies born after this modification ensure safety of the method employed. Taken together, if confirmed in following studies, presented data provide optimistic view on fate of oocytes cryopreserved already by means of slow-freezing method.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Private Fertility Clinic 'nOvum'.
Trial registration number: Retrospective study.
Keywords: oocyte, slow-freezing, sucrose mediated dilution, healthy children

P-232 Does endometriosis affect oocyte and embryo quality: perspectives from in vitro fertilisation cycles

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Study question: Does endometriosis affect oocyte and embryo quality?
Summary answer: In women undergoing IVF, the presence of endometriosis does not affect oocyte quality and its development potential.
What is known already: Endometriosis is known to be detrimental to fertility. This may be related to factors associated with the endometrium and also oocyte/embryo quality. Whilst many studies have explored the endometrium related pathophysiology of endometriosis, very few studies examined the latter. Animal studies suggest that oocyte and embryo development is poorer when exposed to follicular and peritoneal fluid of women with endometriosis. Recent evidence suggests that women with endometriosis have a higher chance of aneuploidy not dissimilar to those with advance maternal age.
Study design, size, duration: We performed a retrospective evaluation of women with endometriosis undergoing IVF in our centre. We reviewed treatment cycle and embryology records via IDEAS™ database from January 2011 to December 2014. Comparative analysis was performed between groups and was expressed as means ± SD or percentages as required.
Participants/materials, setting, methods: Women who were <40-years old who underwent IVF treatment using their own gametes were included. The study group consisted of women who had endometriosis (EN) diagnosed by laparoscopy and the control groups were those with tubal factor (TF) and unexplained (UN) subfertility. We excluded couples with co-existing male factor subfertility who required ICSI. There is no restriction on the stimulation protocols and stimulation drugs used.
Main results and the role of chance: We reviewed 474 IVF cycles, out of which EN; 45, TF; 81 and UN; 130 cycles were analysed. Baseline characteristics were comparable including age (EN; 34 ± 4, TF; 33 ± 4 and UN; 34 ± 3), stimulation protocol and type of FSH between groups. The total number of oocytes collected (EN; 9.2 ± 5, TF; 10.4 ± 7 and UN; 9.6 ± 5), number of normally fertilised oocytes (EN; 6.0 ± 4, TF; 6.5 ± 4 and UN; 6.4 ± 4), and total number of embryos transferred per patient were comparable. With regards to embryology data, cleavage rates were the same for both comparisons. The proportion of good quality embryos per cycle were the same (OR 0.75 95% CI [0.56–0.99]. The number of embryos arresting at D3 and rate of blastocyst per retrieved oocytes were similar in all groups.
Limitations, reason for caution: This study was limited by the small number of patients and a larger cohort of patients is required to give more definitive conclusions. Additionally, women with endometriosis were pooled and not stratified according to disease severity, which may give different outcome to oocyte quality.
Wider implications of the findings: Our results showed oocyte quality and its development were similar in the presence of endometriosis when compared to other indications.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Ministry of Education, Malaysia, Complete Fertility Centre.
Trial registration number: NA.
Keywords: fertilsation, embryo quality, endometriosis, blastulation

P-233 Day 3 transfer using the automated time-lapse enabled EevaTM Test results in similar clinical pregnancy and implantation rates to blastocyst transfer: a longitudinal cohort study

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Study question: The Eeva Test predicts the likelihood that an embryo will form a blastocyst based on automated time-lapse analysis of early cell division timings. Can cleavage stage transfer, following the use of the Eeva Test to improve embryo selection, achieve comparable clinical outcomes of blastocyst stage transfer?
Summary answer: Day-3 transfers using the Eeva Test combined with morphology to select embryos resulted in equivalent implantation rates compared to concurrent blastocyst transfers. The results were consistent in both younger and older cohorts, even though patients using the Eeva Test had fewer eggs and embryos for selection.
What is known already: Blastocyst culture enables embryo self-selection and improves pregnancy and implantation rates. However, this requires optimal culturing conditions and is associated with adverse epigenetic effects. The Eeva Test, using automated time-lapse analysis and a generalizable prediction model, provides predictive information about embryo developmental potential on day-3 to improve the success of cleavage stage transfer. This is the first longitudinal cohort study directly comparing clinical outcomes between cleavage stage transfer using the Eeva Test and blastocyst transfer.
Study design, size, duration: Prospective longitudinal cohort study (September 2012–August 2014) comparing clinical pregnancy and implantation rates between a group of patients who had cleavage stage transfer using the Eeva Test ($N = 341$) and a concurrent parallel group of patients who had blastocyst stage transfer without using the Eeva Test ($N = 203$).
Participants/materials, setting, methods: Women <43 years old with two or more embryos on Day-3 elected for Day-3 transfer with Eeva Test or blastocyst transfer. Clinical pregnancy and implantation rates were compared, and *p*-values were calculated using Fisher's exact test. Logistic regression further assessed the impact of transfer-day on clinical pregnancy.
Main results and the role of chance: In women <37 years old, the Eeva Test and Blastocyst Group ($N = 176$ and 144) showed no statistical differences in CPR (46% vs. 48%, $P = 0.74$) and IR (35% vs. 41%, $P = 0.17$), even though Eeva patients had fewer eggs and embryos. Similar results were found in women 37–42 years old (Eeva Test vs. Blastocyst Group: $N = 165$ vs. 59; CPR: 44% vs. 25%, $P = 0.51$); IR: 18% vs. 20%, $P = 0.88$). Logistic regression revealed that transfer-day did not predict outcome, suggesting that Day-3 embryos selected using the Eeva Test together with morphology had the implantation potential of blastocysts.
Limitations, reason for caution: Patients elected to use the Eeva Test or to have blastocyst transfer; therefore, they were not randomized into the two groups. Nevertheless, the study included a broad population of patients (<43 years old), and the Eeva Test group constituted a poorer prognosis than the control group.
Wider implications of the findings: Our study indicated that cleavage stage transfer, using the Eeva Test to improve embryo selection, can achieve comparable clinical outcomes to blastocyst stage transfer. This provides a viable option for programs with limited resources to perform blastocyst culture to improve their success. For patients with higher risk of cycle cancellation, using the Eeva Test may result in better outcome. Improved implantation for day 3 ET will encourage single embryo transfer practice and reduce potential risks associated with multiple pregnancies and extended culture.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No external funding.
Trial registration number: NA.
Keywords: automated time-lapse, Eeva test, embryo selection, cleavage stage transfer, blastocyst transfer

P-234 Embryo ornithine release: a predicative marker for implantation failure?

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Study question: The selection of embryos with higher implantation potential has been one of the major challenges in assisted reproductive technology (ART). This selection currently based on morphological criteria has been correlated with implantation potential but its accuracy has proved limited. Actually, too many embryo are transferred without capacity of implantation. Thus, rather find a 'successful' marker, we propose to search for a 'failure' biomarker which can be used to eliminate embryo unable to implant.
Summary answer: The non-invasive determination of ornithine in embryo culture may constitute an attractive marker of implantation failure, morphology independent.
What is known already: Metabolomic profiling of culture medium from growing embryos attracted much research. Amino acid turnover were associated with blastocyst development and with clinical pregnancy (Houghton et al., 2002; Brison et al., 2004). But, despite number of metabolomics studies, any biological marker for successful of implantation has emerged. Moreover, a number studies were focused on the 20 amino acids that are constituents of proteins but the profile of non-protein amino acids were not investigated in human.
Study design, size, duration: We performed a retrospective study.
Participants/materials, setting, methods: We analyzed by *liquid chromatography-tandem mass spectrometry* (LC/MS/MS), the ratio consumption/production of a physiological mixture of 10 amino acids constituents of proteins and a non-protein amino acids, ornithine, by growing embryo in culture at days 2 and 3 following the in vitro fertilization. The validation step of the method was performed on 240 single embryo culture medium from 40 patients and a fast and reliable protocol of amino-acid analysis in the culture media was established.
Main results and the role of chance: (1) The analysis on day 2 of the amino acids profile was more discriminative than on day 3 of culture. (2) Amino acids constituents of proteins flux patterns could discriminate morphologically healthy embryos from morphologically unhealthy embryos but were not useful to predict the successful of implantation. But, we also observed amino acid flux patterns distinct between embryos with similar morphological appearance. (3) In a second time, we have analysis media samples from 20 transferred embryo (20 patients). All the embryos displaying an excess of ornithine production towards consumption (ratio >1,2) failed to implant subsequently and so, independently of their morphology in culture.
Limitations, reason for caution: At the time, these potential markers were identified based on metabolomics studies, which needed to be assessed prospectively on large samples.
Wider implications of the findings: Ornithine is a central part of the urea cycle, which allows for the disposal of excess nitrogen and so could be a marker of a poor embryo cell detoxification. Adequate cell detoxification may be of prime importance in the ability of embryo to survive. The non-invasive determination of ornithine in embryo culture may constitute an attractive marker of implantation failure, morphology independent and that due to its swiftness, could be easily used in ART laboratories.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – EA2608-USC INRA. University of Caen Basse-Normandie, FRANCE, Hospital, CHU Côte de Nacre, CAEN, France.
Trial registration number: No concerned.
Keywords: embryo, metabolome, amino Acid

P-235 Details to consider when setting-up dishes and heated stages in the IVF lab: how to minimize the impact of temperature fluctuations outside the incubator

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Study question: How can different set-up of dishes and working conditions affect the temperature in medium droplets when dishes are taken out of the incubator?
Summary answer: In this study we show how several factors related to the setup of culture dishes and the temperature set-point of heated stages directly affect heat transfer in culture medium droplets. All details must be well controlled to minimize the impact of temperature fluctuations outside of the incubator.
What is known already: It is widely known that microtubule spindle is a thermo sensible-structure. Changes in temperature may irreversibly affect spindle integrity, resulting in chromosomal abnormalities in oocytes and embryos. Although during embryo manipulation in routine IVF tasks temperature fluctuations are inevitable, the optimization of the right setup in culture dishes and heated stages may reduce this impact.
Study design, size, duration: Experiments were run at Embryotools between September and December 2014. Set-up was prepared according to the following variables: type of oil (light vs. heavy density), medium droplets distribution (peripheral vs. central), type of culture dish (35 mm, 60 mm, GPS) and temperature set-point (37ºC vs. 40ºC) of heated surfaces.
Participants/materials, setting, methods: Temperature set-point of a heated laminar hood was monitored with external probes (±0.07ºC iButtons, Thermodata). Dishes were taken out of the incubator and placed on the heated surface. A digital fine gauge thermocouple probe (±0.01ºC, TC, Okolab) was used to measure temperature inside culture medium droplets every 30 seconds.
Main results and the role of chance: Different set-up and conditions resulted in greatly varying temperature fluctuations. Whilst some combinations (e.g. central droplets covered with heavy oil in 60 mm or GPS dishes and 40ºC heated stage) succeeded in holding temperature above 36ºC for more than 13 min, others reached excessively warm (>38ºC) or cold (<35ºC) temperatures in few minutes. Overall, heavy oil resulted more competent to slowdown cooling than light oil. Moreover, peripheral droplet distribution proved to cause a faster temperature drop than central distribution. A 40ºC heated surface set-point extended temperature holding times, but resulted in over-heating of culture medium in dishes prepared with light oil. GPS dishes were the most stable on keeping temperature outside the incubator, regardless of the oil type used or temperature set-point of the heated stage.
Limitations, reason for caution: In this study observations are limited to few combinations of culture dishes setup and heated stages set-points. Further studies should be performed to assess temperature recovery when dishes are put back inside the incubator using the same variables, along with the impact of these settings on other parameters like pH.
Wider implications of the findings: This study confirms the impact of several settings (including: type of oil and plastic dish, medium droplets distribution and temperature set-point of heated stages), on temperature fluctuation of medium droplets when dishes are taken out of the incubator. Depending on the settings and disposables used, temperature may behave differently and irreversibly compromise embryo development and quality. Simple details controlled in the lab can minimize stress on embryos and gametes, avoiding unnecessary risks during embryo manipulation.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Embryotools, LifeGlobal.
Trial registration number: NA.
Keywords: culture dish, set-up, quality control, temperature

P-236 Time-lapse evaluation of pronuclear morphology in euploid and aneuplid embryos

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Study question: We hypothesize that during zygote development, morphological and morphokinetcs parameters could be informative of normal/abnormal chromosomal status. An observational study is currently ongoing in our fertility centre, aiming to evaluate embryos at the pronuclear (2PN) stage and to relate pronuclei (PN) appearance to the ploidy status of the embryos.
Summary answer: Preliminary results indicate that PN relative position evaluated at different time-points (T1,T2,T3) could help identify embryos with higher probability of being euploid; PN juxtapposition at T3 (before fading) is significantly more frequent in euploid then aneuploid embryos. A similar relation, although not signficant, is observed at T2 (14h post-ICSI).
What is known already: Morphokinetics studies have focused on the timing of embryo divisions/morulation/blastulation in relation to morphology, ploidy and clinical outcomes. Information lacks on zygote development. Several studies have evaluated PN at a single time point (17–18 h post-ICSI). However, modern time-lapse incubation allows PN recording from appearance to fading. Pronuclei position is distinguished in "distant" (PN not in contact), "near"

(in contact by one single point) or "juxtapposed" (strictly in contact with a plate of adherence between them).

Study design, size, duration: We randomly selected 18 couples (female age 38 ± 5.2 SD) who underwent ICSI and preimplantation genetic screening in May/2013–March/2014. Morphokinetics was retrospectively recorded: time of II-polar body (IIPB) extrusion, PN appearance/fading, PN position at three time-points: T1 = T0 + 6h, T2 = T0 + 12h, T3 = T0 + 18h (T0 being the time of IIPB extrusion, coinciding with end of meiosis).

Participants/materials, setting, methods: Ninety-three embryos were cultured in time-lapse incubators with sequential media. Blastocysts were biopsied from day-5 to day-7 and their ploidy status evaluated by array-CGH; 38/93(=41%) resulted euploid. Pronuclei relative position was categorized as "distant", "near" or "juxtapposed" (based on previous literature). Morphokinetic parameters were compared between aneuploid and euploid embryos.

Main results and the role of chance: No difference was found between aneuploid and euploid embryos in IIPB extrusion (3.6 ± 0.2 and 3.5 ± 0.2 SE), 2PN appearance (10.5 ± 0.3 and 10.6 ± 0.4), fading (24.0 ± 0.4 and 23.6 ± 0.5), duration of 1-cell stage (26.8 ± 0.5 and 26.5 ± 0.5). At T1 (n = 58 records) most embryos had "distant" (39.5% aneuploid, 35% euploid; NS) or "near" (39.5% aneuploid, 45% euploid; NS) PN; only few showed "juxtapposed" PN (21.1% aneuploid, 20.0% euploid; NS). At T2 (n = 37), 71% aneuploid and 89% euploid embryos (NS) had "jusxtapposed" PN; 3.6% aneuploid and 11.1% euploid embryos (NS) had "distant" PN; 25% aneuploid and none euploid embryos had "near" PN (NS). At T3 (n = 41) all euploid embryos (100%) had "juxtapposed" PN versus 66.7% of the aneuploid embryos (OR = 0.14, 95%CI, p = 0.05); 10% aneuploid and non-euploid embryos had "near" PN (NS).

Limitations, reason for caution: Our study is still ongoing and the sample size needs to be enlarged. The time-lapse system does not allow to manipulate the embryos and to rotate them in case the pronuclei appear overlapping. In this case the evaluation of the PN position cannot be accurate and the record is discarded.

Wider implications of the findings: Besides PN relative position other parameters are important for PN evaluation such as nucleolar precursor bodies (NPB) number and position within the PN (data not shown, still in progress). We believe that a more extensive evaluation of zygote and PN appearance and development will help, together with morphokinetics data on cleavage embryos and blastocysts, identifing embryos with higher probability of being aneuploid/euploid. The importance of this evaluation method relies in its non-invasiveness.

Study funding/competing interest(s): Funding by hospital/clinic(s) – No conflicts of interests have to be declared. No external funding was obtained for the present study.

Trial registration number: NA.

Keywords: time-lapse, pronuclei, zygote

P-237 The occurrence of smooth endoplasmic reticulum clusters in metaphase II oocytes and the outcome of ICSI and IVF cycles

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Study question: Is there a difference in fertilization rate (FR), ongoing pregnancy rate (OPR) and life birth rate between ICSI and IVF cycles with and without smooth endoplasmic reticulum clusters (SERC+/SERC-)?

Summary answer: The FR of SERC+ IVF cycles is decreased compared to the FR of SERC+ ICSI cycles and unaffected cycles, which seems partially due to an increased incidence of total fertilization failure (TFF). The OPR and life birth rate of affected ICSI and IVF cycles are not different from SERC- cycles.

What is known already: Due to several alarming reports on increased risks for abnormal neonatal outcome in ICSI cycles containing oocytes with the dysmorphism SERC, it was recommended in 2011 to discard SERC+ oocytes. Recently, authors showed that healthy babies are born from both SERC- and SERC+ oocytes in SERC+ cycles. This dysmorphism occurs in metaphase II oocytes and disappears in case of fertilization. Therefore, embryos from SERC+ oocytes cannot be recognized in IVF cycles and may be transferred.

Study design, size, duration: Observational study between October 2012 and November 2014 of 56 SERC+ ICSI and IVF cycles, containing a total number of 701 oocytes of which 139 are SERC+. TFF, FR, OPR and life birth rate per embryo transfer in SERC+ and SERC- cycles are analyzed and compared.

Participants/materials, setting, methods: ICSI, IVF and mixed cycles from 51 infertile couples treated in the Leiden University Medical Center with at least one oocyte containing SERC observed either after denudation in ICSI cycles or in unfertilized oocytes after PN score in IVF cycles.

Main results and the role of chance: In SERC+ IVF-cycles, the frequency of SERC+ oocytes is lower than in SERC+ ICSI cycles (16,9% vs. 22,8%), which may be explained by the time of observation and disappearance of SERC in fertilized oocytes. Although the FR of SERC- oocytes is slightly higher than that of SERC+ oocytes, the overall FR of affected ICSI cycles is not significantly different from normal ICSI cycles. However, in SERC + IVF cycles the FR is significantly reduced (37,1% vs. 61%). This may be explained by the high incidence of TFF in these cycles (31,8% vs. 5,4%). All three pregnancies from SERC+ oocytes in ICSI cycles resulted in miscarriages. The OPR and life birth rate of both ICSI and IVF SERC+ cycles are not significantly different from SERC- cycles.

Limitations, reason for caution: The frequency of SERC+ cycles may be an underestimation, particularly in IVF cycles, because SERC may be overseen in oocytes during assessment of fertilization. Relatively few embryos from affected oocytes have been selected for transfer, which may explain the lack of life births from SERC+ oocytes in our dataset.

Wider implications of the findings: Our study confirms that life births can occur from SERC+ ICSI and IVF cycles. In case of TFF in a SERC+ IVF cycle, it may be useful to proceed with an ICSI cycle, even if SERC+ oocytes are observed, to increase the FR. Future studies are needed to evaluate whether the high TFF rate in SERC+ IVF cycles is related to the occurrence of SERC.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Leiden University Medical Center.

Trial registration number: NA.

Keywords: smooth endoplasmic reticulum clusters, oocyte dysmorphism, ongoing pregnancy rate, total fertilization failure

P-238 Adding morphokinetics to morphology, is there an added benefit in live births?

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Study question: If embryos are morphologically top quality (TQE), does applying further morphokinetic criteria result in higher birth rates?

Summary answer: TQEs that fulfilled the morphokinetic criteria had a significantly higher live birth rate than TQEs that did not pass the criteria.

What is known already: It is known that morphokinetic information from time-lapse (TL) incubation of embryos can be used to distinguish between the implantation potential of embryos, which has traditionally been done by standard morphological assessment under the microscope. However, there are few studies examining the possibility of added benefit from TL if an embryo is TQE.

Study design, size, duration: This was a retrospective, cohort study of 211 single embryo transfers of TQE on day 2 performed at the clinic between June 2012 until December 2014. Only embryos that had been cultured in the TL EmbryoScope™ incubator from fertilization check until embryo transfer were included in the study.

Participants/materials, setting, methods: 211 embryos that had four even blastomeres, <20% fragmentation and no multinucleation at 44 h, making them TQE were included in this study. 126 passed the additional morphokinetic criteria of no nucleation error at any given time, no cleavage anomalies, and matching the in-house timeline, while 85 embryos did not.

Main results and the role of chance: Of the 211 embryos transferred in the study, 63 resulted in a live birth (29.9%). Of the 126 embryos that fulfilled the additional morphokinetic criteria, 48 resulted in a live birth (38.1%), which was a significantly higher live birth rate compared with the 15 out of 85 embryos (17.6%) that did not pass the additional criteria (p = 0.001). This equates to a relative risk of live birth increase of 116% when applying information obtained from TL in addition to the standard assessment of TQE.

Limitations, reason for caution: Embryos were transferred in a clinical setting, so the distribution sub-characteristics of TQEs in the study may not reflect the overall distributions. This is particularly true for TQEs with observed

nucleation error or cleavage anomaly as such embryos were not preferentially transferred.
Wider implications of the findings: To the best of our knowledge, this is the first study to show that applying morphokinetic criteria in addition to traditional morphological grading results in improved birth rates. It also suggests that using a morphokinetic timeline created specifically for the clinic in question used in combination with morphokinetic deselection criteria further improves rates compared to using just deselection criteria.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Klinikk Hausken.
Trial registration number: NA.
Keywords: time-lapse, top quality embryo, selection, morphokinetics, live birth

P-239 Morphokinetic characteristics of embryos time-lapse-imaged up to the 8-cell stage after polar body diagnosis are not affected by ploidy

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Study question: Can morphokinetic parameters be used to distinguish between euploid and aneuploid embryos that were time-lapse-imaged up to the 8-cell stage after polar body diagnosis (PBD)?
Summary answer: Morphokinetic parameters analyzed in this study cannot be used to distinguish between euploid and aneuploid embryos up to the 8-cell stage.
What is known already: It was previously shown that after 8-cell the time from insemination to the initiation of compaction (tSC), start of blastulation (tSB) and full blastocyst (tB) differ significantly among euploid and aneuploid embryos (eE and aE). Aneuploid embryos showed a delayed tSC, tSB and tB. However, until the 8-cell stage no morphokinetic differences were identified. (Campbell et al., RBM Online, 2013, 26, 477–485).
Study design, size, duration: This retrospective study analyzed 139 embryos (n = 38 cycles, n = 30 patients) undergoing ICSI and PBD in our clinic between 09/2013 and 12/2014). All women had a normal karyogram except for one (mosaic 47XXX/46XX). ICSI was performed on n = 263 oocytes with n = 176 being fertilized and n = 139 included for analysis.
Participants/materials, setting, methods: PBD was performed using FISH against chromosomes 13,16,18,21,22 (n = 57 ooyctes) or 13,16,18,21,22,X (n = 82 oocytes). Time-lapse imaging was performed up to day 3. Data in hours are presented as median (quartiles 25 and 75) and were analyzed by pairwise exclusion using Shapiro-Wilks- and Mann-Whitney-Test (SPSS Version 22). Significance level is $p = 0.05$.
Main results and the role of chance: To identify morphokinetic differences among eE and aE we compared several parameters. Both pronuclei had faded at tPNf(eE) = 24.65 (23.19–27.00) and tPNf(aE) = 24.83 (22.55–28.58) ($p = 0.58$). The time from insemination to completion of division to 2–8 cells (tn) did not differ between euploid and aneuploid embryos. When comparing developmental periods neither the duration of cell cycles (CC) 2 ($p = 0.56$) or 3 ($p = 0.81$), nor the relative proportion of CC2 in relation to the combined durations of CC2 and CC3 differed between euploid and aneuploid embryos ($p = 0.42$). The synchrony of the n^{th} cell cycle (sn) was s2(eE) = 0.67 (0.29–1.08) and s2(aE) = 0.50 (0.17–1.37) ($p = 0.68$), s3(eE) = 4.83 (2.16–12.57) and s3(aE) = 3.83 (2.33–9.10) ($p = 0.89$).
Limitations, reason for caution: Ploidy was assessed by PBD through FISH on just a limited number of chromosomes. In addition, sample size is small.
Wider implications of the findings: Our results are in agreement with previous findings by Campbell et al. (2013) and Kramer et al. (2014) and implicate that morphokinetic parameters up to the 8-cell stage cannot be used to identify aneuploid embryos. Therefore testing for ploidy should be performed by other means, i.e. FISH, ArrayCGH or NGS.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – This study was performed at the Heidelberg University Hospital (Germany) and received no funding. The authors declare no competing interests.
Trial registration number: A trial registration number was not required due to the retrospective study design.
Keywords: embryology, PGS, time-lapse, morphokinetics

P-240 Development of new procedure to reduce the amount of recipient cytoplasm (mitochondria) at the M-II karyoplast transfer for the treatment of mitochondrial diseases

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Study question: Is it possible to decrease the amount of transferred cytoplasma to almost zero to avoid the inheritance of mutated mitochondrial DNA (MT-DNA)?
Summary answer: This newly developed procedure has the potential to become crucial in the treatment of cytoplasmic transfer for mitochondrial diseases.
What is known already: The Mitochondrial diseases caused by mitochondrial DNA mutation are inherited through the maternal cytoplasm. The radical treatment for this is the exchange of patient's cytoplasm for a healthy one (from a donor), even a small amount of transferred patient's cytoplasma may possibly cause mitochondrial disease.
Study design, size, duration: We performed a retrospective study to investigate by how much we could decrease the amount of transferred recipient MT-DNA in 25 donor oocytes. This study was conducted between December 2010 and January 2014.
Participants/materials, setting, methods: The first karyoplast was aspirated using a φ10–12 mm glass pipette. Then the first karyoplast was aspirated into a φ5–6 mm pipette and the M-II chromosome was isolated from the remaining cytoplasm by shaking the pipette. By repeating this procedure mostly M-II chromosome could be isolated (second karyoplast).
Main results and the role of chance: 1. The membrane of the second karyoplast was easily broken and this M-II chromosome was injected directly into the enucleated donor oocyte using a Piezo manipulator (PRIME TECH LTD.: PMM-4G). After 2 h incubation in the medium ICSI was performed. 2. MT-DNA in the first karyoplast and second karyoplast were 7.05 ± 2.31 and 0.76 ± 0.85 in comparison with the set point of 100 in the whole M-II cytoplasm measured quantitative by real time PCR. (211/50). 3. The percentage of successful first and second karyoplast was 100% (25/25), 92.0% (23/25). 4. Fertilization, cleavage and blastocyst rates after ICSI were 65.2% (15/23). 73.3% (11/15) and 27.3% (3/11) respectively.
Limitations, reason for caution: The final amount of cytoplasm in the second karyoplast could not be reduced to zero. It might produce mitochondrial disease through bottle neck phenomenon though this might hardly occur.
Wider implications of the findings: The amount of recipient transferred MT-DNA was reported as 0.5 ~ 0.6% measured by amplification refractory mutation system-quantitative polymerase chain reaction, then it could not be detected at all in a year. It is not clear whether recipient MT-DNA was absorbed or discharged. There is a possibility that the MT-DNA might be detected using more accurate devices. There is clear evidence that the percentage of transferred recipient MT-DNA decreased to almost zero in a year.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Saint Mother Obstetrics and Gynecology Clinic and Institute for ART.
Trial registration number: NA.
Keywords: mitochondrial disease, mitochondrial DNA, heteroplasmy, karyoplast transfer

P-241 Invention of a novel micromanipulator for ICSI, Venus (Vortex-like-movement-Evoked Nicking Upon Stick-site), which enables surer puncture of oocyte membrane with less stress

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Study question: We invented a novel micromanipulator for smoother and less-stressful puncture of oocyte, Venus (Vortex-like-movement-Evoked Nicking Upon Stick-site, PAT.P), which rotates an injection pipette while sticking like a gimlet. We prospectively examined whether Venus improves intracytoplasmic sperm injection (ICSI) outcomes.

Summary answer: Venus provides smoother and surer puncture of oocyte membrane by gimlet-like movement of the injection pipette, consequently improving embryonic growth by reduction of oocyte stress.
What is known already: Non-stress and sure puncture of oocyte membrane is essential for ICSI to achieve successful pregnancies. Conventional procedures of simple stick, Piezo, and aspiration, however, often encounter penetration difficulty, impact-damage, and injection problem due to a stuck pipette, respectively. For surer puncture of oocyte with less stress, therefore, we have invented a novel micromanipulator, Venus, which enables an injection pipette to rotate while sticking like a gimlet.
Study design, size, duration: Prospective randomized studies: a pilot study compares two ICSI ways using Venus (SV and DV, as described below) versus a conventional way (C) and a confirmation study (midway analysis at present) compares SV versus C. Both studies include 51 and 48 oocytes in 3 and 6 ICSI cases, respectively.
Participants/materials, setting, methods: Injection pipette was stuck without penetration, making a dead-end tunnel, and then penetrated through the side-wall of tunnel using Venus (Side-wall Venus, SV) or not (conventional way, C). Alternatively, pipette was penetrated by single stick using Venus (Direct Venus, DV). Stick numbers, survival rate and embryo growth were compared.
Main results and the role of chance: Pilot study: The numbers of sticks until penetration were 3.4 ± 0.5 with C (n = 18), and significantly decreased to 2.5 ± 0.3 with SV ($p < 0.05$, n = 18) and further to 1.4 ± 0.3 with DV ($p < 0.001$, n = 17).With C, SV and DV, oocyte survival rate were 100, 94 and 47% ($p < 0.01$) and development rates to superior blastocysts from surviving oocytes (%SB) were 11, 20 and 0%, respectively. Midway evaluation: With C and SV (n = 24 and 24), oocyte survival were 100 and 100%, and %SB were 24 and 48% (p = 0.1). Superior blastocysts developed significantly more frequently with SV than C (Odds ratio: 4.4, 95% C.I.: 1.01–19.2, logistic). In additional 21 patients with SV alone, 33% resulted in pregnancies.
Limitations, reason for caution: A prospective confirmation study requires 150 oocytes in each group of SV and C to obtain definite conclusions by power analysis. Present pilot and midway analyses showed important beneficial effects of Venus on ICSI outcomes but definite conclusions await further accumulation of data.
Wider implications of the findings: Present study showed beneficial effects of Venus on oocytes derived from 39 years old or younger women. Because weaker oocytes are more vulnerable to ICSI stress, Venus may contribute to aged women and poor responders much more by reduction of puncture stress.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Women's Clinic Jinno.
Trial registration number: UMIN000014600.
Keywords: intracytoplasmic sperm injection, micromanipulator, oocyte membrane, penetration of membrane, embryonic development

P-242 Prediction of embryonic development and clinical outcome following ICSI according to the stages of nuclear maturation division

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Study question: Is it possible to predict embryonic development and clinical outcome according to the stages of nuclear maturation division?
Summary answer: Identification of chromosomal stages has a high potential to predict the embryonic development and clinical outcome by adjusting the preincubation time. Longer pre-incubation (5–6 h) might be beneficial for prometaphase II oocytes to advance to Metaphase II oocytes. Irregularly arranged chromosomes might be a sign of nuclear degradation.
What is known already: Pre-ovulatory oocytes collected after controlled ovarian stimulation are considered mature with the extrusion of the first polar body at ICSI. However, embryonic development varies following ICSI for morphologically mature oocytes.
Study design, size, duration: We performed this retrospective study to investigate the relationship between the clinical outcome and various nucleus stages of M-II oocytes in 53 patients under 38 years of age on 211 cycle
Participants/materials, setting, methods: M-II chromosomes identified using an inverted microscope equipped with Normarski optics. M-II oocytes were divided into four groups, Group A: oocytes that had the M-II chromosomes arranged in two lines, Group B: oocytes at prometaphase II not yet arranged in two lines and Group C: oocytes with irregularly arranged chromosomes.
Main results and the role of chance: 1. The 211 freshly ovulated M-II oocytes were examined. The proportion of grouped oocytes according to the stages of M-II chromosomes were 78.1% (165/211) in group A, 7.1% (15/211) in group B and 14.7% (31/211) in group C. 2. The fertilization, cleavage, blastocyst stage rates and pregnancy, miscarriage and birth rates in four groups are listed in table.

Table 1: Clinical outcome following ICSI according to the stages of nuclear maturation division.

	Fertilization rates (%)	Cleavage rates (%)	Blastocyst rates (%)	Pregnancy rates (%)	Miscarriage rates (%)
A	79.4(131/165)	80.2(105/131)	58.0(76/131)	44.7(34/76)	11.8(4/34)
B	73.3(11/15)	63.6(7/11)	36.3(4/11)	25.0(1/4)	100(1/1)
C	54.8(17/31)	70.5(12/17)	17.6(3/17)	0(0/3)	0

Limitations, reason for caution: Normal embryonic development needs maturation of both of nucleus and cytoplasm. This study investigated whether it is possible to predict embryonic development from the point of view of nuclear condition. However, when maturity of nucleus and cytoplasm do not match this prediction method is not applicable.
Wider implications of the findings: It is very important to collect information to improve the embryonic development just after the oocyte collection.
It is beneficial for embryonic development if oocyte maturity can be adjusted to optimal condition by observing the nuclear maturation division.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Saint Mother Obstetrics and Gynecology Clinic and Institute for ART.
Trial registration number: NA.
Keywords: ICSI, M-II oocyte, nuclear maturation

P-243 Aggregates of smooth endoplasmic reticulum (SERa) in human oocytes: impact on in vitro fertilization (IVF)

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Study question: To determine if patients undergoing IVF and whose oocytes present SERa, a particular morphological feature, have a higher change of negative outcome compared to patients without SERa oocytes.
Summary answer: The present study indicates that, when SERa+ oocytes are excluded from an ICSI programme, women with SERa oocytes (cases) have an higher chance of negative outcome compared to controls. The negative outcome is defined as the absence of suitable oocytes or viable embryos or during the IVF cycle
What is known already: Two reports in literature have associated the use of SERa oocytes IVF to malformation or genetic abnormalities in the newborns. In 2011, based on these data, the Alpha Scientists and ESHRE issued guidelines that, as a precaution, advised against the use of those gametes in IVF. Our unit does not use SERa oocytes and aimed to gather more information on the impact of this limitation on IVF outcome.
Study design, size, duration: A retrospective cross-sectional case-control study. Our hypothesis was that a negative outcome occurs in 30% of cases and 15% of controls. 120 patients is the minimum sample needed, as revealed by a power study (a = 0,05; b = 0,2). The study period ranged from July 2012 till December 2013.
Participants/materials, setting, methods: Cases were IVF patients showing ≥1 SERa+ oocytes at the time of ICSI. Controls were subsequent patients showing no SERa+ oocytes and matched for age, clinical indication to IVF and body mass index (BMI). The ICSI cycle was performed in a standard way, excluding SERa oocytes from insemination.
Main results and the role of chance: The percentage of women experiencing a negative result in their IVF cycle was significantly higher in cases (18%) compared to controls (8%) (p = 0.02) with an Odds Ratio = 2.6 [95%CI = 1.2–5.7]. The Odds Ratio adjusted for age, BMI, basal FSH, duration of infertility, number of retrieved oocytes and used oocytes was 2.6 [95%CI = 1.1–6.5]. Factors that mostly influenced this result were the number of suitable oocytes and basal FSH. A survival function (Kaplan-Meyer method) from pick-up to embryo transfer highlighted that the only significant difference between cases

and controls was the percentage of suitable oocytes: 61% vs. 73% in cases and in controls, respectively ($p = 0.001$). Clinical pregnancy and delivery rates were not statistically different between the two groups (34% vs. 37% and 25% vs. 30%, respectively)

Limitations, reason for caution: The present study has been designed to elucidate an embryological outcome so we cannot draw conclusions about chances of pregnancy or delivery.

Wider implications of the findings: Our results cannot be compared with others in the literature because, differently from other papers, we did not use SERa + oocytes in IVF. Although we have no information regarding the fate of inseminated SERa + oocytes, we can give important data for prognosis of patients showing this particular morphological feature.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Fondazione IRCCS Ca' Granda.

Trial registration number: NA.

Keywords: SER aggregates, smooth endoplasmic reticulum, oocyte, oocyte morphology, ICSI

P-244 Predicted blastocyst development based on the cellular features of day 2 embryos by time-lapse observations

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Study question: Is it possible to reliably predict blastocyst development on day 2 after fertilization in order to reduce the occurrence of monozygotic twinning and to reduce the duration of *in-vitro* embryo culture?

Summary answer: It is possible to predict the likelihood of blastocyst development based on the following criteria. The size of cellular fragmentation is less than 26 mm in diameter, 2PN zygotes undergo normal twofold cell division, cell vacuoles are not larger than 10mm in diameter and reverse cleavage is not observed.

What is known already: It has been reported that blastocyst culture causes a higher rate of monozygotic twinning and a delay in embryo development compared to *in-vivo* embryo growth. The presence of cellular vacuoles (larger than 10 mm) and reverse cleavage are known to diminish blastocyst development.

Study design, size, duration: A retrospective study of 351 2PN zygotes, that were observed by a time-lapse system until day 5 of development conducted at the Hanabusa Women's Clinic from January 2014 to December 2014.

Participants/materials, setting, methods: The size of fragmentation and the presence of vacuoles at the time of the first cleavage were recorded. The rates of blastocyst development and high-grade blastocyst formation (>3AA) were analysed in embryos, with or without normal twofold cell division, and the detection of vacuoles and reverse cleavage.

Main results and the role of chance: The cutoff size of fragments that distinguished normal from abnormal cleaved cells was defined as <26 mm. Based on this criterion, the rate of blastocyst formation from 2PN zygotes with normal twofold cell division without large vacuoles was 97.2% (104/107) and was significantly higher than those with reversed cleavage (50.8% (33/65), $p < 0.001$), erratic cell division into 3–4 cells (56.7% (56/99), $p < 0.001$), or 5 and more cells (13.5%(10/74), $p < 0.001$). The rate of high-grade blastocyst formation (>3AA) from 2PN zygotes with normal twofold cell division without large vacuoles was 42.1% (45/107) and was also significantly higher than those with reversed cleavage (4.6% (3/65), $p < 0.001$), erratic cell division into 3–4 cells (6.1% (6/99), $p < 0.001$), or 5 and more cells (0% (0/74), $p < 0.001$).

Limitations, reason for caution: It is not known whether chromosomes are present in the larger fragments and abnormally cleaved blastomeres. Further studies are required to determine pregnancy rates and take-home baby rates based on the criteria used in the present work.

Wider implications of the findings: As this method can predict blastocyst development with high accuracy from day 2 embryos, blastocyst culture may no longer be necessary for choosing viable embryos. This new method simplifies the procedure for selecting the best embryos, without labor-intensive protocols, such as measuring the time of cleavage and the duration of each cell stage.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hanabusa Women's Clinic.

Trial registration number: NA.

Keywords: blastocyst development, time-lapse observation, fragmentation, twofold cell division

P-245 Should we worry about the clock? Relationship between time for ICSI and reproductive outcomes in cycles with fresh and vitrified oocytes

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Study question: What are the best time interval to perform ICSI with respect to the times of ovum pick-up (OPU), cumulus cells removal (denudation), and vitrification/warming of oocytes in order to maximize fertilization rate, embryo quality, and biochemical, clinical, and ongoing pregnancy rate (PR) in fresh and vitrified cycles?

Summary answer: There are no significant differences in fertilization rate (FR), embryo quality (EQ), and pregnancy rate (PR) of ICSI cycles within a wide range of times employed between OPU and ICSI, including partial time analysis for denudation and recovery from vitrification/warming.

What is known already: During IVF, the oocyte can be subjected to denudation, vitrification/warming, and ICSI. Insufficient interaction with cumulus cells, oocyte ageing outside of the follicle, and improper recovery after warming can all impact the resulting embryo developmental competence; therefore, strictly controlled times between procedures are often implemented. However, most protocols are never tested against reproductive results, and little information is available on the ideal times to be followed to achieve the highest PR.

Study design, size, duration: Data from 3,986 ICSI cycles performed at a fertility center between December 2012 and May 2014 were included (3,178 with fresh and 808 with vitrified/warmed oocytes). Exact times between OPU, denudation, vitrification, warming, and ICSI were recorded automatically by a radiofrequency based system. OPU was performed strictly 36h after trigger.

Participants/materials, setting, methods: ICSI was performed using donor oocytes and either patient's or donor sperm. Univariate differences between manipulation times and PR were tested by Student's t-test; adjusted effect of time was analyzed by logistic regression; linear trend was tested by linear-by-linear test. Effect of time on FR/EQ was modeled by probit/ordinal regression.

Main results and the role of chance: Total OPU-ICSI times ranged from 1 h 25 m to 17 h 13 m (average 4 h 58 m ± 1 h fresh; 9 h 18 m ± 2 h vitrified oocytes). We found no effect of total OPU-ICSI time on FR ($p_{fresh} = 0.39$; $p_{vitrified} = 0.86$;) or EQ ($p_{fresh} = 0.08$; $p_{vitrified} = 0.22$). There was no difference in average total OPU-ICSI times between positive and negative pregnancies (biochemical, clinical, and ongoing) in either vitrified ($p = 0.52$, $p = 0.12$, $p = 0.12$) or fresh ($p = 0.74$, $p = 0.81$, $p = 0.99$) cycles. No effect of adjusted total OPU-ICSI time on PR for either vitrified ($p = 0.59$, $p = 0.20$, $p = 0.13$) or fresh ($p = 0.79$, $p = 0.73$, $p = 0.99$) oocytes was found. Further analysis for linear trend using total OPU-ICSI time categorized in deciles showed that PR do not increase or decrease across deciles. No effect on PR was found for denudation to vitrification, warming to ICSI, and denudation to ICSI times.

Limitations, reason for caution: This is a study with automatically collected times from a high number of ICSI cases; however, its retrospective nature cannot exclude the influence of unaccounted for variables on the results. All women included in the study were ≤35 years old, so findings should not be automatically extended to older women.

Wider implications of the findings: Our results indicate that the effective window of time for insemination by ICSI is wider than previously thought, as total time from OPU to ICSI can be as long as 17 h without developmental competence loss in the resulting embryos. Within appropriate times, the management of cycles in embryology laboratories could be adjusted to accommodate caseloads and workflow without loss of oocyte viability or cycle efficiency.

Study funding/competing interest(s): Funding by hospital/clinic(s).

Trial registration number: NA.

Keywords: ICSI, oocyte, vitrification, timing

P-246 Promoting of early development and implantation competence by secretory leukocyte protease inhibitor in mouse embryos

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Study question: The expression of *Slpi* and its role in preimplantation embryos.
Summary answer: *Slpi* expresses in preimplantation stage embryos and important in implantation competence
What is known already: SLPI is known a uterine implantation factor and expressed in reproductive tracts
Study design, size, duration: 2-cell stage mouse embryo in vitro culture for 72 h (more than 80 embryos per group), recombinant SLPI, ODN, embryo transfer
Participants/materials, setting, methods: CD1 female mice were superovulated with gonadotropins and collected the embryos after hCG 48 h. 2-cell embryos were cultured with or without recombinant SLPI and the expression of Slpi were analyzed with qRT-PCR. The embryos were transferred to the pseudopregnant female. Slpi mRNA specific ODNs were used to knockdown.
Main results and the role of chance: Slpi mRNA was detected in all cleavage stages except morula stage. However, SLPI was localized in all examined stage at the cytoplasm or membrane in a stage-dependent manners. Treatment of SLPI protein speeded up cleavage without the changes of total cell numbers or inner cell numbers of blastocyst. Silencing of *Slpi* expression delayed the embryo development after 8-cell stage without changes in the total cell number or inner cell numbers of blastocyst. The decreased developmental speed and implantation rate by *Slpi* silencing were recovered by the treatment of SLPI protein. The implantation rates were increased significantly by SLPI-treatment in both in vitro developed and *Slpi* silenced embryos.
Limitations, reason for caution: No
Wider implications of the findings: SLPI may regulate the cleavage speed and implantation competence of early-stage embryos.
Study funding/competing interest(s): Funding by University(ies) – Sungshin Women's University.
Trial registration number: NA.
Keywords: secretory leukocyte protease inhibitor, cleavage, implantation competence

P-247 Ongoing pregnancy in vitrified blastocyst transfer is not influenced by the extent of the zona pellucida dissection after thawing, but mainly by the blastocyst re-expansion

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Study question: Has the extent of laser-dissection of the zona pellucida (ZP) after thawing (from <25% to 25–50%) an influence on ongoing pregnancy rates in frozen-thawed single blastocyst transfer cycles?
Summary answer: The dimension of the ZP laser-dissection after thawing (from <25% to 25–50%) has no influence on ongoing pregnancy rates in frozen-thawed blastocyst transfer cycles.

Pre-freeze blastocoele expansion and inner cell mass (ICM) grade and post-thaw blastocoele re-expansion to the original degree are the most significant predictors.
What is known already: The freeze–thaw process might exacerbate the hardening of the ZP, which could impair successful embryonic hatching and implantation. Although still debated, some randomized studies have shown that laser-assisted hatching (AH) may improve the implantation rates in these cases. Historically, concerns have been raised on the length of the AH, because a small sized AH may trap the blastocyst in a typical figure-eight shape. Wider dissection of the zona might overcome this phenomenon.
Study design, size, duration: This was a retrospective study of $n = 258$ patients (258 blastocysts) who underwent a frozen-thawed single blastocyst transfer at our Center between January 2013 and September 2014.
Participants/materials, setting, methods: Blastocoele expansion, ICM and trophectoderm (TE) grade both pre-freezing and post-thawing were recorded. All blastocysts were artificial laser-collapsed before vitrification. After thawing and before the embryo-transfer, the zona was dissected from <25% to 25–50%, depending on the space between the TE and the ZP.
Main results and the role of chance: Multivariate logistic regression revealed no difference in ongoing pregnancy rate between the two groups characterized by different laser-dissection extent (< 25% and 25–50%) after correction for female age, Istanbul Consensus blastocyst morphological parameters and time from thawing (adjusted OR = 1.74, 95% CI: 0.76–4.00, $p = 0.19$). Hatching of blastocyst did not result to be more frequent after a wider dissection (adjusted OR = 0.98, 95% CI: 0.51–1.89, $p = 0.96$). Best pre-freeze predicting factors for ongoing pregnancy were blastocoele expansion (grade 2 vs. 3 adjusted OR = 0.37, 95% CI: 0.14–0.99, $p = 0.047$) and ICM grade (grade C vs. A adjusted OR = 0.27, 95% CI: 0.08–0.88, $p = 0.031$). Among post-thawing morphological parameters, only re-expansion could significantly predict ongoing pregnancy (grade 2 vs. 3 adjusted OR = 0.29, 95% CI: 0.09–0.91, $p = 0.034$).
Limitations, reason for caution: This was a retrospective study; a prospective randomized study that analyzes the influence of the extent of laser-dissection after blastocyst thawing on the ongoing pregnancy rate would be more reliable.
Wider implications of the findings: Blastocysts with higher pre-freeze grades of blastocoele expansion and ICM should be given priority when thawing. Blastocyst re-expansion after thawing should be the most important parameter to be taken into consideration to predict a pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ospedale san Raffaele.
Trial registration number: NA.
Keywords: assisted hatching, vitrification, blastocyst

P-248 The impact of treatment and patient-related factors on timing of embryo development in a cohort of good prognosis patients

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Study question: How much do confounding factors contribute to the variation observed for the morphokinetic parameters proposed as viability markers?
Summary answer: The treatment and patient-related factors explain only a small part of the variation observed for morphokinetic parameters, in particular at the cleavage stage. Variation in timing of embryo development is likely to be related genuine biological differences between embryos.
What is known already: Time-lapse monitoring has been shown to improve clinical outcome. At present, data do not allow for distinguishing between improved culture conditions and selection using time-lapse parameters. Furthermore, several studies report that culture conditions, patients and treatment influence timing of the morphokinetic parameters, which have promoted the perception that each clinic must develop individual models. Whether this is necessary will, however, depend on the individual contribution of the proposed factors, which remains to be investigated.
Study design, size, duration: Infertile patients were prospectively recruited at the Fertility Clinic, Aarhus University Hospital from February 2011 to May 2013. Patients aged <38 years without endometriosis were eligible if ≥8 oocytes were retrieved. Patients were included once. All embryos were monitored for 6 days in a time-lapse incubator (EmbryoScope).
Participants/materials, setting, methods: 1931 embryos (2 PN) from 239 patients were included. The influence of fertilisation method, BMI, maternal age, FSH dose, cause of infertility and number of oocytes retrieved on timing of t2–t5, development of a blastocoel (tEB), and full blastocoel (tFB) was tested in linear regression models for clustered data.
Main results and the role of chance: BMI and number of oocytes retrieved and cause for infertility showed no correlation to timing of t2, t3, t4, t5, tEB, and tFB. Fertilization method affected only t2 significantly. Age and FSH dose significantly affected tEB, and tFB in an unadjusted model, where as only FSH dose showed a significant correlation in an adjusted model. In contrast to the other parameters evaluated, total FHS dose showed a significant correlation to whether t5 and duration of the 2-cell stage (cc2) were in-or outside the time-intervals proposed as optimal in a published hierarchical model (t5 45–55; cc2 9–12 h). The analysis was largely unchanged whether all embryos or only good quality blastocysts were included.
Limitations, reason for caution: Culture media and oxygen concentration were constant during the study period, and the possible effect of these was therefore not analysed.

Wider implications of the findings: Although we can not exclude that is relevant to adjust proposed model to different clinical settings with regard to potential confounders such as fertilisation method, BMI, maternal age, FSH dose, cause of infertility and number of oocytes retrieved, our analysis suggest that the contribution of these external factors to variation in timing of embryo development is less than genuine biological differences between embryos. The impact of FSH dose might reflect the importance of ovarian reserve/quality.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Research at the Fertility Clinic was funded by an unrestricted grant from Ferring and MSD. The authors declare no competing interest.

Trial registration number: NCT01953146 and NCT01139268.

Keywords: time-lapse, embryo selection, culture conditions

P-249 Predictive value of soluble human leukocytic antigen-G levels in embryo culture medium for embryo selection in patients undergoing intracytoplasmic sperm injection

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Study question: Is the measurement of soluble human leukocytic antigen-G (sHLA-G) in the spent culture medium a good predictor of the clinical pregnancy rate (CPR) in infertile patients treated with intracytoplasmic sperm injection (ICSI)?

Summary answer: The measurement of sHLA-G in the culture medium (CM) at 72 h post-insemination is a fair predictor of the CPR in women undergoing ICSI. Its predictive value is lower than the embryo score (ES). However, the combination of both predictors gives a higher predictive value compared to each predictor alone.

What is known already: Researchers have suggested that there is an association between implantation potential and embryonic secretion of sHLA-G. Studies have shown that pregnancy rates were higher when sHLA-G was detected in the spent culture media of day 3 embryos. However, the results were not definitive as pregnancies were also established from the sHLA-G negative embryos. More research is needed in this area to clarify the role of sHLA-G in embryo selection.

Study design, size, duration: A retrospective study of the sHLA-G concentration in the spent CM and the morphology score of embryos (ES) of ICSI-treated women between 1st January and 31st March 2014. Assuming an a value of 0.25 and a 70% probability of detecting a true difference, the minimum sample size was 22.

Participants/materials, setting, methods: The CM of 80 separately cultured embryos from 25 patients undergoing ICSI in our Assisted Conception Center were analyzed for their concentration of sHLA-G at 72 h post insemination using a double monoclonal sandwich enzyme immunoassay assay. The results were compared to the morphological evaluation of the embryos (ES).

Main results and the role of chance: The CPR was 28% (7/25). The mean (±SD) concentration of sHLA-G in the CM of pregnant women was 4.77 (±2.34) U/ml compared to 3.50 (±1.99) U/ml in non-pregnant women ($P = 0.057$). The mean ES in pregnant women was 9.18 (±1.07) compared to 6.24 (±1.41) in non-pregnant women ($P < 0.001$). Receiver operating characteristic (ROC) curves showed that the ES had a higher predictive value for clinical pregnancy (AUC = 0.925; $P = 0.001$) compared to the sHLA-G concentration (AUC = 0.794; $P = 0.025$). The cut-off values were of 5.21 and 3.35 U/ml for both predictors, respectively. The combination of both predictors gave a higher predictive value (AUC = 0.968; $P < 0.001$) compared to each predictor alone.

Limitations, reason for caution: In order to evaluate the clinical usefulness of these findings, a prospective randomized trial (RCT) is needed, where only embryos with a sHLA-G concentration >3.35 U/ml in the culture medium at 72 h post-insemination and an ES >5.21 will be transferred and compared to the classical morphological evaluation method.

Wider implications of the findings: It is concluded that the measurement of sHLA-G in the embryo CM is a fair predictor of clinical pregnancy in women undergoing ICSI. Its combination with embryo scoring gives a higher predictive value compared to each predictor alone. If these results are confirmed by a prospective RCT, this combined method can be used for the selection of embryos to maximize the chances of clinical pregnancy and diminish the incidence of multiple pregnancies.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Alexandria Fertility Center.

Trial registration number: NA.

Keywords: sHLA-G, IVF, ICSI, embryo selection, embryo score

P-250 Is a morphokinetic based selection model for fresh blastocysts transferrable to vitrified blastocysts?

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Study question: Is a novel morphokinetic blastocyst selection model, which ranks fresh embryos according to implantation potential, also valid for vitrified blastocysts?

Summary answer: The selection model – developed and validated in-house to rank embryos according to implantation potential - was effective when applied to vitrified/ warmed blastocysts.

What is known already: Selection models based on the comparison of morphokinetic variables have been used to develop clinical evidence-based embryo selection algorithms. To date these have been used for selection of blastocysts for transfer in fresh cycles only.

Study design, size, duration: An in-house morphokinetic ranking was retrospectively applied to 181 blastocysts warmed and transferred between August 2011 and November 2014 with known implantation data (KID±). KID + rates (KID + /KID + plus KID – ×100%) were compared in relation to clinical pregnancy and live birth.

Participants/materials, setting, methods: Morphokinetic variables were recorded using EmbryoScope™ (Vitrolife, Sweden) in a private IVF setting. A novel morphokinetic embryo selection model was developed in house and tested retrospectively on blastocysts cultured between June 2011 and March 2014. The KID ratio of fresh blastocysts transferred and frozen blastocysts subsequently transferred were analysed.

Main results and the role of chance: For high, medium and low implantation potential warmed blastocysts, defined by the in-house model, KID + rate was 43.3%, 34.2% and 8.3% ($n = 90, 79, 12$). The live birth KID for high, medium and low implantation potential warmed blastocysts, was 33.3%, 27.9% and 0% ($n = 63, 61, 10$). There were no live births from the low potential warmed blastocysts transferred. Although no statistical significance between these KID ratios was seen, they relate well to the KID + rate of previously presented fresh blastocyst transfers in which high, medium and low implantation potential KID + rate was 57.1%, 34.6% and 0% ($n = 273, 153, 8$)

Limitations, reason for caution: The sample size of this preliminary study is small and more data will be included in the study, including more live birth data as it is available. This model may not be transferrable between different settings and confounders of morphokinetic models may exist.

Wider implications of the findings: Validation of morphokinetic models prior to use and consideration of potential confounding factors, such as effect of endometrial preparation and luteal support during frozen embryo replacement cycles. This work supports the use of a morphokinetic blastocyst implantation ranking, assigned at time of vitrification, used prospectively to select blastocysts for warming and transfer.

Study funding/competing interest(s): Funding by hospital/clinic(s) – CARE Fertility.

Trial registration number: NA.

Keywords: time lapse, morphokinetic, vitrified, blastocyst

P-251 An increased trend in pregnancy rate following day 5 transfer of single embryos selected using Eeva Test compared with embryos selected using morphology alone

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Study question: This study aimed to investigate the clinical outcomes of day 5 single embryo transfer (SET) patients whose embryos were selected using the Eeva Test, compared to control day 5 SET patients whose embryos were selected using morphology alone.
Summary answer: Day 5 SET pregnancy rates may be improved when the embryos were selected using the Eeva Test combined with morphology, compared to Day 5 SET patients where the embryo selection was using morphology alone.
What is known already: The Eeva Test combines time-lapse monitoring with automated cell-tracking software providing quantitative information regarding embryo developmental potential. Although the Eeva Test has proven to be a useful aid to embryo selection, most studies to date have evaluated broad patient populations that include day-3 and day-5 transfers. Here, we assess to what extent the Eeva Test impacts embryo selection and clinical outcomes for a population of good prognostic patients who qualify for day 5 SET.
Study design, size, duration: Retrospective, observational cohort study. A total of 501 day 5 patients who underwent elective SET at first treatment cycle were included in this study, with 114 patients using the Eeva Test (Eeva Test group) and morphology for embryo selection and 387 patients using morphology alone (Control group).
Participants/materials, setting, methods: Day 5 SET patients were identified using standard criteria. Eeva Test was an elective option offered to aid embryo selection. All day 5 SET patients during the study period were included. Implantation and clinical pregnancy was defined by presence of a fetal heartbeat.
Main results and the role of chance: There were 114 Day 5 SET patients in the Eeva Test group and 387 patients in the Control group. Whilst there was no significant difference between the number of eggs collected or fertilized for Eeva vs. Control group, patients in the Eeva Test group were significantly older (33.2 ± 3.8 vs. 32.3 ± 4.2, $p = 0.03$). The total number of eggs collected and fertilized for the Eeva test group were 12.4 ± 5.3 and 7.7 ± 4.0 respectively, whereas the control group was 13.4 ± 5.3 and 7.8 ± 5.3. The clinical pregnancy rate indicated a trend towards being increased in those patients whose embryos had been selected through the Eeva Test and morphology against those which had used morphology alone (49% vs. 39%, $p = 0.06$).
Limitations, reason for caution: As this was not a randomized controlled trial, the study groups did not have equivalent patient characteristics. However, control patients were better prognosis than Eeva Test patients due to reduced age, suggesting that patient characteristics did not account for the trend toward improved outcomes in the Eeva Test group.
Wider implications of the findings: Patients whose embryos were selected for SET using the Eeva Test and morphology showed an increased trend in clinical pregnancy compared with control SET patients. Although definitive studies such as randomized controlled trials are necessary, our data indicate that incorporating early cell division timing parameters (Eeva Test) for day 5 blastocyst selection may improve the success of day 5 SET, thus clinics can apply day 5 SET more broadly and further reduce multiple pregnancies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Liverpool Women's Hospital, Liverpool, UK.
Trial registration number: NA.
Keywords: embryo selection, embryo transfer, extended culture, EEVA, time-lapse

P-252 Comparative analysis of twin blastocysts derived from human embryo splitting at cleavage stage

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Study question: Embryo splitting has a potential to reduce number of the embryos needed for research. Our aim was to investigate embryo splitting technique and comparing, at a morphokinetic and molecular level monozygotic twin blastocysts derived from split embryos with normal non-manipulated blastocysts.
Summary answer: The blastocysts derived from split embryos are in general smaller and often have poorly developed or indistinguishable inner cell mass (ICM), containing cells with a dual expression of trophectoderm (TE) and ICM markers.
What is known already: Very limited number of studies investigated splitting of human embryos. Single blastomeres from four cell-stage embryos could develop into blastocysts. The more advanced cleavage stage at which the embryos were split, a higher number of viable twin embryos were able to develop into the blastocyst.
Study design, size, duration: Cleavage stage embryos, donated for research and undergoing splitting, were grouped into two groups based on number of blastomeres that survived thawing. Group 1 consists of embryo with 2–5 ($n = 30$) and the Group 2 of embryos with 6–10 blastomeres ($n = 30$). Non-manipulated matching cleavage stage embryos were used as a control.
Participants/materials, setting, methods: Research embryos were biopsied and half of the blastomeres were transferred into an empty recipient zona pellucida. Resulting twin embryos were cultured in EmbryoScope® for morphokinetic studies. Further, we investigated the expression of early lineage-specific gene markers in the blastocysts derived from split embryos using immunostaining.
Main results and the role of chance: Blastocysts developed from split embryos were smaller than the control non-manipulated blastocysts. In majority of split embryos, ICM was poorly developed or indistinguishable, containing cells with a dual expression of TE and ICM markers. Regardless, splitting human pre-implantation embryos from the Group 2 (6–10 cells) resulted, in general, in more viable and better quality of blastocysts compared to embryos from the Group 1 (2–5 cells). Furthermore, the average time of cell division differs between these two groups. Detailed morphokinetics and immunostaining data will be presented.
Limitations, reason for caution: Original cleavage stage embryos have been donated by different IVF clinics across the UK and their culture condition may differ. After splitting, all twin embryos were cultured only under one condition, which may or may not be different from original.
Wider implications of the findings: The data suggest that although blastocysts can be derived from split embryos, they are inferior in comparison with normal non-manipulated blastocysts and their suitability as a research tool may be questionable.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by national/international organization(s) – The work has been funded by Studentship from Saudi Arabia government and by the incentive funds from the Assisted Conception Unit at Guy's Hospital.
Trial registration number: The work has been done under Human Fertilisation and Embryology Authority research licence number R0075.
Keywords: IVF, blastocysts, monozygotic twin, embryo splitting

P-253 Cytoskeletal analysis by confocal laser scanning microscopy, ultrastructure assessment by transmission electron microscopy and sister chromatid exchange rate in vitrified and fresh human embryos

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Study question: Are there any differences in the incidence of spindle abnormalities, sister chromatid exchange (SCE) rate and mitochondrial and other organelles' structure, number and function in vitrified and fresh embryos?
Summary answer: Differences are observed in the levels of normal vs abnormal spindle chromosome configurations, the chromatid exchange rate and the ultrastructure between fresh and vitrified embryos.

What is known already: Most studies in human vitrified embryos concentrate on assessing the success of the procedure on survival rates and clinical outcomes following transfer. Indeed several clinical trials have confirmed promising results. Limited studies have however examined the effects of vitrification on spindle structure and chromosome alignment, while no studies have looked into the sister chromatid exchange rate (SCE) or investigated in detail the ultrastructure of the cellular organelles in vitrified and fresh human embryos.
Study design, size, duration: This is an ongoing study, initiated in March 2013. Until today, 140 blastocysts, rejected for transfer following day 3 biopsy and PGD, were treated either fresh or following day 5 vitrification with DMSO/EG for cytoskeletal analysis (n = 25 fresh/n = 25 vitrified), Transmission Electron Microscopy analysis (TEM) (n = 25 fresh/n = 25 vitrified) and chromatid exchanges (SCE) (n = 20 fresh/n = 20 vitrified).
Participants/materials, setting, methods: Cytoskeletal analysis by immunostaining with α, γ, acetylated tubulin and DAPI was conducted in an academic hospital with IVF/PGD laboratory. TEM analysis was conducted in a Histology/Embryology Laboratory following fixation in glutaraldehyde osmium, uranyl and immersion in EPON. SCE assessment was performed following treatment with BrdU in a Biology Laboratory.
Main results and the role of chance: Cytoskeletal analysis by confocal laser scanning microscopy revealed that the majority of spindles examined in both groups were normal but the incidence of spindle abnormalities, was significantly higher in the vitrified blastocysts compared to fresh blastocysts ($P < 0.05$). In particular, in the vitrified vs the fresh group, 60.4% vs 73.2% of spindles were normal, 35.8% vs 22.4% were abnormally shaped occasionally with chromosome lagging, bridging or congression failure, while 3.8% vs 4.4 had abnormalities only in the number of poles. The sister chromatid exchange rate was also higher in the vitrified group compared to the fresh group. TEM analysis of vitrified blastocysts (24 h post-warming) showed an increased incidence of lipofuscin droplets (representative of apoptosis), higher number of vacuoles and distension of mitochondria compared to fresh blastocysts.
Limitations, reason for caution: The blastocysts used in this study were all diagnosed with either chromosomal abnormalities or single gene defects following PGD/S. The majority of the cells at the blastocyst stage are in interphase and therefore spindle chromosome configuration analysis and the SCE rate are inevitably based on a small number of mitoses.
Wider implications of the findings: This is the first study to compare the ultrastructure and SCE rate in biopsied fresh and vitrified human embryos. Mechanical stress sustained after exposure to cryoprotectants lead to the increased spindle/chromosome abnormalities observed in the vitrified group. Differences in mitochondrial number/morphology reflect alterations in embryo metabolism, but the increase in lipofuscins may indicate a 'rescue' process for the embryo, which in its attempt to fully recover and continue normal development following vitrification, eliminates damaged/abnormal cells.
Study funding/competing interest(s): Funding by University(ies). Funding by national/international organization(s) – Aristotle University Medical School. EU Social Fund (ESF) and Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Program: Thales.
Trial registration number: A9850.
Keywords: blastocyst vitrification, transmission electron microscopy, mitochondria, cytoskeletal analysis, sister chtomatid exchange rate

P-254 Predicting live birth relies on different kinetic parameters depending on embryo transfer day

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Study question: Is it possible to use the same ranges of morphokinetic variables for the prediction of live birth in patients transferred on different days?
Summary answer: Embryos transferred on day 3 and 4 and blastocysts transferred on day 5 behave differently on kinetic basis when early cleavage parameters are evaluated. Therefore, different models should be used to predict the live birth potential of embryos transferred on different days.
What is known already: Embryo implantation of embryos transferred on day 3 was predicted using the time of division to 5 cells, the time between division from 3 to 4 cells and the time between division from 2 to 3 cells (Meseguer et al., 2011). An aneuploidy risk classification based on the start of blastulation and the formation of a full blastocyst proved beneficial in correlation with live birth when applied to non-biopsied embryos transferred on day 5 (Campbell et al, 2013).
Study design, size, duration: This retrospective cohort study was conducted from October 2011 to December 2013. It included 149 infertile patients and 218 embryos achieving live birth (LB) and incubated in a time-lapse incubator (EmbryoScope™). A total of 66LB embryos transferred on day 3 and 4 and 152LB blastocysts transferred on day 5 were compared.
Participants/materials, setting, methods: The mean age was 33.04 ± 3.98 and 30.75 ± 3.99 for the first and second group, respectively. In the same order, the total number of retrieved oocytes was 8.44 ± 5.09 and 13.03 ± 6.36. Differences in early cleavage kinetics (before t8) were analyzed using a Mann Whitney test. Statistical significance was determined as $p < 0.05$.
Main results and the role of chance: As our policy, embryos were cultured to the blastocyst stage whenever possible (two good quality embryos on day 3 and on day 4). Patients transferred on day 3 and 4 have therefore different clinical characteristics than those transferred on day 5. When analyzing only embryos achieving LB, cleavage timings up to t8 were significantly different for those transferred on day 3 and 4 when compared to those transferred on day 5. Embryos developing into blastocysts are faster and the delay of LB embryos transferred on day 3 and 4 is increasing from t2 to t8, reaching a maximum of 4 h for t7. All p values were found to be below 0.05. The overall live birth rate was calculated as 19.50% for the cohort studied.
Limitations, reason for caution: Blastocyst transfers are obviously done for younger females with a good ovarian reserve. However, this does not change the fact that the embryos achieving the blastocyst stage have significantly different kinetic values beginning from the time to achieve at 2 to t8.
Wider implications of the findings: Separate time ranges should be used when working with absolute time points for cleavage and morulae transfers and blastocyst transfers. Female age and ovarian reserve are also two crucial parameters to take into consideration when building morphokinetic models.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital.
Trial registration number: Approved by the ethical committee of Memorial Sisli Hospital Istanbul Turkey.
Keywords: time lapse, transfer day, live birth

P-255 Using time-lapse to study diploidization of human parthenogenetic haploid embryos

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Study question: Do human parthenogenetic haploid embryos (hPHEs) undergo diploidization through specific cleavage behaviors?
Summary answer: hPHEs can actively undergo cleavage tendency (CT) or blastomere fusion (BF) in the first three divisions to realize self-diploidization.
What is known already: In animal models, partly parthenogenetic haploid embryos were induced to become diploid during preimplantation development, but the underline mechanism is unclear.
Study design, size, duration: A experimental study from August 2012 to May 2014 was conducted. A total of 96 *in-vitro* matured metaphase (MII) oocytes were collected and parthenogenetic activated. Then cleavage behaviors were analyzed by time-lapse. Further, we used Fluroescence in situ hybridization (FISH) to detect chromosome ploidy.
Participants/materials, setting, methods: Immature oocytes from patients receiving ICSI were collected and matured *in vitro* culture. Further parthenogenetic haploid oocytes obtained by calcium ionophore A23187 with puromycin were used for time-lapse observation. Meanwhile, 328 IVF/ICSI embryos were analyzed as the control. 20 hHPEs, occurring CT/BF or not, were tested by FISH in Day 3.
Main results and the role of chance: Compared to IVF/ICSI embryos, hPHEs had higher incidence in CT (18.8% vs. 1.2%, $P < 0.001$) and BF (7.3% vs. 0.9%, $P = 0.001$). Among hPHEs, 63.0% CT/BF behaviors occurred at the first division and 55.6% CT/BF embryos could cleavage normally in the next cell cycle. In addition, the compaction rate of D5 CT/BF embryos was 45.5% while

non-CT/BF embryos was 11.8%. FISH results showed that the hPHEs appeared CT/BF were diploid signals (100%, 9/9), while the non-CT/BF embryos all showed haploid signals (100%, 11/11), besides, when CT/BF occurred in the first division, almost all the blastomeres turned out to be diploid signals. Finally, for artificial diploidization in hPHEs at 2-cell stage, the blastocyst formation rate of electrofusion group was significantly higher than that of non-electrofusion group (39.3% vs 8.7%, $P < 0.01$).

Limitations, reason for caution: In our study, the chromosome ploidy of hPHEs tested by FISH using three-colour fluorescent DNA probes specific for three different chromosomes (16, 18, X) can not represent the whole chromosome ploidy exactly.

Wider implications of the findings: This study gives a better insight into the mechanisms involved in self-diploidization. hPHEs may possess the ability to correct aberrant chromosomal ploidy.

Study funding/competing interest(s): Funding by national/international organization(s) – National Science Foundation of China (No. 81222007).

Trial registration number: NA.

Keywords: parthenogenetic haploid embryos, diploidization, time-lapse, cleavage tendency, blastomere fusion

P-256 Cleavage synchronicity predicts increasingly live birth as women grow older

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Study question: Is the cleavage synchronicity behaves the same regarding female age when predicting live birth?

Summary answer: Cleavage synchronicity from two to eight cells (CS2-8) predicts live birth in retrospective cohort of 537 patients ($p < 0.0001$). When women were categorized in three different age groups (<30; 30–35; 35–40) the live birth ratio increased by 1.86 fold in the young group and 16.2 fold in the oldest group.

What is known already: The relative timing CS2-8 = ((t3-t2) + (t5-t4))/(t8-t2)) has been found to be the best predictor available on day 3 for blastocyst formation and quality when compared to absolute time-points (AUC: 0.786; sensitivity: 83.43; specificity: 62.46) (Cetinkaya et al., 2014). However, the proposed expression need to be further tested in regard to implantation potential and live birth prediction in order to obtain specific models for patients with different clinical characteristics.

Study design, size, duration: This retrospective cohort study was conducted from October 2011 to December 2013. It included 904 embryos having a (t8) and with known live birth data (KLB) from 537 infertile patients. Patients were divided into three categories according to age (Group A: <30; Group B: ≥30 and <35; Group C: ≥35 and <40).

Participants/materials, setting, methods: Incubation was performed in time-lapse incubators (EmbryoScope™). Group A, B and C included 207, 401 and 296 KLB embryos, respectively. Each group was further investigated as having asynchronous (CS2-8 ≤ 0.6) or synchronous (CS2-8 >0.6) cleavages. Differences were then analyzed using a chi-square test. Statistical significance was determined as $p < 0.05$.

Main results and the role of chance: Cleavage synchronicity from 2 to 8 cells ((t3-t2) + (t5-t4))/(t8-t2) reflects the ratio of time the embryo spends from 2 to 4 cells over the time from 2 to 8 cells. Although each blastomere basically behaves independently during mitotic cell divisions, an embryonic synchronicity exists, such that uneven cell stages represent very short time frames during the 5 days of preimplantation embryo development.

The overall live birth rate (LBR) was calculated as 24.11 for the cohort studied. When analyzing by age categories, the LBR increased from 20.9% to 39.0% for group A when asynchronous and synchronous embryos were compared ($p = 0.0422$). Similarly for group B the LBR increased from 11% to 31% ($p = 0.0004$). The trend further increased for group C with a LBR of 1.1% for asynchronous embryos and a LBR of 17.9% for synchronous embryos ($p = 0.0001$).

Limitations, reason for caution: Embryos having a (t8) were exclusively included in the additive model, leaving out evaluation of day 2 embryo transfers. The cohort studied involved only infertile patients (female, male or combined) and is in this respect a heterogeneous population.

Wider implications of the findings: The synchronicity of mitotic divisions is a strong predictor of live birth in this retrospective cohort study. As a conclusion, the effect of synchronous cleavages is more prominent as women grow older, therefore CS2-8 can easily be implemented in the clinical practice when choosing embryos for transfer from time-lapse incubators to obtain high LBR.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital/Turkey.

Trial registration number: Approved by Ethical Committee of Memorial Sisli Hospital, Istanbul.

Keywords: time-lapse, embryo selection, ART, live birth, cleavage synchronicity

P-257 Successful application of a single warming protocol for embryos cryopreserved by either slow freezing or vitrification techniques: can one method fit for all?

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Study question: This study asks whether sucrose gradient-based tri-step warming protocol can be efficiently adapted in a clinical setting as a universal warming protocol for previously cryopreserved and stored embryos by using both slow freezing and vitrification methods.

Summary answer: Our study shows that, irrespective of the methods of cryopreservation as well as freezing date, a sucrose-based common warming protocol can be effectively used and create acceptable cryosurvival as well as pregnancy rates in clinical IVF settings.

What is known already: Recent developments and improvements in cryopreservation technologies created a rapid shift from slow freezing to vitrification protocols in clinical IVF programmes worldwide. For this reason, many clinics have abandoned slow freezing protocols and adopted vitrification as the main technique in their cryopreservation facilities. However, depending on the duration of cryostorage, samples that had been previously cryopreserved by slow freezing method in the past can create problems as well as uncertainties upon warming in embryo viability due to the limited experience and the lack of warming materials/kits for this specific procedures when needed.

Study design, size, duration: This prospective cohort study had been performed between January 2013 and November 2014 in Bahceci Cyprus Assisted Reproductive Technology Center. A total of 497 human embryos from 153 patients that had previously been cryopreserved by slow freezing or vitrification protocols were included in the study.

Participants/materials, setting, methods: In non-donor cycles, a total of 254 embryos in 72 cycles that had previously been cryopreserved by slow freezing (before 2011) and 294 embryos in 81 cycles that had been vitrified (after 2011) were warmed by a single, sucrose gradient-based tri-step warming protocol. Upon warming, embryo and cellular viability was assessed and suitable embryos were cultured/used for embryo transfer. Where available, embryos that were cultured for day 5/6 embryo transfer were also scored for blastocyst development rate.

Main results and the role of chance: No significant differences ($p > 0.05$) were observed in patient characteristics such as female age at the time of cryostorage, BMI, sperm parameters etc. In all procedures, embryos were cultured in conventional human embryo culture media at least 2 h before embryo transfer. Data regarding the embryo survival, blastocyst development as well as pregnancy rates (PR) is shown in table. Although higher blastulation and pregnancy rates are observed in slow freezing/fast warming group, these differences were not statistically significant ($p > 0.05$).

			Survival after warming (%)					
	Cases (*n*)	Embryos (*n*)	100%	≥50%	<50%	0%	Blastulation rate (%)	PR (%)
Slow freezing	72	254	79.9	8.7	9.1	2.4	43.0	68.1
Vitrification	81	294	88.4	4.1	4.8	2.7	35.8	53.1

Limitations, reason for caution: The number of embryos and cycles analysed are the main limitations of our study. Further research and larger sample sizes with the current parameters and technical setting are required to confirm our findings. Slight variations in slow freezing procedures can also be addressed in a large study.

Wider implications of the findings: Current data on using a universal warming protocol for oocytes/embryos in different cryopreservation cycles are scarce. Our data, although underpowered, show important and acceptable embryo development and pregnancy results that can be created by a single, universal warming protocol that is easy and adaptable in any clinical setting.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study received no funding and no conflicts of interests to be declared.
Trial registration number: This study was not an RCT and therefore there is no registration number.
Keywords: embryo cryopreservation, slow freezing, vitrification/warming, clinical outcome

P-258 Similar implantation and pregnancy rates using a single medium versus sequential media for embryo culture: a randomized controlled trial

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Study question: Are implantation rates different after embryo culture in a single medium compared to sequential media?
Summary answer: Implantation rates are similar after embryo culture in a single medium compared to sequential media.
What is known already: Sequential media and single media represent two fundamentally different approaches for embryo culture, in principle and formulation. Several studies randomizing sibling oocytes showed that single media are associated with higher blastocyst formation rates compared to sequential media. However, despite the remarkable interest in the use of single media, reliable data on implantation and pregnancy rates are limited as available studies with patient randomization are currently scarce.
Study design, size, duration: RCT performed at Eugonia Assisted Reproduction Unit in Athens, between January 2014 and June 2014. A total of 100 patients were randomly allocated to embryo culture in ether a single medium (Global; $n = 50$ patients) or sequential media (ISM1/BlastAssist, Origio; $n = 50$ patients). Primary outcome was implantation rate.
Participants/materials, setting, methods: Patients ≤40 years, with ≤2 previous attempts were treated with GnRH antagonist protocol. Embryos were group-cultured in the assigned medium (Global or ISM1/BlastAssist) and transfers were performed on Days 2, 3 or 5. Media were changed on Day 3. Doctors and patients were blinded to the type of medium used.
Main results and the role of chance: Baseline patient characteristics were similar in the two groups. Implantation rates [35.6 ± 38.5% versus 35.8 ± 39.2%], positive hCG rates [72.7% (32/44) versus 75% (36/48)], clinical pregnancy rates [61.4% (27/44) versus 58.3% (28/48)], and ongoing pregnancy rates [50% (22/44) versus 50% (24/48)] per embryo transfer did not differ statistically in single and sequential media, respectively. No significant differences were observed in blastocyst formation rates per 2PN [55.9% (123/220) versus 54.3% (133/245)], embryo utilization rates per 2PN [63.7% (216/339) versus 58.9% (218/370)], number of 2PN [7.2 ± 4.9 versus 7.4 ± 4.8], number of good quality embryos [2.8 ± 1.8 versus 3.0 ± 2.6], and number of embryos transferred per patient [2.5 ± 0.7 versus 2.5 ± 0.7] in single and sequential media, respectively. All outcomes were similar when evaluated for Day-2, Day-3 and Day-5 transfers separately.
Limitations, reason for caution: The present study was powered to compare implantation rates as a marker of embryo viability. Powered RCTs reporting on live birth rates in single versus sequential media are necessary to verify the present findings.
Wider implications of the findings: Implantation and pregnancy rates, as well as blastocyst formation and embryo utilization rates were similar in single (Global) and sequential (ISM1/BlastAssist) embryo culture media. Both types of media appear to adequately support *in vitro* preimplantation embryo development, yielding embryos of similar viability and developmental competence, and similar reproductive outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by commercial/corporate company(ies) – Eugonia Assisted Reproduction Unit, LifeGlobal.
Trial registration number: NCT02048527.
Keywords: single media, sequential media, implantation rates, pregnancy rates, embryo culture

P-259 Exploring mathematical vectors to predict embryo implantation: a model using the start of blastulation and the time to reach the expanded blastocyst stage

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Study question: Can mathematical vectors be a useful tool when modeling and predicting embryo implantation from tEB (time from insemination to expanded blastocyst) versus tSB (time from insemination to start of blastulation)?
Summary answer: A geometric approach was used to calculate the distance and angle of coordinates derived from tEB versus tSB in 599 KID (Known Implantation Data) blastocysts belonging to 385 patients. An increase of 2.29 fold was calculated when the lowest category was compared with the highest implantation group (24.16% vs. 55.41%, respectively).
What is known already: Since time-lapse has entered the IVF laboratory many models using kinetic expressions, e.g., cleavage timings, time intervals and time ratios have been proposed and have tried with variable reproducibility to predict blastocyst formation, implantation and live birth. However, predicting blastocyst implantation is a difficult objective to achieve since if embryos are able to develop into blastocysts of fair quality then the implantation potential is obviously driven by extrinsic factors like, among others, endometrial receptivity.
Study design, size, duration: This retrospective cohort study was conducted from October 2011 to November 2014, including 599KID blastocysts. For each embryo, a vector was drawn from the origin to the tEB vs. tSB intersection point and the length of the vector was determined. Also, the angle of this vector to the tEB axis was evaluated.
Participants/materials, setting, methods: Incubation was performed in time-lapse incubators (EmbryoScope™). Cut-offs were calculated by an area under curve (AUC) analysis (145.35 for the length of the vector and 0.72 for the angle of the vector to the x axis). Differences were then analyzed using a chi-square test. Statistical significance was determined as $p < 0.05$.
Main results and the role of chance: Time points that define precise embryo cleavage events may not be generalized to infertile patients with different etiologies, and may depend on the conditions applied in IVF units. Therefore, exploring unorthodox ways to approach embryologic developmental timings may give the flexibility required for each individual embryo's evaluation in relation to its implantation potential, by allowing an associative analysis of many time-points with each other instead of strict cut-off values resulting in grouping dissimilar embryos. In the study conducted, the overall KID rate was calculated as 37.73. When dividing the data into four quartiles according to the cut-off values obtained, the KID rate increased from 24.16% to 55.41%, with the two intermediate groups having a KID rate of 37.01% and 40.96% ($p < 0.0001$).
Limitations, reason for caution: Embryos cultured to the blastocyst stage were exclusively included in the additive model, leaving out evaluation of day 3 embryo transfers.
Wider implications of the findings: The use of mathematical vectors, although unconventional, is an approach that deserve to be explored as an option that may efficiently predict the implantation potential of blastocysts when integrated to time-lapse incubation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This retrospective study was funded by ART and Reproductive Genetics Center, Memorial Sisli Hospital, Istanbul, Turkey.
Trial registration number: This retrospective study was approved by the Ethics Committee of the Memorial Sisli Hospital, Istanbul, Turkey.
Keywords: ART, implantation, morphokinetics

P-260 Is everything about the morula? The time to achieve a morula and the period at which the embryo stays a morula predict implantation

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Study question: Are the time to achieve a morula and the period at which the embryo stays a morula important predictors of implantation?
Summary answer: Five classes of implantation have been established based on time from insemination to a full morula (tM) and time period between a full

blastocoele where the blastocyst has not yet started expansion (tB) and tM. The KID rate (Known Implantation Data) was increased by nearly two-fold between the first and the fifth group.

What is known already: The aneuploidy status of embryos was related to the start of blastulation and the formation of a full blastocyst. The aneuploidy risk classification built proved beneficial in a correlation with live birth when applied to non-biopsied embryos (Campbell et al., 2013). Relative timings were found to be better indicators of blastocyst formation and quality when compared to absolute time-points (Cetinkaya et al., 2014). Therefore, time intervals and relative ratios may allow high predictivity of implantation.

Study design, size, duration: This retrospective cohort study was conducted from October 2011 to June 2014. It included 681 KID embryos having achieved the blastocyst stage and belonging to 427 infertile patients transferred on day 5. KID blastocyst were divided into five categories according to four parallels to the trendline of KID = 1 embryos.

Participants/materials, setting, methods: Incubation was performed in time-lapse incubators (EmbryoScope™). Group I, II, III, IV and V representing each 20% of the whole cohort included 103, 108, 113, 107 and 108 KID embryos, respectively. Differences were analyzed using a chi-square test. Statistical significance was determined as $p < 0.05$.

Main results and the role of chance: Time points that define precise embryo cleavage events may not be generalized to infertile patients with different etiologies, and may depend on the conditions applied in ART units. However, using relative equations such as time intervals generating a spectrum-like distribution may give the flexibility required for each individual embryo's evaluation, by avoiding impractical hierarchical classifications based on strict cut-off values that result in only a few subgroups in which more than one embryo can be located. When plotting tM against (tB-tM), five KID categories were defined. The KID rate was calculated as 46.21%, 42.96%, 34.07%, 31.79%, 23.48% for group I, II, III, IV and V, respectively ($p = 0.0010$). The relative increase between groups I–III and I–V was of 35% and 96%, respectively.

Limitations, reason for caution: The cohort studied involved only infertile patients (female, male or combined) and is in this respect a heterogeneous population.

Wider implications of the findings: The relation between the time to achieve a morula and the time the embryo stays as a morula is a predictor of implantation in this retrospective cohort study. Therefore, using time intervals in conjunction with selected developmental stages may allow an individualized ranking of embryos in relation to implantation for each patient.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital.

Trial registration number: NA.

Keywords: ART, morphokinetics, morula, implantation

P-261 A prognostic model with two selected morphokinetic parameters accurately predicts the implantation potential of human embryos cultured in EmbryoScopeTM

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Study question: Is it possible to predict the implantation potential of human embryos using morphokinetic parameters?

Summary answer: The logistic prediction model composed of synchrony of the second cell cycle (s2) and the period of time between the beginning of blastocyst formation and full blastocyst stage (Ssbb) has a powerful diagnostic capability for prediction of embryo implantation.

What is known already: Many candidate time-lapse markers have been studied regarding the selection of good quality embryos and improvement of pregnancy rates. The results of these studies highlight an adequate reproducibility about the successful implantation for only a few specific parameters, such as eight cells time point (t8), blastocyst formation time (tB), morula formation time (tM) and synchrony of the second cell cycle (s2). However, new sets of target parameters and better predictive models need to be developed.

Study design, size, duration: This retrospective study was conducted from January 2012 to November 2014. A total of 352 embryos from 223 patients were studied. 36 morphokinetic variables were analyzed.

Participants/materials, setting, methods: Embryos were cultured and analyzed in EmbryoScope™. The selection of variables was done by two-tailed *t*-test and backward stepwise regression. A logistic regression model was build and the discrimination and calibration was done by ROC analysis and Hosmer-Lemeshow test, respectively. All analyses were performed in SPSS19 and SigmaStat3.5 statistical software.

Main results and the role of chance: After two-step selection of all morphokinetic variables s2 and Ssbb were found to be the most significant parameters to be included in the model (Likelihood Ratio Test Statistic: 52,597 ($P = <0,001$)). The recall of the obtained model was 82.75%, while the fall-out was 82.85%.

Limitations, reason for caution: The developed prognostic model is not appropriate for embryos cultured until second or third day after ICSI procedure, because one of the selected parameters (Ssbb) is connected with later stages of embryonic development.

Wider implications of the findings: The use of morphokinetic parameters (synchrony of the second cell cycle and the time between the beginning of blastocyst formation and full blastocyst stage) would improve the selection of embryo with better implantation potential. This process will optimize the selection of embryos for single embryotransfer and could lead to reduction of negative effects observed in multiple pregnancies.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Nadezhda Fertility Clinic.

Trial registration number: NA.

Keywords: prognostic model, EmbryoScope, implantation rate, time-lapse markers

P-262 Monopronucleated ICSI zygotes: *in vitro* development and morphokinetic evaluation

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Study question: What is the development capability of embryos derived from monopronucleated zygotes? Are kinetic parameters of ICSI monopronucleated (1PN) zygotes similar to those of normal zygotes (2PN)?

Summary answer: 1PN zygotes that reach blastocyst stage have similar kinetic behaviour to 2PN zygotes that give rise to pregnancy but different from those 1PN that arrest their development in earlier stages.

What is known already: Embryos from 1PN zygotes are not usually considered for embryo transfer due to possible anomalies during the fertilization process and a high incidence of aneuploidy.

The incorporation of time-lapse methodologies in ART laboratories provides constant information throughout embryo *in vitro* culture. The frequency of image capture and the number of focal planes are selected according to particular interest. The results obtained allow the identification of anomalies occurring during pronuclear formation, embryo cleavage and blastocyst formation.

Study design, size, duration: A prospective observational cross-sectional study performed between September 2012 and June 2014 with a total of 117 monopronucleated ICSI zygotes in the study group and 124 normal fertilized ICSI zygotes that resulted in ongoing pregnancies in the control group.

Participants/materials, setting, methods: Embryos were cultured in a tri-gas EmbryoScope™. Images were acquired at five focal planes every 15 min. Times of pronuclei appearance/disappearance, cleavage and cell cycles (Day 0 to Day 3) were analysed comparing both groups. Blastocyst formation was assessed in the study group. Statistical analysis was by Mann-Whitney/Chi-square test.

Main results and the role of chance: 21 monopronucleated ICSI zygotes (17.9%) developed to blastocyst stage and 96 (82.1%) arrested in early stages. Differences (median; $p < 0.05$) were observed between these two subgroups in time of PN disappearance (22 vs 24.4 h) and cleavage time to 2, 4, 6 and 7 cells (26.2 vs 29.6 h; 40.3 vs 47.2 h; 52.7 vs 57.7 h; 55.7 vs 62.4 h).

Cleavage times of monopronucleated ICSI zygotes that reached blastocyst stage were similar to those of the control group. Differences were only observed in time of PN disappearance (22 vs 24.2 h) and in the duration of the third cell cycle (11.4 vs 13.3 h).

Kinetics of 1PN ICSI zygotes that arrested their development were slower than those of both 1PN zygotes that reached blastocyst stage and 2PN zygotes that produced ongoing pregnancies.

Limitations, reason for caution: Despite the large number of monopronucleated zygotes included in the study, the subgroup of zygotes that reached blastocyst stage was limited. The control group was not followed up to the blastocyst stage and no comparison of kinetic parameters after Day 3 was done.
Wider implications of the findings: Not all 1PN zygotes have the same kinetic behaviour and developmental capability. Monopronucleated zygotes that achieve the blastocyst stage have similar kinetics to normal fertilized 2PN zygotes. In view of the results of this study, a new strategy could be considered rather than simply discarding such embryos. PGS of 1PN blastocysts would make it possible to determine their chromosomal constitution and thus their suitability for transfer or cryopreservation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – "Cátedra de Investigación en Obstetricia y Ginecología" of the Department of Obstetrics, Gynecology and Reproduction, Hospital Universitario Quiron Dexeus.
Trial registration number: NA.
Keywords: monopronucleated ICSI zygotes, morphokinetic evaluation, time-lapse

P-263 Cilostazol improves oocyte competence and IVF outcomes in mice: ovulation of immature oocytes with higher developmental rates

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Study question: Can temporal arrest of oocyte maturation in superovulated mice synchronize oocyte meiotic and cytoplasmic maturation and improve oocyte competence and IVF outcomes?
Summary answer: Temporal arrest of oocyte maturation *in vivo* results in synchronization of oocyte meiotic and cytoplasmic maturation. The latter was observed to improve IVF outcomes and oocyte competence in superovulated mice.
What is known already: Temporal arrest of oocyte meiotic maturation *in vitro*, using phosphodiesterase 3A (PDE3A) inhibitors, can synchronize cytoplasmic and meiotic maturation and improve IVF outcomes. However, the beneficial effect of *in vivo* synchronization of oocyte maturation on IVF outcomes has not yet been addressed. Cilostazol is a PDE3A inhibitor with an established record of safe long-term use in patients. Cilostazol caused mice to ovulate immature oocytes at different stages based on time, dose, or frequency of administration.
Study design, size, duration: This study was designed as a mouse superovulated model for women undergoing hyperstimulation and IVF. No less than 10 mice were used in each group. This study was started in 2012 and finished in 2014.
Participants/materials, setting, methods: Swiss Webster mice were used in this study, and all materials were purchased from Sigma (St. Louis, MO). This study was of university laboratory setting and as follow: Ovulated or ovarian mature or immature oocytes were collected from superovulated mice treated with different doses of cilostazol.
Main results and the role of chance: Ovulated germinal vesicle (GV) oocytes had significantly higher rates of advanced chromatin configuration and cortical granule distribution than did ovarian GV oocytes collected from large antral follicles of hyperstimulated mice. Ovulated GV oocytes had lower levels of cAMP and consequently higher rates of germinal vesicle breakdown, first polar body emission, *in vitro* fertilization (IVF), and blastocyst formation than did ovarian GV oocytes ($P < 0.0001$). Ovulated MI oocytes had higher rates of normal spindles and chromosomes aligned at the metaphase plates than did ovarian MI oocytes collected from preovulatory follicles of superovulated mice ($P < 0.003$). Mice ovulating MI oocytes produced litter sizes greater than those observed in control mice ovulating mature oocytes ($P < 0.002$).
Limitations, reason for caution: The positive impact of CLZ on oocyte maturation and IVF outcomes need to be confirmed using other animal models that are more predictive to human reproduction.
Wider implications of the findings: The ovulated immature oocytes may substitute for ovarian immature oocytes and become an additional research resource. More importantly, the capability of a clinically approved medication to increase oocyte fertilization rates and litter sizes in mice, at doses extrapolated from human therapeutic doses, suggests the potential scenario of the inclusion of CLZ in human hyperstimulation programs to increase the live birth rate of IVF babies.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Texas A&M University and BARZ IVF Center for Embryo Research and Infertility Treatment.
Trial registration number: NA.
Keywords: cilostazol, immature oocytes, oocyte competence

P-264 Time-lapse analysis of 1264 embryo compaction: how many cells are necessary?

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Study question: The aim of our study was to evaluate the morphokinetic parameters of embryo compaction and to observe how many cells were included in the compaction using the EmbryoScope®, a time lapse monitoring system.
Summary answer: In this study we showed that embryo compaction can occurred from the 3-cell stage to the ≥9-cell stage but the early cleavage development differs between groups and that obtention of good quality blastocyst is correlate with early cleavage development as well as the number of cells included in the compaction.
What is known already: The onset of compaction can occur on the third to the 4th day of embryo development that is concomitant with the activation of the embryonic genome. At this stage, blastomeres begin to compact tightly and cell boundaries progressively disappear until the fully compaction.
Study design, size, duration: We retrospectively analysed all the embryo compaction obtained after ICSI cycles performed with the EmbryoScope® between February 2011 and November 2014 in our IVF unit.
Participants/materials, setting, methods: Immediately after ICSI, oocytes were placed into the EmbryoScope®. Each embryo was investigated by detailed time-lapse analysis measuring the exact timing of the developmental events in hours after ICSI procedure. We particularly observed the time of compaction and the number of cells involved.
Main results and the role of chance: We observed that beginning of compaction occurred between 3-cell and ≥9-cell stage. Most of time, compaction occurred at the ≥9-cell stage (87.12%). Only 78 compaction occurred at the 8-cell stage (6.17%) and 84 occurred before the 8-cell stage (6.65%). When compaction happened with less than 8 cells we showed that PN visibility is longer (time between PN appearance and PN fading), 2 cell-stage (t2) to t7 are more precocious and that embryos stayed longuer at the morula stage until blastulation. Irregular division mostly occurred in <8-cell group and we observed more good quality blastocyst in ≥9-cell. 52.81% of ≥9-cell group were good quality embryos (transferred or cryopreserved), 39.74% in 8-cell group and 14.46% in <8-cell. Finally, pregnancy rate in higher in ≥9-cell group.
Limitations, reason for caution: Evaluation of early cleavage stage is easy but after the 9-cell stage, the precision to count cells is not relevant.
Wider implications of the findings: Time-lapse systems are known to optimize embryo culture thanks to uninterrupted culture and continuous surveillance. In this study we reported that the kinetic developmental pattern of embryo compaction depended of the number of cells at the initiation of compaction and that it also have an impact on the chance to obtain a good quality blastocyst.
Study funding/competing interest(s): Funding by University(ies) – CHU de Nantes.
Trial registration number: NA.
Keywords: time lapse, compaction

P-265 Outcome of vitrified-warmed blastocysts derived from poor-quality cleavage stage embryos

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Study question: Do supernumerary cleavage stage embryos that do not fulfil the freezing criteria on day 3 have the capacity to develop into good-quality blastocysts on day 5/6 and what is their implantation potential in blastocyst warming cycles?
Summary answer: One third of the embryos unsuitable for cryopreservation on day 3 were vitrified at the blastocyst stage. The clinical outcome of the warming cycles of blastocysts derived from these poor-quality embryos on day 3 is comparable to the outcome of warmed blastocysts derived from good-quality day 3 embryos.
What is known already: Embryo transfer on day 5 provides a better embryo selection and a higher implantation rate compared to day 3 transfer. In day 3 transfer and cryopreservation programs, often applied for poor prognosis patients, supernumerary embryos not fulfilling the day 3 cryopreservation criteria are discarded. Postponing vitrification of supernumerary day 3 embryos till the blastocyst stage and analysis of their clinical outcome in warming cycles might have implications for the cryopreservation policy after day 3 transfer.
Study design, size, duration: Retrospective study of 876 day 3 transfer cycles comparing extended blastocyst culture of 3918 supernumerary embryos suitable and not suitable for cryopreservation on day 3 from January 2010 until December 2013. The clinical outcome of 631 blastocyst warming cycles until November 2014 was analysed.
Participants/materials, setting, methods: Main day 3 cryopreservation criteria were ≥6 cells, ≤20% fragmentation, no multinucleation and no severe deviation in blastomere size. Clinical outcome parameters were positive hCG, clinical pregnancy rate (CPR), CPR with foetal heart beat (FHB) and implantation rate with FHB (IR). Chi-square test was performed for statistical analysis.
Main results and the role of chance: Out of 773 embryos that did not fulfil (group A) and 3145 that did fulfil (group B) the day 3 cryopreservation criteria, 247 (32%) and 1680 (53.4%) were vitrified on day 5/6, respectively ($p < 0.0001$). Survival rates after warming were comparable for both groups (87.2% and 89.1%). SET of warmed blastocysts of group A ($n = 67$) and group B ($n = 346$) resulted in 22.4% and 34.4% positive hCG, 19.7% and 28.8% CPR and 16.7% and 24.3% CPR or IR with FHB, respectively. Un-mixed DET cycles of group A ($n = 12$) and group B ($n = 206$) resulted in 41.7% and 42.2% positive hCG, 36.4% and 33.5% CPR, 36.4% and 29.9% CPR with FHB and 27.3% and 18.5% IR. The clinical outcome was not significantly different between both groups.
Limitations, reason for caution: The current findings are based on a retrospective analysis and, therefore, could not account for other potential confounding factors.
Wider implications of the findings: Poor-quality cleavage stage embryos that would normally be discarded on day 3 may form good blastocysts with acceptable implantation rates after warming. Therefore, we may consider postponing our selection of embryos for cryopreservation to the blastocyst stage in order to rescue embryos with implantation potential. The percentage of embryos suitable for cryopreservation on day 3 is almost reduced by half when cultured to day 5/6. The clinical significance of this loss requires further investigation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – UZ Brussel.
Trial registration number: NA.
Keywords: embryo quality, vitrification

P-266 Follicular fluid biomarkers for oocyte quality in human *in vitro* fertilization: proof of principle

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Study question: Is the peptide profile in individual follicular fluid (iFF) associated with oocyte quality in IVF/ICSI and can we use peptidomic techniques to identify potential biomarkers for oocyte quality in the future?
Summary answer: Our data demonstrate a set of 23 peptides that could discriminate fertilized oocytes and non-fertilized oocytes. It is also confirmed that using peptidomic techniques to identify potential biomarkers for oocyte quality is feasible.
What is known already: Follicular fluid (FF) is available during oocyte aspiration and theoretically represents an optimal source for non-invasive biochemical predictors of oocyte quality. Several studies have been published on the levels of peptides, with a known endocrinological function, in follicular fluid and their value for predicting embryo quality. Nonetheless, it is unlikely that a single peptide would be predictive. Peptide profiling of iFF and its potential use as an biomarker for oocyte quality has never been reported.
Study design, size, duration: A total number of 67 individual follicular fluid samples from couples undergoing IVF/ICSI treatment at the Leuven University Fertility Center were analyzed in 3 independent training groups and divided in two classes: 'fertilized'($n = 8$, 7 and 16) and 'non-fertilized' ($n = 9$, 11 and 16).
Participants/materials, setting, methods: iFF samples were collected during oocyte aspiration. Peptides were extracted using a filter assisted sample preparation and reverse solid phase extraction. Samples were dissolved in 10 ml of 5% acetonitrile in water and evaluated via LC-MS/MS (Orbitrap QExactive).The mean signal intensity was compared between fertilized and non-fertilized oocytes.
Main results and the role of chance: To avoid overfitting, three training groups were analyzed independently. A total number of 162 candidate peptides were found in the 1st training group, classifying 94.1% (16/17) of the iFF correctly. In the 2nd experiment, 236 candidate peptides were found, discriminating 88.2% (16/18) of the iFF. In the 3rd training group, 395 candidate peptides were found, with an accuracy of 84.4% (27/32). Of all the candidate peptides, 23 were confirmed by all the experiments, discriminating 88.23% (15/17), 83.33% (15/18), and 87.5% (28/32) iFF blindly in principle component analysis.
Limitations, reason for caution: To promote one or more of the candidate peptides as a biomarker(s) for oocyte quality and fertilization (relative) quantification has to be elaborated.
Wider implications of the findings: This study, for the first time, presents a set of peptides in iFF can be used as biomarker capable of predicting oocyte quality and fertilization. It also reveals that peptide profiling in individual follicular fluid samples can be a new promising innovative, non-invasive approach to predict oocyte quality and fertilization in clinical IVF practice.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck Serono Grant for Fertility Innovation.
Trial registration number: NA.
Keywords: peptide profile, human follicular fluid, oocyte quality, IVF outcome

P-267 Sperm DNA integrity and its effect on embryo development: a retrospective time lapse study

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Study question: Is there a correlation between the degree of sperm DNA fragmentation as measured by Sperm Chromatin Structure Assay and early embryo development?
Summary answer: There was no correlation between the degree of sperm DNA fragmentation as measured by Sperm Chromatin Structure Assay and of embryo development assessed by Time Lapse videography.
What is known already: A negative correlation has been shown between sperm DNA fragmentation and IVF and ICSI fertilisation rates. To our knowledge, no trial evaluating the correlation between the degree of sperm DNA fragmentation as measured by Sperm Chromatin Structure Assay (SCSA) and early embryo development has been undertaken.
Study design, size, duration: In this retrospective study oocytes from women assigned to IVF or ICSI were cultured in EmbryoScope and monitored by time lapse videography. Sperm sample was collected for SCSA.
Participants/materials, setting, methods: The study included 4000 oocytes from 697 couples treated by IVF or ICSI. Embryos were cultured in an EmbryoScope and monitored by time lapse videography until day 6. The semen was collected on the same day as ovum pick up and the sperm DNA fragmentation assessed by SCSA, expressed by DFI.
Main results and the role of chance: Oocytes/embryo were categorized into four groups according to DFI level (0–10%, 10.1–20%, 20.1–30%, and >30%). Mean values for all parameters were tested in a linear regression analysis model with the respective parameters as dependant and the four DFI categories as independent factors. For all endpoints; fertilization rate (2PN), extrusion of second polar body (2PBe), pronuclear appearance (PNa), pronuclear fading (PNf), early cleavage (t2), blastocyst development (tB) and blastocyst rate the mean numbers were equal between all four groups. No statistical difference between the groups was detected.

Limitations, reason for caution: None.
Wider implications of the findings: The combination of Time lapse technology and assays like SCSA may gave us insight in early embryo development. Since there is a correlation between sperm DNA fragmentation and outcomes of IVF and ICSI, one could speculate that there is a paternal influence on early embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funded by Skane University Hospital, Malmö, Sweden.
Trial registration number: NA.
Keywords: DNA fragmentation, SCSA, time-lapse, EmbryoScope, embryo development

P-268 Are TrpV3 channels present in human oocytes to mediate strontium-induced artificial activation?

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Study question: Is the TrpV3 channel present and functional in mediating strontium-induced artificial activation in human oocytes as was recently reported in mouse?
Summary answer: The TrpV3 channels are distributed in *in vitro* matured (IVM) human oocytes. However, neither strontium nor the agonists of TrpV3 channels are able to induce calcium rises or activation in human oocytes.
What is known already: Calcium (Ca^{2+})-influx is the main signal for the start of oocyte activation in mammalian eggs. Selective activation of Transient receptor potential channel (TrpV3) by agonists promotes Ca^{2+}-entry and induces mouse oocyte activation. The absence of TrpV3 channels in mouse oocytes failed to provoke strontium-induced activation. Strontium is sometimes used to overcome fertilisation failure after ICSI in human, but its efficiency is controversial and the mechanism how it mediates the Ca^{2+}-influx has not been studied.
Study design, size, duration: The distribution of the TrpV3 channels was investigated in IVM metaphase I-II (MI-MII) human oocytes after 3 or 24 h of maturation and in *in vivo* matured (IVO) mouse MII oocytes. The TrpV3 agonists-induced Ca^{2+}-oscillations, activation rate and subsequent embryonic development was analysed in both human and mouse MII oocytes.
Participants/materials, setting, methods: MII oocytes from B6D2F1 mice as well as IVM human oocytes were used. The distribution of TrpV3 channels was determined by confocal microscopy and Ca^{2+}-pattern was measured by time-lapse imaging after exposure to TrpV3 agonists for 1 h. Embryo developmental potential was assessed after artificial activation with these agonists.
Main results and the role of chance: The distribution of TrpV3 channels in IVM human oocytes showed a similar homogeneous pattern as was observed in IVO mouse MII oocytes. In mouse oocytes, both agonists of TrpV3 (200 μM 2-aminoethoxydiphenyl borate (2-APB) and 200 μM of carvacrol) promoted a single broad Ca^{2+}-peak in the first hour of exposure. However, no Ca^{2+}-influx and release could be observed in IVM human oocytes after the exposure to these agonists. Moreover, the use of 200 μM 2-APB activated 85% of mouse MII oocytes with 83% of them developing to blastocyst stage. In contrast, neither activation nor blastocyst formation was observed in human IVM oocytes.
Limitations, reason for caution: IVO human MII oocytes were not available for research. The distribution and function of TrpV3 channels could be different in IVO and IVM human oocytes.
Wider implications of the findings: The dysfunction of TrpV3 channels might explain the inconsistency that is reported in human oocytes to induce artificial activation using strontium to overcome fertilisation failure.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). China Scholarship Council and Special Research Fund from Ghent University (Bijzonder Onderzoeksfonds, BOF).
Trial registration number: NA.
Keywords: TrpV3 channels, Ca2+-release, strontium, human oocytes

P-269 Time-lapse versus bench incubator – a prospective randomized study comparing embryo outcome of sibling oocytes

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Study question: To determine whether culturing in the integrated EmbryoScope time-lapse incubator (TLI) with low oxygen improves embryo quality and blastulation rate in comparison with low oxygen bench incubator (LOI).
Summary answer: Cleavage stage embryo and blastocyst quality were comparable, whereas blastulation rate was significantly higher in the TLI system. The percentage of blastocysts suitable for freezing was similar in both groups.
What is known already: Previous retrospective studies showed higher pregnancy rates using embryos from TLI compared to standard incubators with atmospheric oxygen. Prospective randomized study comparing implantation and ongoing pregnancy found significant increased rates and decrease pregnancy loss with TLI system than with standard incubator, both with atmospheric oxygen supply. The authors admitted that they had no idea how much of this contribution was due to improved culture conditions.
Study design, size, duration: A prospective randomized sibling oocytes split study. Patients undergoing ICSI with ≥8 oocytes were enrolled between July and September 2014. Oocytes were randomly allocated into two groups after injection. Group A include 301 oocytes cultured in EmbryoScope, Group B 225 oocytes cultured in K-System. Oxygen in both systems was 6.0%.
Participants/materials, setting, methods: Oocytes were cultured in separate droplets of one step medium till D6. Group A oocytes were left in TLI till end of culture. In group B, evaluation of fertilization, Day 2, 3, 5 and 6 was done. Day 3 transfer was when <8 good quality embryos (I–II) were present. The suitable D3, D5 and D6 embryos for freezing were vitrified.
Main results and the role of chance: The percentage of D3 good embryos (6–10 cells, degree I–II) was borderline significant: in group A 27.0% of the embryos were with good quality and 36.5% in group B ($p = 0.0501$). The blastulation rate was statistically significantly increased in group A (51.8%, versus 30.3% in group B, $p < 0.001$). Blastulation rate on D5 was 30.4% and 14.7% ($p < 0.005$) and 21.4% versus 15.6% ($p = 0.294$) on D6. Day 3 supernumerary embryos of good quality were frozen: 8.3% of all embryos in group A and 13.3% in group B. ($p = 0.138$). Blastocysts were evaluated according to Gardner classification. Thirty good quality blastocyst out of 87 (34.5%) were frozen in group A and 11 of 33 (33.3%) in group B ($p = 0.923$).
Limitations, reason for caution: The size of the study group is limited, and so, conclusions and significance may not be well established. Implantation of embryos as a primary end point is impossible in this size of series, and thus, morphokinetics was not considered.
Wider implications of the findings: Our study compares, for the first time, the outcome of culture in TLI with this in conventional incubator, having the same a priori qualities, meaning dry chambers, same gas control conditions, low oxygen and same medium. The only investigated parameter was the exposure of embryos for evaluation with LOI. Under these conditions the percentage of good quality embryos was marginally better with LOI system, blastulation rate was significantly higher with TLI. However, the good quality blastocysts suitable for freezing were comparable in both systems. The advantages of TLI have still to be proven.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Assaf Harofeh Medical Center.
Trial registration number: NA.
Keywords: time lapse, blastulation, embryo culture, sibling oocytes

P-270 A higher cumulative pregnancy rate in elective cohort embryo freezing than fresh transfer with subsequent frozen embryo transfer in IVF/ICSI cycles in high responders

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Study question: Cumulative ongoing pregnancy rates (OPR) between elective cohort embryo freezing (freezing-all strategy) and first-cycle fresh embryo transfer (ET) with subsequent frozen ETs (FETs) for high responders in *in-vitro* fertilization/intracytoplasmic sperm injection (IVF-ICSI) cycles remain unclear.
Summary answer: Elective cohort embryo freezing could increase the cumulative second-trimester ongoing pregnancy rates in high responders.
What is known already: Elective cohort embryo freezing in high responders with subsequent FETs at better endometrial status is a safe and effective strategy for IVF-ICSI treatments. However, fresh ET used to be a norm in IVF/ICSI cycles considering the possibility of cryo-damage and its less procedure-related cost and time.

Study design, size, duration: This is a retrospective, cohort study. Analysis was performed on 180 patients with high response to ovarian stimulation (≥20 follicles bigger than 10 mm) with their first IVF/ICSI cycle during 2011–2014. The primary outcome measurement was cumulative second-trimester OPR. The secondary outcome was incidence of severe ovarian hyperstimulation syndrome (OHSS).
Participants/materials, setting, methods: Our study group consisted of 92 patients using elective embryo cryopreservation and subsequent FETs. The control group composed of 88 patients using fresh embryos at their first cycle of ETs, who would have another FETs if no successful ongoing pregnancy was achieved at the first cycle.
Main results and the role of chance: The ages were comparable between the two groups. Comparing to the control group, the study group had higher percentage of D5 ET (76.7% vs 32.2%, $p < 0.05$), and higher implantation rate (49.1% vs 29.8%, $p < 0.05$) in the first ET cycle. There were significant differences in clinical pregnancy rate (75.6% vs 67.8%, $p < 0.05$) and OPR (67.8% vs 56.3%, $p < 0.05$) between the study group and control group in the first ET cycle. In addition, a higher cumulative OPR (90.0% vs 82.8%, $p < 0.05$), especially noted in women with 20–25 follicles ≥11 mm (100% vs 81.5%, $p < 0.05$), and more surplus embryos were stored for further use in the study group comparing to the control group. Two cases (2.1%) of severe late OHSS developed in the control group.
Limitations, reason for caution: The sample sizes available in some analyses were small, limiting the power the study.
Wider implications of the findings: The use of cohort freezing might increase the chance of second-trimester ongoing pregnancy, especially for women with expected 20–25 oocytes. Therefore, when counseling to high-responders, we could offer them these data as well as the benefit of OHSS reduction.
Study funding/competing interest(s): Funding by University(ies) – Funding by National Taiwan University Hospital. All authors declare no conflicts of interests.
Trial registration number: NA.
Keywords: cumulative pregnancy, cohort freezing

P-271 Time-lapse monitoring and morphokinetic parameters predictive of embryo implantation: the lack of inter cohort reproducibility

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Study question: Are Time-lapse monitoring parameters described as predictive of implantation in the literature able to predict implantation when applied in another center?
Summary answer: In this study, few morphokinetic parameters described as predictive of embryo implantation in previous retrospective studies were carried out to our database to check if they were still relevant when used out of their original laboratory context. As result, none was predictive of embryo implantation when applied to our database.
What is known already: Since time-lapse monitoring (TLM) had become a daily tool for many laboratories, numerous studies attended to highlight morphokinetic parameters correlated the best with embryo implantation. However, no evident-based consensus on which parameter to use exists and groups using TLM don't have similar practice, resulting variability in embryo selection among them.
Study design, size, duration: Eight morphokinetic parameters (defined in the results) described in recent studies were applied retrospectively to a database of 342 single transferred embryos (no matter the stage) resulting of ICSI cycles which culture was performed between February 2011 and July 2014 with the EmbryoScope® in the CHU of Nantes.
Participants/materials, setting, methods: Each embryo were previously prospectively annotated during the IVF process and selected with these criteria to be transferred. Implantation prediction was tested for each morphokinetic parameter, using a positive hCG blood test as relevant of embryo implantation. Statistical analysis consisted in Chi² and Student tests, performed in R using Rcmdr.
Main results and the role of chance: None of the following parameters showed itself as predictive of embryo implantation: PN fading <20.45 h ($n = 342$ embryos, $p = 0.49$), duration of 2-cells stage <11.9 h ($n = 336$ embryos, $p = 0.0503$), duration of 2-cells stage >5 h ($n = 336$ embryos, $p = 0.1507$), duration of 3-cells stage <0.76 h ($n = 182$ embryos, $p = 0.1559$), duration of third round of cleavage (from the 3-cells to 5-cells stage) between 9.7 and 21 h ($n = 323$ embryos, $p = 0.1925$), time to 5-cells stage between 48.8 and 56.6 h ($n = 330$ embryos, $p = 0.3538$), time to 8-cells stage ($n = 257$ embryos, $p = 0.1768$) and time to early blastulation ($n = 36$ embryos, $p = 0.3905$).
Limitations, reason for caution: These parameter had only been applied to a monocentric database making the results not as strong as if being found with several ones from different IVF centers, above the embryo growing speed can change depending on the media used.
Wider implications of the findings: In spite of TLM is now recognized as an innovative tool in the embryo assessment, this study reinforces that a large prospective study is needed to standardize the use of TLM and to highlight the most predictive morphokinetic parameters or algorithms which could be adaptive to any of ivf laboratory working with different culture media and/or incubators and gas conditions.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CHU de Nantes.
Trial registration number: NA.
Keywords: IVF, time-lapse, embryo selection

P-272 Impact of postovulatory oocyte aging on initiation of genome activation in murine 2-cell embryos

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Study question: Does postovulatory aging of oocytes affect genome activation of murine 2-cell embryos after *in-vitro*-Fertilization (IVF)?
Summary answer: Fertilization of postovulatory aged oocytes has no impact on the number of resulting 2-cell embryos, but impairs transcriptional activation of the embryonic genome.
What is known already: Transcription in oocytes arrests during meiotic progression. Re-activation of transcription is essential for embryonic development and takes place in the 2-cell embryo in mice. Postovulatory aging of oocytes has been shown to reduce fertilization and implantation rates as well as embryonic development. So far, it is not known whether transcriptional activation in 2-cell embryos is impaired by postovulatory aging.
Study design, size, duration: Embryonic genome activation was determined in murine 2-cell embryos generated from control oocytes harvested and fertilized 14 h after ovulation induction ($n = 36$), and from oocytes after additional 4 h ($n = 32$), 6 h ($n = 34$) or 8 h ($n = 82$) of postovulatory aging.
Participants/materials, setting, methods: IVF was performed with oocytes from C57Bl/6J females. 24 h later, 2-cell embryos were counted and treated with fluorescence labelled BrUTP. The amount of incorporated BrUTP in nascent mRNA was evaluated by quantification of fluorescence intensity using Image J. Statistics were performed with Jarque-Bera and Mann-Whitney *U*-test.
Main results and the role of chance: Rate of 2-cell embryos after IVF was about 70 % in controls and remained stable after additional postovulatory aging of oocytes for up to 8 h. BrUTP incorporation was measured 24 h after IVF in early 2-cell embryos. Though fluorescence intensity of incorporated BrUTP did not change significantly in 2-cell embryos generated from aged oocytes, a decrease in number of embryos showing transcriptional activation was observed after 6 and 8 h of additional postovulatory aging compared to controls.
Limitations, reason for caution: Though the decrease in number of embryos showing genome activation was not significant, an effect of postovulatory aging on BrUTP incorporation can be assumed since concordant effects were seen after 6 as well as 8 h of postovulatory aging in two independent experiments.
Wider implications of the findings: Since genome activation is essential for embryonic development, postovulatory aging-induced reduction of transcriptional activation may lead to developmental defects of the embryo. Postovulatory aging of oocytes may occur in the course of assisted reproduction techniques. Thus these results could be of clinical relevance.
Study funding/competing interest(s): Funding by national/international organization(s) – Funded by DFG: GR 1138/12-1, HO 949/21-1, EI 199/7-1.
Trial registration number: None.
Keywords: postovulatory aging, 2-cell embryo, embryonic genome activation, IVF

P-273 Zona pellucida mRNA expression in human oocytes is related to oocyte maturity, zona inner layer retardance and fertilization competence

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Study question: Is the mRNA expression of zona pellucida genes, ZP 1, 2, 3 and 4, in oocyte and cumulus cells related to the zona pellucida structure as observed by the Polarized Light Microscopy (PLM) and the oocyte quality as observed by the oocyte's maturity and fertilization capacity?
Summary answer: The ZP mRNA expression in human oocytes is related to oocyte maturity, zona inner layer retardance and fertilization competence. ZP3 expression in cumulus cells (CC) is related to the oocyte maturity.
What is known already: ZP structure and birefringence could provide useful information on oocyte cytoplasmic maturation, developmental competence for embryonic growth, blastocyst formation and pregnancy. In order to understand the molecular basis of morphological changes in the ZP, in the current study the PLM analysis was combined with the analysis of the expression of the genes encoding for ZP1, 2, 3 and 4, both in the oocytes and in the surrounding cumulus cells.
Study design, size, duration: This is a retrospective study comprising 98 supernumerary human cumulus oocyte complexes (COC) (80 MII, 10 MI and 8 GV) obtained from 39 patients after controlled ovarian stimulation. Single oocytes and their corresponding CC were analysed individually and results are corrected for the potential impact of patient and cycle characteristics.
Participants/materials, setting, methods: Patients were stimulated in a long protocol with GnRH-agonist and recombinant FSH. Mature and immature oocytes were examined using PLM. QPCR was performed for ZP1, 2, 3 and 4 in these individual oocytes and their CC. EFNB2 mRNA was measured in CC as control. Data were analysed using one-parametric and multivariate analysis.
Main results and the role of chance: PLM analysis revealed that both the inner layer (IL)-ZP area and thickness were significantly lower in mature oocytes (p = 0,016 and p = 0,002 respectively) compared to those of immature oocytes. Oocytes contained ZP1/2/3 and 4 mRNA while in CC only ZP3 was quantifiable. When comparing mature and immature oocytes or their corresponding CC, ZP1/2 and 4 expression decreased in mature oocytes (p = 0,02; p = 0,008 and p = 0,02 respectively) and ZP3 expression decreased in the CC of mature oocytes (p = 0,01). In MII oocytes IL-ZP retardance was significantly related with ZP expression in oocytes (e.g., ZP1 = p = 0.016). ZP3 expression in the MII oocytes was the main predictor for the fertilization capacity next to IL-retardance, EFNB2 expression in CC and the ovarian sensitivity.
Limitations, reason for caution: This is a retrospective study and the relation to fertilization capacity is indirect as oocyte gene expression analysis required the lysis of the oocyte.
Wider implications of the findings: Overall relations between PLM observations, gene expression changes and intrinsic oocyte competence were successfully documented. As such the PLM and cumulus cell gene expression are confirmed as valuable non-invasive techniques to evaluate oocyte competence.
Study funding/competing interest(s): Funding by University(ies) – University of Torino.
Trial registration number: NA.
Keywords: zona pellucida, ZP genes, polarized light microscopy, oocyte competence

P-274 Non-invasive metabolomic and chemometric analysis of human embryo culture medium at low oxygen pressure

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Study question: Selecting the best embryo with the greatest potential to implant is the crucial step in all IVF centers. Metabolomic approach of embryo culture medium, together with the morphological criteria, has not produced satisfactory results. One of the major limitations is the technical complexity and cost.
Summary answer: We have not identified any metabolite in the culture medium at low pressures that is correlated with an increase/decrease in the pregnancy rate.
What is known already: Recent randomized controlled trials using commercial instruments failed to show a consistent benefit in improving pregnancy rates when metabolomics is used as an adjunct to morphology (Uyar and Seli, 2014). The High Pressure Liquid Chromatography with Mass Spectrometry (HPLC-MS) data have been analyzed with an approach developed in our group (Marhuenda-Egea et al., 2013) in order to found a relation between the implantation success and the metabolite concentration in embryo culture medium.
Study design, size, duration: Prospective study. We evaluated the metabolites presented in the culture media at low pressures of 121 embryos coming from 76 patients performed an IVF treatment with oocyte donation in our center during 2013.
Participants/materials, setting, methods: The embryos were cultured individually from day 3 to day 5 in 50 ml of Blastocyst Medium (Cook Medical, Ireland). The media were collected after embryo transfer and analyzed by HPLC-MS. The chemometric models were performed with Robust Principal Component analysis (RobustPCA) for samples from both, non-pregnancy and pregnancy cycles.
Main results and the role of chance: The global live birth rate of the recipients was 51.3%. The scores plot obtained by RobustPCA did not showed differences between the non-pregnancy and pregnancy samples. The distribution of the samples in the scores plot were determined by the loadings of the RobustPCA. These loadings were conditioned by the metabolite concentration.
Limitations, reason for caution: Although the use of metabolomics as a tool for embryo selection does not provide satisfactory results, the number of cases should be increased in order to check our detection method.
Wider implications of the findings: It seems that in the group of best reproductive prognosis (oocyte donation) and culture at low pressures which mimics the maternal environment, metabolomics is not useful. It would be interesting to test this analytical method in patients with poor reproductive prognosis like repeated implantation failure or recurrent pregnancy loss. Other steps could be the analysis of the metabolites in the uterus, since in this ambient could be the key to understand the pregnancy success.
Study funding/competing interest(s): Funding by University(ies). Funding by hospital/clinic(s) – Universidad de Alicante. Instituto Bernabeu Reproductive Medicine.
Trial registration number: None.
Keywords: metabolomic, embryo selection, ovodonacion, low O_2 pressures

P-275 Preserving the integrity of the corona-oocyte-complex (COC), during *in vitro* preimplantation embryonic development, enhances embryonic quality and increase pregnancy and implantation rates

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Study question: Can we improve embryo quality and potential by preserving the biological interactions between the corona cells and the oocyte/embryo?
Summary answer: Keeping the integrity of the COC significantly increases embryo quality and improves implantation and pregnancy rates in embryos obtained in IVF procedures.
What is known already: The bi-directional communication existing between the human oocyte and COC is essential for the production of competent oocytes. The COC transmits signals by cytoplasmic extensions contacting oolemma through gap junctions. Despite this, little is known about the interactions of the COC and preimplantation embryos. COC has been used only as feeder cells, in a co-culture system.
Study design, size, duration: We conducted a prospective study involving 160 patients (from July 2012 to September 2013) who were candidates for *in vitro* fertilization due to different female factor. We excluded couples with severe male factor, history of fertilization failure and those who had less than 6 fertilized oocytes.
Participants/materials, setting, methods: When evaluating fertilization, zygotes were divided in two groups: Study group (GR+; n = 80), in which

COC were preserved; and Control group (GR-; $n = 80$), in which zygotes were completed denuded. In each group embryo quality was determined at the time of transfer. Quality embryo, pregnancy and implantation rates were compared.

Main results and the role of chance: Both groups of patients were comparable in age (36.9 vs. 36.5) and number of embryos transferred (2.15 vs. 2.22). Referred to embryo quality, the percentage of top quality embryos (grade I) was significantly higher in the GR+ group than in the GR- group (72% vs 28%, $p < 0.001$), while among grade II embryos, no significant differences were observed (54% vs. 46%, $p = 0.8156$). Meanwhile, the percentage of poor quality embryos (grade III and IV) was significantly higher in the GR– group (60% vs 40%, $p < 0.001$ and 71% vs. 29%, $p < 0.001$, respectively). Regarding pregnancy rates and implantation, both were significantly increased in the study group (46% vs. 31%, $p = 0.050$ and 24% vs 16%, $p = 0.034$, respectively).

Limitations, reason for caution: Our main limitation is that the better results in the study group could be related to the absence of stress experienced when zygotes are completely denuded. Furthermore, we analyzed only embryos from IVF procedures, discarding ICSI procedures.

Wider implications of the findings: This study demonstrated that maintaining the association between corona cells and oocyte improve the results of *in vitro* fertilization treatments. We suggest that these CS exert a trophic effect on embryo development, resulting in a higher embryo quality. These improvements in embryonic development induce an increase in the values of both implantation and pregnancy rates. We are currently conducting a similar study but in couples undergoing ICSI procedures in order to corroborate the results obtained.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Procrearte.

Trial registration number: None.

Keywords: granulose cells, embryo quality

P-276 Which parameters affect the incidence of monozygotic twin pregnancies after single embryo transfer?

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Study question: Does the manipulation of gametes or embryos during assisted reproductive technologies (ART) increases the chance of monozygotic twinning?

Summary answer: The overall monozygotic twin (MZT) pregnancy rate after fresh single embryo transfer (SET) was 2.7%. Blastocyst transfer was associated with an increased risk of MZT, while zona pellucida manipulation by ICSI, or day-3 embryo biopsy for pre-implantation genetic diagnosis (PGD) did not increase the risk for MZT pregnancy.

What is known already: Monozygotic twins have a higher risk for perinatal complications than dizygotic twins or singletons. Although an increased incidence of MZT pregnancies after ART (ranging from 1.5 to 5.6% vs 0.4% in the general population) has been previously reported, conflicting data regarding the possible impact of different laboratory procedures [e.g., ICSI, assisted hatching (AH), and prolonged embryo culture] have been described.

Study design, size, duration: All 4678 clinical pregnancies (CP; defined as the visualization of a gestational sac) after ART with fresh SET carried out in our IVF centre between 2004 and 2013 were retrospectively analysed for the incidence of MZT pregnancies. In addition, the effect of different laboratory procedures on the incidence of MZT was evaluated.

Participants/materials, setting, methods: MZT rates (MZTR) were assessed using multivariable logistic regression adjusting for oocyte age, the day of embryo transfer and the following laboratory procedures: type of insemination (conventional IVF vs ICSI), embryo biopsy (always ICSI and day-5 transfer) and AH on day-3 or day-5.

Main results and the role of chance: The overall MZTR was 2.7%. No significant differences were found between the MZT/no-MZT groups regarding oocyte age, use of ICSI or embryo biopsy. AH was performed in 35 cases without ever resulting in MZT. Blastocyst transfer was significantly higher in the MZT group (74.4% vs 59.3%, $p = 0.001$) and it was the only variable associated with an increased MZT risk (OR 2.08, CI 95% 1.35–3.20) after multivariable logistic regression.

Univariate subgroup analyses showed higher MZTR after day-5 compared to day-3 transfers (3.3% vs 1.8%, $p < 0.001$). Embryo biopsy did not increase MZTR when compared to day-5 ICSI without biopsy (3.3% vs 3.3%). Performing ICSI also did not have any significant impact on MZTR when compared to IVF in both day-3 (1.6% vs 2.6%) or day-5 transfers (3.3% vs 4.6%).

Limitations, reason for caution: This retrospective, monocentric study is limited by the fact that monozygosity was not confirmed by genetic testing. Furthermore, larger studies should be performed to investigate the influence of AH on MZT rates. When comparing our findings to other studies, variations in the definition of CP should be taken into account.

Wider implications of the findings: As long-term culture is associated with a higher risk of monozygosity, more studies on the effect of prolonged culture (both culture media composition and culture duration) should be performed to identify the possible mechanisms responsible for the induction of monozygosity after ART.

Study funding/competing interest(s): Funding by hospital/clinic(s) – University Hospital Brussel-UZ Brussel.

Trial registration number: NA.

Keywords: monozygotic twins, single embryo transfer, blastocyst transfer

P-277 Comparison of microRNA expression in mouse ovarian follicle culture before and after cryo-preservation; A pilot study

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Study question: Does the cryopreservation affect the profile of microRNA in mouse ovarian follicle during *in vitro* culture?

Summary answer: Cryopreservation procedure may induce change in microRNA expression of mouse ovarian follicle.

What is known already: Cryopreservation of ovarian follicle has led to a great deal of interest as an alternative for fertility preservation. MicroRNAs are known to repress target genes at post-transcriptional level and play important roles in development and maturation of cell. However, the expression profiles and roles of microRNA in ovarian follicle during cryopreservation procedure have not been fully elucidated.

Study design, size, duration: Ovaries from 20 of 2-week-old C57BL6 mice were removed and preantral follicles were mechanically isolated. Recruited ovarian follicles were evenly divided into fresh (CTL) and vitrification (VT) groups.

Participants/materials, setting, methods: Total RNA was extracted from fresh ovarian follicles in CTL group and from vitrified and thawed ovarian follicles in VT group. The profiles of microRNA expression between two groups were analyzed by Exiqon miRNA array. Survival rate after *in vitro* culture of ovarian follicle was compared between CTL and VT groups.

Main results and the role of chance: Survival rate of CTL group was higher than that of VT group (72.2% vs. 24.4%, $P < 0.001$). Vitrification group showed 174 of over-expressed and 186 of down-expressed microRNAs compared to fresh group. Mmu-miR-182-5p, -5623-5p and -3473a were over-expressed and mmu-miR-878-3p, -375-5p and -138-2-3p were down-expressed in vitrification group compared to fresh group by more than 1.5-fold.

Limitations, reason for caution: The experiment of target gene confirmation for searched microRNAs is necessary as further study.

Wider implications of the findings: Our results suggest that vitrification of mouse ovarian follicle may induce change in microRNA expression and affect competence during *in vitro* culture. Further studies must be necessary to determine the effect of microRNA on survival rate of ovarian follicle after cryopreservation procedure.

Study funding/competing interest(s): Funding by University(ies) – Bumsuk Academic Research Fund in 2012.

Trial registration number: NA.

Keywords: microRNA, ovarian follicle culture, cryopreservation, mouse

P-278 Embryological KPIs significantly improved using SAGE 1-Step™ compared with the standard culture system in use, yet no subsequent difference in pregnancy rate was observed

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Study question: Introducing a new medium into the laboratory requires benchmarking against the current system in use. What parameters should be used as

benchmarks within a clinic? How does improvement in embryology outcome influence applied clinical outcome?
Summary answer: Improvements made in medium composition may improve some aspects of *in vitro* embryology including blastocyst rates and utilization rates (proportion of embryos either transferred or cryopreserved), yet this was not demonstrated to result in an increase in implantation rate.
What is known already: The composition of embryo culture media requires regular updating and subsequent clinical evaluation. Commonly, clinics will have a set of key performance indicators (KPIs) which they will benchmark new culture media against. KPIs need to, at a minimum, be maintained after changes in a culture system are introduced. However, the ultimate goal is to increase the baby take home rate of a clinic.
Study design, size, duration: This was a 2 arm, retrospective study comparing SAGE 1-Step™ medium (ORIGIO, Denmark) to the standard operational protocol currently used by the laboratory (Global Total, IVFOnline). Differences between the SAGE 1-Step™ ($n = 45$) and Global Total ($n = 83$) media systems were compared using a chi-square statistic.
Participants/materials, setting, methods: All patients undergoing IVF with stimulated cycle over a 2 month period in a busy IVF center were randomly allocated for culture in SAGE 1-Step™ medium or Global Total medium. All procedures were identical and ICSI was performed in all cases irrespective of sperm quality.
Main results and the role of chance: No difference was identified between treatment groups regarding the mean age (36,4 vs. 37,6) or the mean number of oocytes retrieved per patient (11,4 vs. 10,8) for the SAGE or Global groups, respectively, using a two-sample *t*-test. The cleavage (306/308 vs. 473/487), blastocyst (149/306 vs. 90/473) and embryo utilization (220/512 vs. 157/893) rates were all improved ($p > 0.001$) when embryos were cultured in SAGE 1-Step™ medium, compared to the currently validated system used by the clinic. Despite this improvement in embryology, no difference in implantation rate was observed between the two groups following embryo transfer (21/45 vs. 38/83; $p = 0.9$).
Limitations, reason for caution: Unequal replication across the two treatment groups; non-sibling design; and randomization according to time period could reduce the power of this comparison due to uncontrolled treatment factors (patient and environmental variations). The study should be repeated in different sites to look for developing trends, or extended with the above factors accounted for.
Wider implications of the findings: In conclusion, the compositional differences between SAGE 1-Step™ and Global Total media were shown to significantly improve standard embryological KPIs. Despite no difference in early pregnancy outcomes, improving any aspect of culture should be seen as a positive validation parameter for benchmarking the performance of new media products. While the ultimate aim of clinics is to improve the take-home baby rate (assuring healthy offspring) the clinical pregnancy rates may not accurately demonstrate improvements in laboratory outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Embryogenesis IVF Unit, Greece.
Trial registration number: NA.
Keywords: SAGE 1-step, culture system

P-279 Polar body analysis by immunofluorescence staining in human multiple pronuclear embryos

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Study question: What is the cause of multi pronuclear (multi-PN) formation in human embryos after ICSI?
Summary answer: The main causes of multi-PN formation after ICSI are probably related to maternal chromosomal fragmentation or the failure of second polar body (PB) extrusion. However, in 14.3% of multi-PN embryos, insemination of immature oocytes was a cause of multi-PN formation as polar bodies were not present in the perivitelline space (PVS) of multi-PN embryos.
What is known already: Polyspermy, failure of second polar body formation and chromosomal fragmentation are regarded as the main causes of multi-PN formation. In our laboratory, 4.1% embryos showed multi-PN after ICSI. The causes of multi-PN formation differ across patients, and normal microscopical observation alone does not provide sufficient information to assess this phenomenon. In this study, we examined the cause of multi-PN formation by immunofluorescence staining especially focused on the existence of PB.
Study design, size, duration: From August 2013 to May 2014, we investigated, using immunofluorescence staining, 95 embryos which showed multi-PN (over 3 PN) after ICSI. The immunofluorescence results of these embryos confirmed that there was a first PB (or first PB-like cytoplasm) in PVS before insemination by ICSI. The Ethics Committee at our clinic approved this study before it started.
Participants/materials, setting, methods: Multi-PN embryos were stained with an anti-dimethylated lysine 9 on histone H3 antibody to detect the female chromosome, and DAPI to detect female and male chromosomes. After staining, embryos were observed using a CV1000, a box-type confocal microscopic system.
Main results and the role of chance: After immunofluorescence staining of 95 multi-PN embryos, 44 embryos (45.8%) had over 2 PB, 37 embryos (37.1%) have a single PB, and 14 embryos (14.3%) did not have a PB. Moreover, all multi-PN embryos used in this study had a single male pronucleus and multiple female pronuclei (over 2 pronuclei).
Limitations, reason for caution: Using the above techniques it was possible to ascertain the existence of PB and pronuclear sexing was possible. However, whether aneuploidy was present in PN embryos was not ascertained. Further studies are warranted to determine aneuploidy status of these multi-PN embryos.
Wider implications of the findings: First polar body presence is used as criteria of oocyte maturation. However, caution should be applied for assessing oocyte maturation by the existence of first PB, especially in patients with low rates of oocyte maturation, because there are PB-like cytoplasmic bodies in human oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – There was no specific funding for this study. The authors have no conflicts of interest to declare.
Trial registration number: NA.
Keywords: polar body, multiple pronuclear embryos, ICSI, immunofluorescence staining

P-280 Human oocyte exposure to 1,2-propanediol obtains similar results compared to the use of dimethyl sulfoxide during vitrification by cryotop method

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Study question: Does the presence 1,2-Propanediol (PrOH) mixed with Ethylene Glycol (EG), used as permeable cryoprotectants, impact negatively over clinical outcomes after human oocyte vitrification and warming by the cryotop method when compared to dimethyl sulphoxide (DMSO) and EG mix as components of the medium?
Summary answer: This study suggest that the exposure of human oocytes to PrOH obtains similar results to DMSO in terms of survival, fertilization, pregnancy, implantation and miscarriage rates as well as blastocyst formation when used during vitrification process by the cryotop method.
What is known already: Several studies conducted in mouse oocytes have previously reported that the use of PrOH as a component of the vitrification media induces zona pellucida hardening, cellular degeneration and proteome alterations that could result in higher risk of aneuploidies after mouse oocyte warming, compared with vitrification solutions containing DMSO instead PrOH.
Study design, size, duration: This retrospective study analyzed 536 vitrified and warmed donor oocytes during two periods. The first (2008–2009) employed homemade equilibration medium containing 7.5% PrOH and 7.5% EG followed by a 15% PrOH and 15% EG for vitrification. During the second period (2009–2010), PrOH was substituted by DMSO.

Participants/materials, setting, methods: 49 couples showing normal seminal parameters (WHO, 2010) were divided into two groups: 265 warmed oocytes from the first period were designated to 24 patients (Group A) and 267 warmed oocytes from the second period were designated to 25 patients (Group B). Survival, fertilization, pregnancy, implantation and miscarriage rates were evaluated.
Main results and the role of chance: No statistical differences ($P > 0.05$) were observed between treatments regarding the mean of designated oocytes per warmed cycle [265/24 (11.0) vs. 267/25 (10.6)], survival rate after oocyte warming [230/265 (87%) vs. 238/267 (89%)] and fertilization rate [175/230 (76%) vs. 187/238 (78%)]. Moreover, the mean of transferred embryos [50/24 (2.1) vs. 49/25 (1.96)] as well as pregnancy [16/24 (66%) vs. 17/25 (68%)], implantation [17/50 (34%) vs. 19/49 (38%)] and miscarriage rates [2/16 (12.5%) vs. 3/17 (17%)] were similar between both groups. The number of embryos that reached at least the blastocyst stage at day 5 of embryo development before transfer were also similar in both groups [79/175 (45%) vs. 95/187 (51%)].
Limitations, reason for caution: The impact of PrOH to human oocytes was only evaluated in terms of clinical outcomes as an indirect parameter of oocyte quality after warming. The obtained results from this study may not necessarily reflect its lack of toxicity to human oocytes.
Wider implications of the findings: The results of the present study suggest that human oocytes can be vitrified successfully using a mixture of PrOH and EG, expecting similar clinical results to those using DMSO, used commonly as permeable cryoprotectant in the commercial vitrification kits. Further analysis must be conducted to demonstrate these findings.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Ingenes, IVF Lab, Mexico City, Mexico. Center for Reproductive Medicine Glickman Urological & Kidney Institute Cleveland Clinic, IVF Lab, Cleveland OH, U.S.A. Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán." No financial support was received for this study, and there are no potential conflicts of interest."
Trial registration number: None.
Keywords: oocyte, cryoprotectants, 1.2 – propanediol, vitrification, warming

P-281 The role of *in vivo* and *in vitro* maturation time on oocyte nuclear-cytoplasmic dyssynchrony

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Study question: Although intracytoplasmic sperm injection (ICSI) largely achieves fertilization independent of spermatozoa characteristics, complete fertilization failure (CFF) occurs, albeit rarely. We question whether the timing of *in vivo* and *in vitro* maturation influences oocyte nuclear-cytoplasmic asynchrony.
Summary answer: Cycles with CFF were more likely to have a shorter time interval between human chorionic gonadotropin (hCG) trigger and oocyte retrieval, hCG trigger and removal of cumulus cells, and oocyte retrieval and removal of cumulus cells. Shorter *in vivo* and *in vitro* maturation time contributes to oocyte nuclear-cytoplasmic dyssynchrony.
What is known already: The ability of ICSI to overcome almost all forms of male infertility has enabled couples that would otherwise remain childless to achieve parenthood. Successful fertilization is not guaranteed, however; and in rare cases, CFF occurs even in ICSI cycles. CFF may possibly be attributable to delayed maturation of the ooplasm in relation to completed nuclear maturity.
Study design, size, duration: Retrospective, paired-comparison of ICSI cycles with CFF to index cycles with successful fertilization in the same patient during a 10-year period. Cycles that involved surgically retrieved spermatozoa or donor oocytes were excluded. Semen parameters, specifically spermatozoa count and motility, were comparable and within norm between CFF and index cycles.
Participants/materials, setting, methods: Eighteen ICSI cycles were analyzed for potential inclusion. Of these, 3 (16.7%) cycles were excluded due to the use of donor oocytes. Controlled ovarian stimulation (COS) using long or short protocols, hCG trigger, oocyte retrieval, semen analyses and processing were performed as per our standard protocols.
Main results and the role of chance: Fifteen patients met inclusion criteria. There was no difference in age, body mass index, total duration of COS, total dosage of gonadotropins, hCG trigger dose, peak estradiol level, or mature oocytes retrieved in cycles with CFF compared to cycles with successful fertilization. The overall fertilization rate was 77.7% in the index cycles. Compared to index cycles, cycles with CFF showed a shorter time interval between hCG trigger and oocyte retrieval [2029.0 (±15.5) min vs. 2195.0 (±9.5) min; $P < 0.001$], hCG trigger and removal of cumulus cells retrieval [2201.4 (±15.3) min vs. 2309.0 (±22.6) min; $P < 0.001$], and oocyte retrieval and removal of cumulus cells [171.8 (±15.4) min vs. 114.0 (±13.1) min; $P < 0.001$]. The interval between hCG trigger and ICSI, however, was comparable between the two groups.
Limitations, reason for caution: Our findings reveal novel patterns in the time intervals between hCG trigger, oocyte retrieval, removal of cumulus cells and ICSI. However, the retrospective design and small sample size are limitations. This study's insights may help to mitigate the devastating effect of CFF.
Wider implications of the findings: The occurrence of CFF despite ICSI with adequate spermatozoa and number of eggs suggests that there is an optimal timing for cumulus-bound *in vivo* and *in vitro* maturation. In this context, modulating the time intervals between hCG trigger, oocyte retrieval, removal of cumulus cells and ICSI to grant fertilization seems feasible. Altering hCG trigger doses or utilizing an adjuvant gonadotropin-releasing hormone agonist trigger in a prospective setting may be utilized to prevent CFF.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine.
Trial registration number: NA.
Keywords: IVF, ICSI, oocyte nuclear-cytoplasmic dyssynchrony

P-282 An examination of embryo morphokinetics, utilization and pregnancy rates in single-step and sequential culture media systems

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Study question: Do embryo morphokinetics, blastocyst formation (BFR), utilisation rates (UR) and pregnancy rates differ between single-step and sequential culture media? Ultimately, this information will aid in the development of environment specific embryo scoring algorithms (ESAs) using time-lapse imaging provided by the EmbryoScope®.
Summary answer: These data suggest that embryos develop significantly faster in single-step medium. In addition, a higher number of 'usable' embryos were created from those in single-step medium with an increased BFR and UR. These preliminary results suggest that the biochemical (BPR) and clinical pregnancy rates (CPR) are equivalent.
What is known already: Two broad types of culture media are currently used in the IVF laboratory; single-step and sequential. Their effectiveness has been called into question owing to the existence of two theories regarding embryo nutrient use; 'let the embryo decide' and 'back to nature.' At present, both are used to varying degrees throughout IVF laboratories but their effect on embryo development in terms of morphokinetics remains to be elucidated.
Study design, size, duration: Embryos cultured in time-lapse from 09-06-14 to 08-09-14 were used to analyse sequential media use (group 1; $n = 3000$). Those from 09-09-14 to 08-12-14 were used to analyse single-step media (group 2; $n = 3392$). BFR, UR and morphokinetic parameters were analysed. BPR and CPR were analysed where data was available.
Participants/materials, setting, methods: Embryos from patients undergoing treatment within the Hewitt Fertility Centre were included. Embryos were cultured using either Vitrolife™ sequential media (G1 Plus™ and G2 Plus™) or single-step GTL™ medium at 5% O_2, 6% CO_2, 37°C throughout, in EmbryoScope® instruments. Morphokinetic data were derived retrospectively by manually annotating the captured images.
Main results and the role of chance: Group 1;4126 oocytes, 3000 embryos, 443 embryo transfers, average patient age; 35.1 ± 4.77, IVF:ICSI ratio; 1.10:1.0. Group 2; 4727 oocytes, 3392 embryos, 446 embryo transfers, average patient age; 34.5 ± 5.04, IVF:ICSI ratio; 1.12:1.0. All morphokinetic parameters revealed significant differences ($p < 0.0001$, unpaired *t*-test) with embryos cultured in single-step media developing faster; t2 (27.65 ± 0.09236 ($n = 2012$), 27.16 ± 0.08857 ($n = 2275$)), t3 (38.91 ± 0.1159 ($n = 1953$), 37.88 ± 0.1065 ($n = 2153$)), t4 (40.40 ± 0.1321 ($n = 1929$), 39.37 ± 0.1198 ($n = 2103$)), t5 (50.99 ± 0.1447 ($n = 2011$), 52.09 ± 0.1614 ($n = 1873$)). Analyses revealed the following results for group 1 and group 2, respectively; total number of

embryos cultured to day 5, 2797 vs 3153; 1616 vs 1935 reaching blastocyst, 915 vs 1188 good quality blastocysts (GQBs), BFR; 57.78% vs 61.37%, blastocyst UR; 37.33% vs 41.17%, overall UR; 39.3% vs 42.81%, BPR; 50.33% vs 50.67%, CPR; 37.47% vs 33.63% ($p > 0.5$, Chi-square test).
Limitations, reason for caution: Morphokinetic analyses are, by their nature, subjective therefore caution should be taken when considering the morphokinetic results. In addition, this study is a retrospective analysis and prospective trials are required to clarify the effect of different culture systems on embryo development.
Wider implications of the findings: These data suggest that an embryo's environment can affect its morphokinetic development adding to the increasing pool of evidence that environment-specific embryo scoring algorithms (ESAs) should be developed when time-lapse systems are in use. The use of published ESAs should be done so with caution and in-house derivation and validation is strongly advised. Embryo quality, as determined by UR, is also higher where single-step medium is used supporting the 'let the embryo choose' hypothesis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The Hewitt Fertility Centre.
Trial registration number: NA.
Keywords: morphokinetics, single-step media, sequential media, time-lapse

P-283 Successful *in vitro* fertilization and normal early embryo development after cryopreservation using a novel vitrification technique for epididymal spermatozoa

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Study question: Is vitrification of epididymalspermatozoa as effective in maintaining normal functions, including successful IVF and embryo development to blastocysts, as slow cooling.
Summary answer: Epididymal sperm vitrification using fibreplugs showed lower motility and viability post-warming but similar embryo developmental outcomes following IVF compared to slow-cooled or fresh spermatozoa. Vitrification with raffinose resulted in higher motility and viability scores than with sucrose, but sucrose vitrification gave better DNA integrity than slow cooling and vitrification with raffinose.
What is known already: Slow-cooling cryopreservation protocols for human spermatozoa are well established but not effective for small volumes. Vitrification shows promise for small volumes and is most effective when only non-penetrating cryoprotectants (CPAs) are used. Mouse sperm cryopreservation is challenging as the spermatozoa are very sensitive to chilling. In our preliminary study, epididymal sperm was vitrified using 0.25 ml straws, glass capillaries, fibreplugs (Cryologic) and open pulled straws but post-warm motility could only be achieved with fibreplugs.
Study design, size, duration: Four groups of mouse epididymal sperm samples ($n = 5$) were used and assessed for motility, viability (HOS) and DNA damage (TUNEL assay): fresh control (FC); slow freezing with 18%w/v raffinose (RS); and vitrification with 18%w/v raffinose (RV) or 0.25M sucrose (SV). Three groups were then selected (FC, RS and RV) to assess fertilization and embryo development.
Participants/materials, setting, methods: Prior to cryopreservation or IVF, epididymal sperm was prepared by swim up into KSOM + 1%w/v BSA (FC and SV) or into 18%w/v raffinose + 3%w/v skim-milk (RS and RV). Mouse eggs ($n = 267$) were inseminated using RV ($n = 102$), RS ($n = 86$) and FC spermatozoa ($n = 79$) and numbers of 2-cell embryos and blastocysts assessed.
Main results and the role of chance: Sperm motility [14 ± 1.4% (SV) and 30 ± 1.6% (RV) vs. 46.0 ± 3.0% (RS), $p < 0.01$, ANOVA, post hoc *t*-test] and viability (23 ± 4.2% (SV) and 39 ± 0.6% (RV) vs 57 ± 2.0% (RS); $P < 0.05$) were significantly lower after vitrification than slow-cooling. However, sperm vitrified with SV gave the lowest incidence of DNA fragmentation (15 ± 1.8% (SV) vs. 26 ± 2.8% (RV) and 27 ± 1.2% (RS), $p < 0.01$). The percentage of two–cell embryos produced by RV (66.7%), RS (61.6%) and FC (51.9%) spermatozoa was not significantly different. The proportion of blastocysts that developed *in vitro* from 2-cells, however, was significantly higher in FC (65.9%) than RV (36.8%, $P < 0.05$, Chi-square), but similar to the RC (50.9%). Expanded blastocyst rates were highest using RV spermatozoa (33.8%) than RS (17%, $P < 0.05$) and FC spermatozoa (19.5%).
Limitations, reason for caution: The tolerance to CPAs maybe specie-specific, therefore the type and concentration of CPAs selected for vitrification must be tested before translation of these results to clinical practice. Also, mouse embryo transfer trials are needed to further demonstrate the efficacy of each technique.
Wider implications of the findings: Vitrification using fibreplugs is simple, fast, and cost-effective and the warmed samples can be used directly for IVF or ICSI without further preparation, thus increasing the recovery rate of motile sperm. Fibreplugs are already used clinically for oocyte and embryo vitrification and thus can be conveniently and safely used for small volume vitrification of sperm. Also, the device does not come directly in contact with liquid nitrogen and thus is considered a safe technique.
Study funding/competing interest(s): Funding by University(ies) – Monash University.
Trial registration number: NA.
Keywords: vitrification, sperm

P-284 Higher pregnancy rate of embryos with synchronous transition from the 2-cell stage to the 4-cell stage (t4-t3)

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Study question: How do different morphokinetic parameters affect the pregnancy rate? Is there any difference between stimulation protocols?
Summary answer: The duration of the 3-cell stage (t4-t3) is shorter in embryos that lead to a pregnancy in both the agonist and the antagonist group, being significant in the latter.
What is known already: The EmbryoScope™ allows the selection of embryos with the highest implantation potential based on diverse morphokinetic markers. Some of them, like the time of cleavage from the 2- to the 3-cell stage (t3), the time of cleavage from the 3- to the 4-cell stage (t4) and the duration of the 3-cell stage (t4-t3) are associated with high blastulation or implantation rates after transfer on day 3.
Study design, size, duration: We performed a retrospective analysis of the time-lapse annotations of 178 Patients undergoing ICSI from September 2013 to December 2014.
Participants/materials, setting, methods: Using the EmbryoScope™ (Unisense Fertilitech, Denmark) as a time lapse imaging device, we analysed different morphokinetic parameters (t3, t4 and t4-t3) of all transferred embryos depending on the stimulation protocol (antagonist vs. agonist). Transfers were performed either on day 3 or 5.
Main results and the role of chance: Embryos with a high implantation rate present a significantly shorter duration of the 3-cell stage (1.7 ± 1.6 h vs. 4.1 ± 4.9 h, $p = 0.027$) in the antagonist group (mean age 34.1 years). A similar trend was observed in embryos of the agonist group (mean age 37.2 years) without reaching statistical significance (2.0 ± 2.3 h vs. 2.6 ± 2.8 h, $p = n.s.$). This difference is probably due to the distinctly lower mean age of patients in the antagonist group.
Limitations, reason for caution: The embryo numbers are small and the study results are based on a retrospective time lapse movie evaluation that can be associated with certain inter and intra-observer variation.
Wider implications of the findings: The analysis of morphokinetic parameters of the early embryonic development allows a non-invasive selection of embryos with the highest implantation potential, even after prolonged culture until day 5 of development. Further studies are needed to investigate the effects of different stimulation protocols on morphokinetic parameters of early human embryogenesis *in vitro*.
Study funding/competing interest(s): Funding by University(ies) – None.
Trial registration number: NA.
Keywords: time-lapse, morphokinetic

P-285 Vitrif-augmentation: cumulative live birth rate per oocyte retrieval at first IVF cycle in a single embryo transfer policy

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Study question: The aim of this retrospective cohort study was to assess the cumulative live birth rate per oocyte retrieval in first IVF cycle using vitrification and a single embryo transfer policy (e-set).
Summary answer: Our study shows a cumulative rate of live birth of 38% after the third thawed embryo transfer in women undergoing a first IVF cycle with vitrification of embryos and a strict e-set policy.
What is known already: Embryos survival rate after vitrification is better than with slow cooling. An e-set politicy allows reduction in multiple pregnancies and better maternal and foetal outcomes with no reduction in the pregnancy and/or delivery rate in IVF program.
Study design, size, duration: Our study is a retrospective monocentric cohort study that included all the couples having a first oocyte retrieval from the 1st of January 2012 to the 31st of August 2013 at the ART center of Schiltigheim France. The main outcome was the cumulative live birth rate per oocyte retrieval.
Participants/materials, setting, methods: 541 patients undergoing a first oocyte retrieval for an IVF program and eligible for e-set (mandatory if <38 years old) were included. Exclusion criteria were oocyte or sperm donation, pre-gestational diagnosis and embryo cryopreservation. A closed vitrification system was used to freeze supernumerary embryos.
Main results and the role of chance: Women mean age was 32 years old [21–42]. Out of the 541 undergoing their first oocyte retrieval 93 (17%) had no embryo transfer (no embryos or no oocytes) and 348 couples had at least one frozen embryo. The live birth rate after the fresh embryo transfer was 30% per transfer and 25% per oocyte retrieval. The cumulative live birth rate after fresh and 3 thawed embryo transfer was 38%. The embryo survival rate after warming was 94.4%. The mean number of transferred embryos at frozen transfers was 1.14.
Limitations, reason for caution: Both the retrospective and the monocentric design of our study are limitations. In our study 26 women underwent a freeze all policy and thus with no fresh transfer (ovarian hyperstimulation syndrome or high progesterone level at ovulation induction) could not be included regarding our main objective. Another bias was the missing datas about the remaining frozen embryos. Only eight patients were lost in follow-up.
Wider implications of the findings: This evaluation needs to be completed with multiple pregnancy rates, to show the benefit of e-set policy. Over the past few years, e-set protocol has already spread widely in European recommendations. We believe that the parallel development of vitrification and the outstanding embryos survival rate after warming, support this policy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hôpitaux universitaires de Strasbourg.
Trial registration number: NA.
Keywords: vitrification, cumulative live birth rate, e-set

P-286 Rapid growth and grading system for assisted-hatching blastocysts to predict clinical outcome

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Study question: Gardner's grading system is popular for the assessment of blastocyst. It employed scoring hatching status and therefore may be less useful to clinical practice where all blastocysts performed assisted hatching. Hence, we reanalyzed morphometric measurements to developed a new grading system to predict developing competency of hatched blastocysts.
Summary answer: Receiver operating characteristic (ROC) analysis showed that our criteria consisting of a time to reach blastocysts (category A), duration from blastocyst formation to full expansion (category B), and cell number of trophectoderm on the maxim cross-section (category C) was superior to Gardner's scoring system to discriminate the live birth pregnancy.
What is known already: Gardner's criteria includes degree of expansion and hatching status, where all hating or hatched score is rated as the maximum score six. This is developed for spontaneous hatching and thus considering assisted hatching. Hence, it remains to be determined whether the criteria is useful for assisted-hatching blastocysts.
Study design, size, duration: We chart-reviewed 2335 blastocysts from infertility patients who performed IVF-ET on year 2013. Relationship between morphological assessment conducted just prior for cryopreservation and outcome (live birth rate: LBR) were analyzed by multiple logistic analysis and predictability of the criteria was assessed by area under the curve (AUC) of ROC analysis.
Participants/materials, setting, methods: Patients who collected oocytes were performed natural cycle or minimally stimulated cycle IVF. All cases after blastocyst transfer compared LBR by three new categories and Gardner's criteria (degree of expansion, inner cell mass grading, and trophectoderm grading) among each three groups.
Main results and the role of chance: We compared LBR into three categories; 45.9%, 40.5%, and 28.3% in category A, 52.5%, 39.8%, and 21.7% in category B, and 39.6%, 42.5%, and 31.2% in category C. There were significant difference among three groups into each category ($p < 0.01$). On the other hands, there was no correlation between LBR and the average diameter of blastocysts. In ROC analysis, including patient's age and endometrial thickness, the AUC was higher in new categories than in Gardner's criteria (0.738 vs. 0.697). In multiple logistic analysis, we found that patient's age, endometrial thickness, the duration from blastocyst to good blastocyst (category B), and cell number of trophectoderm on the maxim cross-section (category C) become independent factors. On the other hands, Gardner's grading system was not independent factors.
Limitations, reason for caution: This study was a retrospective study. Our cases were only cryopreserved-warmed blastocyst transfer using Cryotop method and all blastocysts were performed by laser assisted hatching before embryo transfer.
Wider implications of the findings: These findings suggest that the criteria of blastocyst quality needs not only inner cell mass and trophectoderm grading but also the duration from blastocyst to good blastocyst. The growth speed until expanded blastocyst formation may represent embryo quality.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Shinbashi YUME Clinic.
Trial registration number: NA.
Keywords: blastocyst, grade

P-287 Evaluation of the effect of various cryoprotectants and protocols on donor oocyte survival and embryo viability based on HOPE registry data

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Study question: To characterize oocyte survival rate (OSR), embryo quality, and implantation and pregnancy rates for donor oocytes cryopreserved by either slow freezing (SF) or vitrification (VIT), according to cryoprotectant use in the Human Oocyte Preservation Experience (HOPE) Registry in the US.
Summary answer: OSR (% of oocytes thawed/warmed that undergo intracytoplasmic sperm injection) and implantation rates (IRs) (% of embryos transferred developing foetal sacs) differ by cryoprotectants used during SF/VIT and thawing/warming.
What is known already: Oocyte cryopreservation is used for different indications including: fertility preservation (for medical or social reasons); for IVF patients as an alternative to embryo cryopreservation; and oocyte donation via donor banking. Efficiency of oocyte cryopreservation has been improved in recent years, but more clinical data on the effect of cryoprotectants on oocyte cryopreservation outcomes are warranted.
Study design, size, duration: This post-hoc analysis of the Phase IV prospective, multicentre (16 US centres), observational HOPE Registry data evaluated cryoprotectant use. Data from 136 patients (enrolled between June 2008 and September 2010), 18–50 years, receiving donor oocytes cryopreserved by either SF ($n = 41$, 302 oocytes) or VIT ($n = 95$, 704 oocytes) were analyzed.
Participants/materials, setting, methods: The HOPE Registry was established to collect clinical and laboratory information on ART cycle outcomes using cryopreserved oocytes (autologous and donor). We compared outcomes by various parameters in cycles using donor oocytes. Six centres (involving 85/145 [58%] patients) were audited and regular monitoring across all centres ensured clean patient data.
Main results and the role of chance: For SF, OSR and IR (both mean [SD]) differed significantly among cryoprotectant combinations (both $p < 0.05$); respectively, PROH/sucrose (91.9% [11.8%], 26.4% [28.3%]) vs PROH/sucrose/

synthetic serum substitute (54.1% [30.8%], 6.25% [17.7%]) or PROH/sucrose/choline (53.0% [22.2%], 0% [0%]). For VIT, OSR and IR differed significantly among cryoprotectant combinations (both $p < 0.05$); respectively, DMSO/sucrose/ethylene glycol (88.3% [17.6%], 58.7% [44.8%]) and PROH/ETOH/sucrose/other (88.0% [13.3%], 37.0% [48.4%]) vs ETOH/DMSO/sucrose (46.7% [25.4%], 0% [0%]). For thawing, with SF, OSR differed significantly among cryoprotectant combinations ($p = 0.0004$); PROH/sucrose, (85.2% [20.0%]) vs PROH/sucrose/synthetic serum substitute (54.1% [30.8%]) or sucrose (39.2% [20.1%]) (both $p \leq 0.05$); respectively, IRs were 20.1% [27.1%] and 6.3% (17.7%) or 0% [0%] (p = not significant). For warming following VIT, sucrose was used in 95.7% of cycles, with a mean (SD) OSR and IR of 87.5% (15.6%) and 52.7% (45.5%), respectively. Higher OSR and IR translated into higher pregnancy rates.

Limitations, reason for caution: General registry limitations include selection bias due to non-sequential patients, observational data only and missing data. Specific limitations include changes over time in cryopreservation techniques following the start of the registry. No corrections for multiple comparisons were performed on these analysis of variance results.

Wider implications of the findings: Recently, VIT has become a more widely used cryopreservation technique than SF. Effects of different cryoprotectants used could potentially influence outcomes from assisted reproductive technology cycles that use SF-thawed/VIT-warmed oocytes.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – EMD Serono, Inc., Rockland, MA, USA (a subsidiary of Merck KGaA, Darmstadt, Germany).

Trial registration number: NCT00699400.

Keywords: cryoprotectants, oocyte survival rate, vitrification, slow freezing, assisted reproductive technology

P-288 Miscarriage rate tends to increase after fresh embryo transfer in hyper responders (>20 oocytes): a prospective observational study comparing fresh transfer vs freeze-all strategy

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Study question: Does the freeze-all strategy enhance the chance of ongoing pregnancy in case of ovarian hyper response without clinical hyper stimulation?

Summary answer: The clinical outcome is similar between the first frozen ET (FRET) in the freeze-all group (A) and the fresh transfer (FT) group (B). The miscarriage rate (MR) is higher after FT with no statistical significance due to sample size. The MR is significantly reduced in FRET cycles of group B compared to the FT.

What is known already: It has been suggested that controlled ovarian hyperstimulation adversely affects clinical outcome in ART cycles with FT in comparison with the outcome obtained after FRET. Kyrou et al. (2009) demonstrated that patients with high estradiol concentrations have significantly higher progesterone levels and significantly more oocytes. The abnormal concentrations of these hormones alter endometrial receptivity. A meta-analysis of published data by Roque (2013), demonstrated that IVF outcomes may be improved by the freeze-all strategy compared with FT.

Study design, size, duration: Prospective observational study from January 2012 to September 2014, between two groups of patients ($n = 93$ in each) with ovarian hyper response and recovery of >20 oocyte at the time of pick-up. In group A all the embryos underwent vitrification. In group B a FT was performed and spare embryos were vitrified.

Participants/materials, setting, methods: Age, indication, rank and stimulation regimen were similar between groups. FRET were performed in natural cycles after vitrification. Pregnancy rate (PR) and MR were compared in the first FRET vs FT. Cumulative outcomes were compared between groups, inside group B (FRET vs FT) and in FRET cycles in group A + B vs FT.

Main results and the role of chance: PR and MR were similar between group A and B when comparing the first FRET to the FT (PR 32.3% vs 31.2%, $p = 0.87$ and MR 16.7% vs 31.0%, $p = 0.19$). Cumulative PR and MR were comparable between the groups when including all the FRET cycles (PR 55.9% vs 52.7%, $p = 0.66$ and MR 13.5% vs 20.4%, $p = 0.35$). In group B, FT yielded significantly higher MR compared to consecutive FRET cycles (31.0% vs 5.0%, $p = 0.05$). Computing the outcome of all the FRET cycles (group A + B) compared to FT showed a significantly higher MR after FT (11.1% vs 31%, $p < 0.02$).

Limitations, reason for caution: The absence of statistical difference in MR between the two groups is due to the small sample size. However, as there is a clear tendency to higher MR rate after FT in group B, the trial is still ongoing and would enroll more patients.

Wider implications of the findings: Adopting the freeze-all policy in case of high oocyte recovery (>20) in hyper responders yields the same clinical outcome compared to fresh transfer. Nevertheless, data suggest that the rate of take home baby may be enhanced by the freeze-all approach by reducing the abortion rate and the transfer of embryos in a more physiological environment with a better embryo-endometrium synchrony.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Laboratoire Eylau-UNILABS, Paris.

Trial registration number: NA.

Keywords: freeze-all, miscarriage, hyper responders

P-289 Metabolomic analysis of culture media suggests a medium-chain fatty acid as an alternative energy source in preimplantation development

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Study question: Which medium-chain fatty acids (not listed on the composition list of the product data sheet) are present in commercial embryo culture media?

Summary answer: Five medium-chain fatty acids were identified in one-step culture media and especially, Octanoate is likely used as an alternative energy source in mitochondria throughout the preimplantation stages.

What is known already: Improvements in culture media formulations have led to an increase in the ability to maintain mammalian embryos in culture throughout the preimplantation stages. Recent development of culture media for human embryos is based on a characteristics switch in energy source preference. However, little has been reported regarding the direct measurements of global metabolites in preimplantation embryos.

Study design, size, duration: We studied metabolomic profiling of Mouse and Human culture media. The following four commercial human embryo culture media were included in this study: Continuous Single Culture Complete from Irvine Scientific, Sydney IVF Blastocyst Medium from Cook Medical, ONE STEP medium from NAKA medical and SAGE 1-step from ORIGIO.

Participants/materials, setting, methods: (1) 100 mouse embryos were cultured in KSOM (Millipore) until the blastocyst stage at the 120 h post hCG. These culture medium samples were measured by Capillary Electrophoresis Time-of-Flight Mass Spectrometer (CE-TOFMS). (2) All human media samples were analyzed by Gas Chromatograph Mass Spectrometer (GC-MS).

Main results and the role of chance: (1) 5 metabolites (pyruvate, lactate, glutamine, 5-Oxoproline, and octanoate) were significantly detected in both of the negative control and the embryo group. Compared to the negative control group, the embryo group showed significantly lower concentration of octanoete, suggesting it as an important energy substrate. Octanoate is not found in the component list of Millipore and is supposed to be carried into culture media by BSA. No embryos incubated in energy-depleted culture media reached the blastocyst stage. However, 76.9 ± 3.0% embryos incubated with octanoate alone reached the blastocyst stage. (2) 5 medium-chain fatty acids (octanoate, palmitate, palmitoleate, alienate and linoleatewere) were identified in all four commercial human embryo culture media.

Limitations, reason for caution: For four of the commercial embryo culture media only one batch was analyzed. However, this does not affect the overall conclusions.

Wider implications of the findings: Octanoate is likely used as an alternative energy source in mitochondria throughout the preimplantation stages. This study reveals metabolomic profiling of culture media in which preimplantation embryos were grown to understand metabolic demands of embryos towards further optimization of culture condition and exploration of a biomarker for embryo selection.
Study funding/competing interest(s): Funding by University(ies) – Keio University.
Trial registration number: NA.
Keywords: ART, medium, fatty acid, metabolome, culture

P-290 The insulin-like growth factor 1 receptor (IGF1R) expression is related to the activation of the embryonic genome during early bovine development *in vitro*

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Study question: The aim of the present study was to analyze the Insulin-like growth factor 1 receptor (IGF1R) expression during early bovine embryonic development *in vitro* on mRNA and protein level and to find out possible relations to the activation of the embryonic genome.
Summary answer: The mRNA and protein expression of the insulin-like growth factor 1 receptor showed a similar pattern during bovine early embryonic development. The stage-specific expression indicates a link to the maternal-embryonic transition, which takes place between the 8-cell and 16-cell stage in bovine embryos.
What is known already: The insulin-like growth factor 1 (IGF1) is a key regulator in early embryonic development, influencing physiological processes and stimulating growth and development. The signal transduction of IGF1 is performed by its binding to the insulin-like growth factor 1 receptor. On mRNA level, the IGF1R was already detected throughout preimplantation embryonic development and could be identified as a potential marker of good quality embryos. However, data on protein level is rare.
Study design, size, duration: *In vitro* derived embryos of different stages (2-cell, 4-cell, 8-cell, 16-cell stage, morula, blastocyst and expanded blastocyst) were either directly subjected to immunofluorescence staining or frozen at –80°C for use in RT-qPCR. Data were analysed by one-way ANOVA followed by a Tukey test using SigmaStat 3.5 Software.
Participants/materials, setting, methods: The mRNA expression of the IGF1R was analyzed by RT-qPCR using rabbit globin mRNA as an external standard. Immunofluorescence staining was performed with a peptide antibody against two peptide sequences of the bovine IGF1R alpha unit. Pixel intensity was measured and mean gray value was calculated using cellsens® software.
Main results and the role of chance: The detection of the IGF1R mRNA and protein was possible in all stages of embryonic development beginning at the 2-cell stage up to the expanded blastocyst. The maximal mRNA expression could be observed in 2- and 4-cell embryos. It significantly decreased to the 8-cell stage, followed by an increase up to the expanded blastocyst. The IGF1R protein was mainly localized in the plasma membrane of single blastomeres. Mean gray values were highest in the 2-cell stage, showing a significant decline up to the 16-cell stage and an increase again until the expanded blastocyst. The mRNA and protein expression showed similar patterns during early embryonic development, indicating a link to the maternal-embryonic transition.
Limitations, reason for caution: The analysis of gene expression by RT-qPCR and immunofluorescence staining in *in vitro* produced bovine embryos showed clearly that IGF1 plays an important role. But there are indications for differences to *in vivo* derived embryos, which have to be analyzed in future experiments.
Wider implications of the findings: The present study gives insides into basic knowledge of the IGF-system, which is influenced by the activation of the embryonic genome. Effects of IGF1 as conveyed by IGF1R play an important role in early embryonic development, as it is expressed throughout all stages.
Study funding/competing interest(s): Funding by national/international organization(s) – H. Wilhelm Schaumann Foundation (Hamburg, Germany).
Trial registration number: NA.
Keywords: insulin-like growth factor 1 receptor, gene expression, bovine embryo, embryonic genome activation, protein expression

P-291 *In vitro* culture of human embryos with micro-vibration increases take-baby-home rates: data of 4303 patients during four years

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Study question: Can *in vitro* culture of embryos with micro-vibration increase take-baby-home rate?
Summary answer: *In vitro* culture of embryos under micro-vibration conditions significantly increases take-baby-home rate for patients of 30 years old and older.
What is known already: The *in-vitro* culture of human embryos in a medium subjected to regular short intervals of micro-vibrations leads to increased development rates. This type of treatment tries to mimic the conditions which take place in the nature whereby oviductal fluid is mechanically agitated by the epithelial cilia.
Study design, size, duration: This work was performed from August 2010 to December 2013. Written informed consent was obtained from all the participating couples for the culture of non-fertilized and pronuclear oocytes and embryos. Patients were distributed on four age-groups: <29 years old, 30–34 years old, 35–39 years old and >40 years old.
Participants/materials, setting, methods: 4303 patients (mean age 35,5 + 4.6) prepared to IVF-/ICSI-cycles. The culture of oocytes/embryos performed under following conditions: micro-vibration with 20 Hz over 5 s/h (2152 patients, 4304 embryos) or static (2151 patients, 4302 embryos). Embryos transferred on Day 3/5 after oocytes retrieval. The data analyzed by ANOVA for categorical variables using the CATMOD Procedure (SAS).
Main results and the role of chance: The following take-baby-home rate was detected for Groups <29 years old, 30–34 years old, 35–39 years old and >40 years old, respectively (static vs. vibration): 29.3% vs. 32.9% ($P > 0.1$, increasing on the level of tendency), 27.1% vs. 38.7% ($P < 0.05$), 23.8% vs. 28.9% ($P < 0.05$), 9.1% vs. 14.5% ($P < 0.05$).
Limitations, reason for caution: This is a retrospective analysis and not all confounding factors could be considered.
Wider implications of the findings: Taking into account that *in vivo*, just after ovulation oocyte begins to be mechanically agitated, this agitation should be used also for IVF/ICSI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Praxisklinik Frauenstraße, Frauenstr. 51, 89073 Ulm, Telefon: 0731/966510, Fax: 0731/9665130, E-Mail: info@kinderwunsch-ulm.de, Website: www.kinderwunsch-ulm.de.
Trial registration number: N/A.
Keywords: oocytes, embryo, micro-vibration, *in vitro* culture, static culture

P-292 Oocyte vitrification before or after *in vitro* maturation for fertility preservation? A cohort prospective comparative study

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Study question: The developmental competence of the *in vitro* matured human oocytes is more compromised when they are vitrified at the germinal vesicle (GV) or at the metaphase II (MII) stage?

Summary answer: There is no significant difference on the survival rate of the post thaw germinal vesicle (GV) and metaphase II (MII) stage oocytes. Still, the developmental competence of the GV-state human oocytes seems to be compromised by the vitrification procedure.
What is known already: Cryopreservation procedure compromises the developmental competence of the oocytes applied either before or after *in vitro* maturation (IVM) compared to the developmental competence of the post thaw *in vivo* matured human oocytes and to the *in vitro* matured human oocytes that are directly fertilized.
Study design, size, duration: A total of 408 GV sibling oocytes in three different groups of study, from 120 consenting IVF patients were included in the present cohort prospective comparative study performed from December 2013 until November 2014. The first group was the "GV vitrification" group, the second was the "MII vitrification" group and the third one was the "NO vitrification" group.
Participants/materials, setting, methods: The study was conducted in a private ART unit. The source of GVs was IVF cycles of patients younger than 38 years from which were retrieved at least three GVs. A commercial IVM medium (Sage, Copper Surgical, USA) supplemented with 75 mIU/ml FSH and LH was used. For vitrification the FertiPro media and the closed Vitrisafe device were used.
Main results and the role of chance: The results of the present study indicate that though the survival rate of the oocytes after vitrification at different developmental stages is the same (81,25% vs. 80,35% for group 1 and 2 respectively), the developmental competence of the oocytes vitrified at the GV stage is strongly compromised. The maturation rate is lower in the "GV vitrification" group compared to the "MII vitrification" group (44,23% vs. 87,5% respectively). Moreover, the fertilization and the blastocyst formation rate seem to be influenced by the vitrification procedure at the GVs. On the other hand, the developmental potential of the oocytes vitrified after IVM seems to be similar to those that have not been vitrified.
Limitations, reason for caution: the source of the GVs was stimulated cycles, while it's possible usage would be from unstimulated ones.
Wider implications of the findings: Cryopreservation of immature or *in vitro* matured oocytes derived from unstimulated cycles is an option for fertility preservation in cases of patients undergoing therapeutic treatments for malignant and non-malignant diseases. The developmental stage of the oocytes to vitrify is an important parameter for the success of the procedure.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Iakentro.
Trial registration number: NA.
Keywords: GV, vitrification, *in vitro* maturation

P-293 Establishment and validation of a model for hCG stimulation in human mural granulosa cell culture

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Study question: To establish a standardized protocol for the culture of MGCs as a model for hCG effect.
Summary answer: In order to examine the effect of hCG on MGCs in culture, hCG should be administrated after 4 days in culture with daily medium exchange.
What is known already: Oocyte re-entry into the meiotic cell cycle and completion of maturation are triggered by increase in LH which is crucial for the final stages of oocyte maturation. Cell culture techniques of human mural granulosa cells serve as a major *in vitro* tool for understanding molecular signaling. However, the use of luteinized MGCs has major limitations due to their luteinized state hence a validated uniform model should be conducted in order to examine the effect of hCG in culture.
Study design, size, duration: luteinized MGCs were cultured with daily medium exchange. Cells were harvested and total RNA was purified for qRT-PCR analysis. Cell medium was obtained in order to examine progesterone secretion.
Participants/materials, setting, methods: Materials: Mural granulosa cell culture. Methods: qRT-PCR, ELISA for progesterone.
Main results and the role of chance: Our results indicate that after 4 days in culture with daily medium exchange MGCs demonstrate an increase in mRNA expression levels of LH downstream genes, Amphiregulin and Epiregulin and a decrease in sFRP4 mRNA expression. mRNA expression levels of the examined steroidogenesis genes, 3bHSD, 11bHSD and COX2 were also upregulated as a result of hCG administration on day 4 with daily medium exchange. We showed that luteinized MGCs response to hCG stimulation by upregulating LH downstream and upregulating steroidogenesis mRNA genes expression after 4 days of culture. On day 4, maximal effect of hCG on examined genes expression was observed 4–6 h after hCG administration. Progesterone secretion to the medium was increased as a response to hCG stimulation and reached the highest levels on day 4.
Limitations, reason for caution: MGCs were luteinized hence already exposed to hCG.
Wider implications of the findings: This novel model may provide an important standardized research tool for examining the effect of hCG on molecular processes occurring during the latter stages of follicular development in the human ovary.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Sheba Medical Center.
Trial registration number: 821/13/ANIM.
Keywords: mural granulosa cells, hCG, human, culture model

P-294 The effects of late ICSI on embryo quality

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Study question: What are the reasons of decreased implantation potential of embryos derived after delayed ICSI?
Summary answer: If oocytes were fertilized by ICSI at 42 h or more after ovulation trigger the obtained embryos showed impaired developmental ability. This was revealed by reduced blastocyst quality, increased irregular division rate and abrupt morphokinetic pattern.
What is known already: Studies published so far defined empirically that prolonged storage of oocytes post retrieval is related to reduced embryo quality and significantly lower pregnancy rate. However the detailed explanation of this phenomenon is still missing.
Study design, size, duration: This cross sectional study included time-lapse embryo culture data from 410 fresh embryo transfer cycles, where oocytes originated from patient younger than 35 years. Of those in 140 cases comprehensive genetic screening was performed. Data was collected during 2 years in a private IVF centre.
Participants/materials, setting, methods: Oocyte fertilization was performed by ICSI or IMSI if needed. Time lapse culture was performed in EmbryoScope (Unisense Fertilitech, Denmark) and imaging data analysed with manufacturers software. Timings of development, presence of faulty cleavage patterns, morphological appearance were scored and compared depending on time spent by oocytes prior to fertilization.
Main results and the role of chance: Oocytes fertilised 42 h or more post ovulation triggering constituted 37% of cohort. The implantation rate in this subgroup was not significantly lower probably due to improvement of embryo selection with time-lapse embryo culture. Interestingly, after comparison of embryo division timings of the 'late ICSI' subgroup the faster dynamics was revealed. Additionally such embryos possessed increased incidence of poor prognosis predictor events such as divisions of blastomeres into three or more cells or production of two unevenly sized blastomeres. Although in the 'late ICSI' cohort transferred blastocysts were of comparable morphological quality the blastocyst formation rate was lower. Also, amongst all blastocysts obtained the percent of blastocyst having highest scores was reduced for this subgroup. No difference was observed regarding ploidy of embryos from delayed ICSI.
Limitations, reason for caution: This is a retrospective study and thus can be subject of bias. Amongst embryos derived by late fertilization there is a significant fraction with proper development dynamics and morphology. The efficiency of implantation of those can be the subject to further studies.
Wider implications of the findings: Our study reveals the importance of fertilization timing and complement previously published data. The accelerated cleavage of lately fertilized embryos can signify the existence of relation between the timing of oocyte maturation and subsequent embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Clinic of Reproductive Medicine 'Nadiya', Kiev, Ukraine.
Trial registration number: NA.
Keywords: ICSI, IMSI, embryo quality

P-295 Large scale comparison of morphokinetic timings of over 12,500 IVF and ICSI embryos from insemination to blastulation

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Study question: Do IVF and ICSI embryos have different morphokinetics?
Summary answer: Mean time of pronuclear fading, following insemination, was 2 h delayed in IVF compared with ICSI but subsequent timings, durations and synchronicity of cell cycles, duration of compaction and blastulation did not differ between the two methods.
What is known already: Time lapse monitoring allows the detailed comparison of morphological and dynamic variables of development between embryos. Information comparing IVF and ICSI embryos using time lapse imaging is limited although one study suggests that the initial delay to early morphokinetic variables, in IVF compared with ICSI, is short-lived and that mean values for IVF embryos, reaching consecutive cell stages, align with those of ICSI embryos after the 8 cell stage.
Study design, size, duration: Morphokinetic data was retrospectively collected from 12939 embryos using EmbryoScope™ time lapse imaging from multiple sites using standardised laboratory practice and culture conditions (5%O_2; 5.5%CO_2), between May 2011 and December 2013. Insemination times were recorded: IVF time when gametes were mixed and ICSI time midway through the ICSI procedure.
Participants/materials, setting, methods: Mean values in hours (h) and standard deviations were calculated for the following: pronuclear fading (PNf); time to 2 cells (t2) from PNf (d1); t3-t2 (cc2a); t4-t3 (s2); t5-t4 (cc3a); t8-t5 (s3); duration of late stages (time to start compaction (tSC) from t8) (dLS); duration of compaction (dCom) and blastulation (dB).
Main results and the role of chance: A difference in means of 2.3 h between ICSI and IVF was observed in PNf. The mean (h) and standard deviation (in brackets) for ICSI and IVF PNf was 23.7 (5.1) (n = 9619) and 26.0 (4.2) (n = 3320) respectively. Ongoing morphokinetics were similar when comparing cell cycle, synchronicity and durations through to blastulation. Mean values and standard deviations were respectively as follows for ICSI: d1 3.0 (2.4); cc2 9.2 (5.2); S2 2.7 (4.8); cc3 9.5 (6.6); S3 11.3 (9.1); dLS 28.6 (11.2); dCom 12.3 (7.7); dB 11.2 (5.5) and IVF: d1 3.3 (2.6); cc2 9.3 (4.8); S2 2.7 (5.1); cc3 10.1 (6.6); S3 11.0 (9.1); dLS 28.1 (10.2); dCom 10.3 (6.3); dB 10.8 (5.0).
Limitations, reason for caution: Recording of insemination time may lack precision, particularly following ICSI where several oocytes are inseminated over several minutes, compared with IVF which is performed at a definitive time.
Wider implications of the findings: Morphokinetic algorithms, in order to be applicable to both IVF and ICSI embryos should avoid using time of insemination (t0), or direct derivatives of this, to assure accuracy. Durations, or relative time from pronuclear fading, provide more reliable variables for time lapse algorithm development considering the inevitable, but now measurable, delay in IVF embryos observed up to pronuclear fading.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CARE Fertility Group.
Trial registration number: N/A.
Keywords: time lapse, morphokinetics, ICSI, IVF

P-296 S-Biopsy: a simplified, less invasive embryo biopsy technique for day 3 preimplantational genetic screening (PGS)

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Study question: To report a stripper based, less invasive method for preimplantational genetic screening, comparing it to the conventional technique used for blastomere biopsy (aspiration of a blastomere by micromanipulation) to assess if this technique can obtain similar outcomes while making PGS more approachable to inexperienced personnel within the IVF laboratory.
Summary answer: When compared to the conventional biopsy method, the stripper based biopsy (S-biopsy) technique successfully removed the desired blastomeres for PGS while considerably reducing the invasiveness to the biopsied embryos and the performance time in which embryos are manipulated outside their incubators, even without extensive experience from the user.
What is known already: There is currently enough class I evidence provided by a series of randomized controlled trials to support preimplantational genetic screening (PGS) as a reliable tool to improve clinical outcomes by selecting chromosomally integral embryos for transfer. PGS requires nucleated cells for analysis; however, the conventional technique used for day three biopsy is extremely hard to master and has been noted to adversely impact the viability of the biopsied embryos if performed without extensive experience.
Study design, size, duration: 368 good quality day 3 embryos (7 or 8 cells, <10% fragmentation, no statistical differences) from PGS cycles were randomized within their own IVF cycles to be biopsied either by the conventional technique or S-biopsy to form two groups that were compared for blastomere integrity and blastocyst formation rate.
Participants/materials, setting, methods: S-Biopsy was performed using a laser to create a thin funnel in the zona pellucida next to the desired blastomere, which was then extracted by aspiration and release of the embryo with a 140 mm stripper capillary. The conventional method was performed as described in the literature.
Main results and the role of chance: Group A (188 embryos) was biopsied by the conventional aspiration technique, while Group B (180 embryos) was biopsied by S-Biopsy. All biopsied embryos were allowed to reach the blastocyst stage and a chi-square for concurrence was used to determine statistical differences (Alpha level: 0.05). There was no statistical difference for both groups regarding intact blastomeres (Group A: 95.7%, 180 embryos vs Group B: 95.0%, 171 embryos) or blastocyst formation rate (Group A: 70.7%, 133 blastocysts vs Group B: 72.7%, 131 blastocysts). However, there was a significant difference in performance times: 4 minutes (±30 seconds) for S-Biopsy vs 15 minutes (±60 seconds) for the conventional technique (3 biopsied embryos were considered as a mean in both techniques for all biopsies in each group, preparation and material set up times were included).
Limitations, reason for caution: The S-Biopsy has been proven to be safe. However it isn't completely risk free, as the embryo or the blastomere can be damaged if the technique is not performed properly (e.g., hitting the blastomere with the laser, doing a bigger or incomplete funnel, using a wrong sized capillary).
Wider implications of the findings: There are other aspects that add more value to the technique as it is extremely easy to learn and perform; and it doesn't involve the use of a micromanipulator, holding or biopsy pipettes or PGS media (Ca++ Mg++ free). It can also be performed within the culture dish without increasing embryo manipulation. This makes blastomere biopsy for PGS easier, cheaper, less time consuming and more accessible to a wider array of IVF programs.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Ingenes. No financial support was received for this study and there are no potential conflicts of interest.
Trial registration number: None.
Keywords: IVF, day 3 biopsy, PGS, embryo

P-297 The impact of abnormal mitoses and fragmentation from zygote to blastocyst on potential of blastocyst to result in a pregnancy

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Study question: Do abnormal mitoses, large fragments and high fragmentation rate occur in embryos, which are able to develop to blastocysts with good morphology and do these blastocysts result in a pregnancy?
Summary answer: Abnormal mitoses, as direct cleavage (DC) and failed mitosis (FM) are compatible with pregnancy when occurring from 2nd cleavage and the blastomeres are integrated in blastocysts. High fragmentation (HF) and large fragments (LF) are frequently seen in embryos resulting in pregnancies.
What is known already: Blastocysts with a good morphology have high pregnancy rate. Embryos showing DC at the 1st cleavage can develop to blastocysts,

but their potential to result in a pregnancy is limited. The presence of a nucleus makes it possible to distinguish blastomeres from fragments. It is conventionally accepted to use a cut-off of 45 mm in diameter considering all cells less than 45 mm as a fragment. High fragmentation rate (>25%) is considered detrimental for embryo potential.
Study design, size, duration: Cohort study including 97 IVF/ICSI treatments. On day 2, the best embryo was transferred and remaining embryos were cultured to day 5. Blastocysts were vitrified and transferred after thawing in a subsequent cycle between January 2013 and October 2014. Pregnancy was assessed by ultrasound at gestational age 7 + 0.
Participants/materials, setting, methods: Embryos able to develop in good morphology blastocysts on day 5 were retrospectively assessed. Assessment was performed observing every mitosis and every nucleus in time-lapse. We recognized DCs; FMs (disassembling and reassembling of the nuclei without cytokinesis); LF (>45 µm); HF (>25%); and related to outcome.
Main results and the role of chance: Twenty-eight Blastocysts Resulted in a Pregnancy (BRP), 72 Blastocysts resulted in No Pregnancy (BNP). At the 1st cleavage (from 1 to 2 cells), DC and FM occurred respectively in 1 BNP and none BRP. Compared to 1st cleavage, FM occurred more frequently at the 2nd (2–4 cells), 3rd (4–8 cells), and 4th (8–16 cells) cleavages, in both BRP and BNP (2nd 2 vs. 5; 3rd 4 vs. 12; 4th 3 vs. 5). DC occurred less frequently than FM at any stage in both BRP and BNP (1st 1 vs. 2; 2nd 1 vs. 2; 3rd 0 vs. 0). LFs were released at 1st and 2nd cleavages in 9 BRP and 17 BNP. HF occurred in 1 BRP and 3 BNP. Fragments were never integrated in the blastocyst, while most of the blastomeres resulted after FM and DC were included.
Limitations, reason for caution: Blastocysts were vitrified. Few blastocysts did not survive the thawing, potentially selecting the ones with the highest potential. Fresh transfer blastocysts could have higher rate of abnormal mitosis. Abnormal mitoses could be underestimated at the 4th cleavage, due to the difficulties to assess this stage.
Wider implications of the findings: Embryos presenting DC and FM can result in good morphology blastocysts and, except at the 1st cleavage, in pregnancies. Embryos may have some self-repairing or recovering process. The occurring of DC and FM could be more frequent at earlier stage and that embryos select themselves when cultured till blastocyst at day 5. This supports the transfer of blastocysts instead of earlier embryo stage, in which the self-selection has not occurred yet.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Holbaek Regional Hospital.
Trial registration number: Approval number from the National Ethical Committee of Medical Science of Denmark: SJ-250.
Keywords: blastocyst, direct cleavage, fragmentation, vitrification, pregnancy

P-298 The activities of aminopeptidase N (APN) and embryo quality

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Study question: The objective is to establish the relationship between the activity of aminopeptidase N (APN) enzyme and embryo quality.
Summary answer: The alterations in the metabolism of APN might be one of the factors which affect embryo quality.
What is known already: The sperm potential is determined by different factors and physiological processes. Most of these processes are regulated by biochemical signals necessary for cellular communication. Many of these molecules are peptides which act on receptors located on the sperm membrane. The effect of peptidases can be controlled by enzymatic hydrolysis. APN pathway is considered to be of great importance in the degradation of peptidases.
Study design, size, duration: 89 sperm samples of patients belonging to the program of assisted reproduction of the IVI Bilbao were analyzed.
Participants/materials, setting, methods: These samples were treated to obtain three different fractions (sperm cells, seminal liquid and prostasomes). Embryo quality (number of cell and fragmentation of the embryos) was evaluated by qualified embryologist.
The activities of APN were measured using fluorimetric aminoacid derivatives and were correlated with embryo quality.
Main results and the role of chance: The higher activity of APN in seminal liquid was negatively correlated with the number of cells on day 2 but positively correlated with the percentage of fragmentation. For the activity of APN in prostasomes the same correlations with above mentioned variables were founded.
Limitations, reason for caution: Therefore, the study of the metabolism of peptidases may be useful as a biochemical tool for the diagnosis of human fertility, but need to be more studied with a larger number of samples.
Wider implications of the findings: Previous studies about the metabolism of the APN and its influence on the embryo quality weren't founded. We suggest that mentioned enzyme could be a good predictor of the embryo quality.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). University of the Basque Country. Spain. IVI Bilbao. Spain.
Trial registration number: CEISH/61/2011/IRAZUSTA ASTIAZARAN.
Keywords: embryo quality, peptidases

P-299 Euploid-blastocyst formation and blastocyst implantation rate is higher for Eeva high than for low embryos: a multicenter blinded study in PGS and non-PGS trophectoderm-biopsy patients

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Study question: Do Eeva Test results, generated through the automated time-lapse analysis of early cell division parameters, correlate with the likelihood of euploid blastocyst formation? Furthermore, do Eeva Test results correlate with implantation after blastocyst transfer?
Summary answer: In patients seeking preimplantation genetic screening (PGS) of embryos derived from IVF, euploid blastocyst formation for Eeva High embryos was significantly higher than for Eeva Low embryos. In non-PGS patients, blastocyst implantation was significantly higher for Eeva High embryos than for Eeva Low embryos.
What is known already: Time-lapse imaging parameters have been correlated to aneuploidy risk (Chavez et al 2012, Campbell et al 2013) and shown in a recent RCT to improve IVF outcomes (Rubio et al 2014). The Eeva Test is an automated time-lapse analysis and embryo classification system that reduces the time needed to measure time-lapse parameters manually. Here, we perform an evaluation of the correlation of Eeva Test results with euploid blastocyst formation, and with blastocyst implantation.
Study design, size, duration: A total of 96 PGS trophectoderm biopsy patients from two centers (2012–2013) were prospectively enrolled to study the correlation between time-lapse parameters and embryo ploidy status. Known blastocyst implantation was retrospectively assessed in a separate group of 171 non-PGS blastocyst transfers from four centers (2011–2014).
Participants/materials, setting, methods: For PGS patients, blastocyst culture, biopsy and PGS were performed per standard protocols. Embryos selected for transfer were based on comprehensive-chromosomal-screening results. For non-PGS patients, standard morphological grading was used for embryo selection. Time-lapse images were collected using the Eeva System, but all Eeva results were blinded to embryologists.
Main results and the role of chance: In 96 PGS patients, 36% of the total 982 embryos were euploid blastocysts. Euploid blastocyst formation rates for Eeva High, Medium and Low embryos were 45% (95/213), 44% (115/263) and 27% (139/506), respectively. The difference in euploid blastocyst formation rates between Eeva High and Low embryos was statistically significant ($p < 0.0001$). In 171 non-PGS patients, 39% of the 233 transferred embryos with known implantation status implanted. Day 5 known implantation rates for Eeva High, Medium and Low embryos were 44% (43/98), 45% (28/62) and 26% (19/73), respectively. The difference in day 5 known implantation rates between Eeva High and Low embryos was statistically significant ($p = 0.02$).

Limitations, reason for caution: This was not a randomized, controlled study. Implantation was calculated using known implantation confirmed by ultrasound at 6–8 weeks. Known implantation may underestimate overall implantation rates by excluding some implanted embryos from multiple embryo transfer cases.
Wider implications of the findings: This multicenter blinded study demonstrated a positive correlation between Eeva Test results and two highly relevant clinical outcomes: euploid blastocyst formation and blastocyst implantation. Eeva Test results may therefore reflect the chromosomal integrity of human embryos and add valuable information to improve embryo selection and facilitate the trend towards eSET. The positive correlation between Eeva Test results and euploid blastocyst formation may also provide potential decision support for patients considering PGS trophectoderm biopsy.
Study funding/competing interest(s): Funding by hospital/clinic(s). Funding by commercial/corporate company(ies) – Auxogyn, Inc and participating clinics. M. D. VerMilyea has provided technical consultation to Auxogyn, Inc.
Trial registration number: ClinicalTrials.gov # NCT01635049, NCT01369446.
Keywords: automated, Eeva Test, Embryo selection, morphokinetics, time-lapse

P-300 Modified rapid thawing of oocytes can assure higher survival rates maintaining subcellular integrity

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Study question: Can a faster thawing method result in a higher survival rate using slow freezing? Is the morphology of the oocyte preserved?
Summary answer: There is a significant increase in the survival rate within siblings using a faster thawing method. Rapid melting of ice crystals prevents oocyte degeneration. Cell morphology appears preserved by confocal microscopy of cellular structural elements. Together, these findings suggest that rapid thawing methods have potential for wider clinical application.
What is known already: In the clinical practice, oocyte cryopreservation is mainly used for fertility preservation and social freezing as well as egg donation programs.

Nevertheless, in the literature, there only have been few improvements in slow freezing protocols mainly due to the widespread use of vitrification procedures. In the past decade however, both survival and implantation rates have increased significantly due to a more balanced combination of sucrose, protein supplementation and exposure timing.
Study design, size, duration: This study involved 40 women undergoing *in vitro* fertilization (IVF) treatment who cryopreserved their oocytes and latter elected to donate these gametes for research. The mean age was 35 years old (range: 25–42). The operator froze all the spare metaphase II oocytes within 3 h post retrieval using slow freezing.
Participants/materials, setting, methods: Two warming methods were evaluate in sibling oocytes as follow: Group A) 30 sec air, 40 sec in a 30°C water bath Group B) 5 sec air, 5 sec in a 37°C water bath Survived oocytes were then fixed for tubulin and DNA fluorescence staining and imaged by confocal microscopy.
Main results and the role of chance: A total of 186 oocytes were divided in two groups of 93 oocytes to avoid inter individual variability bias. Sixty out of 93 eggs (64%) survived (group A) while 75/93 (group B) (81%) ($P < 0.025$). Two hours later survival was checked and 2 and 4 eggs were lysed in groups A and B respectively (P = ns). Confocal microscopy on a smaller number of the survived eggs of each group revealed similar mitotic spindle and chromatin distribution as shown by tubulin and DNA staining, which are compatible with previously reported data.

We tried to minimize the role of chance by splitting sibling oocytes for the thawing procedure. We speculate that a faster warming helps to minimize ice crystal formation and improve post thaw survival.
Limitations, reason for caution: The main limitation to widespread application of the current thawing methods is the lack of data on egg development therefore further evaluation of egg health is required before these procedures can be utilized in routine practice.
Wider implications of the findings: Oocyte cryopreservation techniques are associated with satisfactory clinical outcomes, but the long-term implications for egg health remain unknown. This study demonstrates that slow freezing protocols can be further improved. These findings may influence the choice of oocyte cryopreservation method and guide future optimization of different procedures.
Study funding/competing interest(s): Funding by hospital/clinic(s) – N/A.
Trial registration number: NA.
Keywords: IVF, cryopreservation

P-301 Comparative investigation of the effect of sage® media versus lifeglobal® media on human in vitro fertilization outcome after transfer of cryopreserved embryos

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Study question: The aim of this study was to compare the effectiveness of IVF cycles performed with Sage® media and LifeGlobal® media by assessing fertilization and blastocyst rates as well as outcomes of frozen embryo transfers (FET).
Summary answer: In our investigation the use of LifeGlobal® has shown better results comparing to Sage® medium, namely an increase in blastocyst rate, clinical pregnancy and implantation rates, thereby increasing the overall effectiveness of IVF program.
What is known already: Preimplantation embryos are able to develop in different culture media, which vary notably in their composition. On the other hand, in a number of experimental studies it has been shown that early embryo culture conditions might affect embryonic, fetal and newborn health.
Study design, size, duration: In the present retrospective study 442 treatment cycles from couples undergoing IVF or intra-cytoplasmic sperm injection (ICSI) from January 2011 to June 2014 were distributed between culture media Sage® and LifeGlobal®.
Participants/materials, setting, methods: A total of 438 patients aged between 20 and 39 years, not suffering from genetic infertility or cryptozoospermia were included. According to Gardner blastocyst grading system only embryos of good quality were cryopreserved using Vitrification Kit (Cryotech®) for subsequent transfer.
Main results and the role of chance: The 438 patients fitted the inclusion criteria; 213 of them were assigned to Sage® and the other 225 to LifeGlobal® media. Numbers of oocytes recovered (16.3 ± 8.1 for Sage® vs. 17.6 ± 8.5 for LifeGlobal®), an average number of transferred embryos (1.1 per FET) and fertilisation rates (77.8 ± 15.1 for Sage® vs. 76.0 ± 15.9 for LifeGlobal®) were similar between the two groups (P = NS). The same number of FET (n = 275) was perfomed in both groups. The blastocyst rate was significantly higher in the LifeGlobal® group (62.9 ± 21.5 vs 54.2 ± 23.3, $P < 0.05$) as well as the percentage of FET resulted in positive b-hCG (54.9 vs. 43.3%, $P < 0.01$), Clinical Pregnancy Rate (48.0 vs. 38.9%, $P < 0.05$) and Implantation Rate (46.1 vs. 36.8, $P < 0.05$). The difference in miscarriage rate before 12 weeks was not significant.
Limitations, reason for caution: Current study does not include take home baby and late term pregnancy loss rates as these data are still being collated on account of the ongoing pregnancies and patients lost to follow-up.
Wider implications of the findings: Due to the conflicting reports of the culture media effectiveness and the fact that manufacturers do not fully disclose their media composition it is reasonable to conduct an independent research in order to improve human embryo culture conditions. Having started with a simple comparison of embryological and pregnancy outcomes the investigation should be continued and deepened which might lead to better understanding of interactions of different aspects affecting embryos health in vitro.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Altravita IVF clinic.
Trial registration number: Not RCT.
Keywords: embryo culture, embryo, IVF

P-302 Fragilis, Stella and Blimp1 gene expression progressively decreases in the ovaries as mice get older

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Study question: How Fragilis, Stella and Blimp1 gene levels change in mice ovaries according to the animals' age?
Summary answer: Fragilis, Stella and Blimp1 gene levels decrease in mice ovaries from neonatal to 65 days old and then decrease more when reaching 240 days.
What is known already: Fragilis, Stella and Blimp1, genes that are known to be involved in germ line cells differentiation, are expressed in the early development of mice ovaries and also during the adult life.
Study design, size, duration: Ovaries from Balb-c mice at different ages – neonatal (nn), 65 days (65d) and 240 days (240d) old – were evaluated regarding the expression of Fragillis, Stella, Blimp1 genes; the study lasted 3 months.
Participants/materials, setting, methods: RNA from ovaries of 10 animals of each mentioned age were isolated, the RNAs were reverse transcribed into cDNA, and gene expression levels obtained by real time PCR using relative quantification (RQ) method. B2m was the endogenous control. We used One Way ANOVA and Tukey's test to analyze the results (RQs).
Main results and the role of chance: Means of RQs and standard deviation for each gene were: Fragilis (nn: 3.16 ± 0.59; 65d: 1.98 ± 0.27; 240d: 1.45 ± 0.27); Stella (nn: 15.05 ± 1.55; 65d: 7.78 ± 2.82; 240d: 1.62 ± 1.11); Blimp1 (nn: 11.98 ± 2.18; 65d: 4.02 ± 1.19; 240d: 3.08 ± 1.77). We used One Way ANOVA and Tukey's test to analyze the results. The genes showed to be statistically different according to the mice ages at $p < 0.01$.
Limitations, reason for caution: This is a gene expression study and investigations regarding protein levels must be carried on.
Wider implications of the findings: Immediately after birth the three genes showed to be expressed at high levels compared to adult life; as the mice get older their expression decreases. This dynamic follows the decreasing fertility related to age in mammals, what may suggest that although adult ovaries harbor oocyte precursor cells, the reduction of their function along time may not support gametogenesis at older ages.
Study funding/competing interest(s): Funding by University(ies) – funding by national/international organization(s) – USP- University of São Paulo- Brazil – CNPq- National Counsel of Technological and Scientific Development- Brazil – CAPES-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brazil.
Trial registration number: CEUA (Ethics Committee in the use of Animals) - FMRP/USP-60/2014.
Keywords: fragillis, stella, Blimp1, gene expression, reproductive age

P-303 The category of embryo potential afforded by the automated time-lapse enabled EevaTM Test is strongly associated with the speed embryo development

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Study question: The Eeva Test predicts the potential of an embryo to form a blastocyst based on automated time-lapse analyses of early cell divisions by determining 3 categories of embryo. How do these categories relate to cell number and developmental pace at assessment on day 3?
Summary answer: 'High potential' (HP) embryos showed a distribution of cell numbers strongly favouring rapid development with cell numbers greater than 7 predominating. In contrast 'low potential' embryos (LP) showed a distribution of cell numbers mostly less than 8 cells. 'Medium potential' (MP) embryos showed a mixed distribution of cell numbers.
What is known already: The Eeva Test, comprising automated time-lapse analyses of early embryo development, is able to indicate the potential of an embryo to develop into a blastocyst by categorisation to HP, MP or LP embryos. This categorisation has been shown to aid embryo selection achieving higher fresh pregnancy rates by comparison with use of morphology alone.
Study design, size, duration: This was a prospective examination of all 1470 embryos from 257 successive IVF and ICSI cycles in women < 40 years old, over 2 years in a single centre.
Participants/materials, setting, methods: Embryos were assessed on day-3 for cell count, regularity and degree of fragmentation, and the Eeva Test category was recorded. The blastomere count was categorised: 'less than 8 cells' (L8c), 8 cells (8c) or 'more than 8 cells' (M8c) and the 3 Eeva test categories were also recorded.
Main results and the role of chance: The distribution of categorisation of all embryos showed: L8c = 44% of the total, 8c = 42% and M8c = 14%. For the 720 LP embryos the distribution was L8c = 63%, 8c = 27% and M8c = 10%. For the 339 MP embryos the distribution was L8c = 35%, 8c = 55% and M8c = 10%. For the 411 HP embryos the distribution was L8c = 19%, 8c = 57% and M8c = 24%. These distributions differ significantly ($P < 0.01$) and imply that when the cell division timings lie within the ranges predictive of blastocyst formation (HP), the developmental capacity of embryos is more rapid – increased proportions of 8c and M8c embryos. This has implications for selection of embryos for transfer at the cleavage stage.
Limitations, reason for caution: This is a valid observational study of unselected embryos which may have implications for embryo selection in the absence of time-lapse evaluations. However, any such concept would require prospective controlled evaluation.
Wider implications of the findings: The timings of the initial cell divisions of an embryo appear to be critical for the pace of further development. Furthermore, as blastocyst formation appears to be associated with faster growing embryos, selection policies for cleavage stage embryos (whether to transfer 1 or 2 embryos) may take this into account.
Study funding/competing interest(s): Funding by hospital/clinic(s) – no external funding.
Trial registration number: NA.
Keywords: automated time-lapse, Eeva test, embryo selection, cleavage stage transfer

P-304 Mitochondrial oxygen consumption rates skyrocket at the blastulation in spite of constant mitochondrial DNA number during preimplantation development

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Study question: How does it change that mitochondrial DNA (mtDNA) copy number and mitochondrial function in human embryos during preimplantation development?
Summary answer: Mitochondrial oxygen consumption rates (mtOCRs) rise sharply at the blastulation in spite of constant mtDNA number during preimplantation development. In addition, mitochondria became mature and the CCO activity was detected at blastocyst stage.
What is known already: OCRs relate to the quantity of ATP production and mitochondrial respiration may be an effective index of mammalian embryo quality. The number of mtDNA has been shown to be a marker of mitochondria number. The number of mtDNA remains constant within preimplantation murine embryos, but not in cattle and pigs decreasing sharply from 2-cell to 4/8-cell stages. Moreover, the changes of mtOCRs of human embryos remain unknown.
Study design, size, duration: This experimental study was performed after obtaining informed consent of patients and an approval of ethical committee of JSOBGY using 21 MII oocytes, 12 day 3 embryos, 20 day 4 embryos and 20 blastocysts. We assessed the relationship among embryo developmental stage, their mtDNA number, and their mtOCRs.
Participants/materials, setting, methods: The mtOCRs and the mtDNA copy number of each sample were measured simultaneously using scanning electrochemical microscopy combined with a mito-toxin (cyanide) and by real time PCR, respectively. Morphological changes and cytochrome c oxidase (CCO) activities of mitochondria were assessed using 8 eggs. Data were compared using Tukey–Kramer method.
Main results and the role of chance: The copy number of mtDNA kept constantly from mature oocytes to blastocyst stage (155,702-222,511 copies). The mtOCRs didn't change from mature oocytes to day 4 embryos (0.5-1.5 fmol/sec). However, the mtOCR in blastocyst (4.3) was higher ($P < 0.05$) than others.

Round- or oval-shaped mitochondria transformed into elongated tubular forms, developing well-defined transverse cristae structure toward blastocyst stage at transmission electronic microscopy (TEM) observation. The mitochondrial CCO activity was detected at blastocysts stage based on TEM analysis, not but mature oocytes and cleavage embryos.

Limitations, reason for caution: Our data showed the number of mtDNA is constant during preimplantation development. This result is similar to those obtained in murine studies, but not bovine and porcine studies. Further studies need to clarify the species barrier and the relationship between the mtOCRs and mtDNA copy number in mammalian embryos.

Wider implications of the findings: Data of the present study showed mtOCRs rise sharply at the blastulation, that mitochondria became mature and that the CCO activity was detected at blastocyst stage. Blastulation requires more ATP depending on Na/K-ATPase. Thus, it is reasonable that mitochondria become mature and increase their OCRs at the timing of blastulation. This study provided new insights on the implications of a behavior of mtDNA copy number and mitochondrial function during human embryo development.

Study funding/competing interest(s): Funding by hospital/clinic(s) – no competing interests are declared.

Trial registration number: 142.

Keywords: embryo development, mitochondria, mitochondrial DNA, mitochondrial function

P-305 Is morphologically selected sperm injection (IMSI) useful in the case of performing IMSI and ICSI of sibling oocytes at the same time?

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Study question: Is there any difference of outcome between morphologically selected sperm injection (IMSI) and ICSI in non-selected patients? Is there any difference of outcome between IMSI and ICSI in patients with teratozoospermia, high sperm DNA fragmentation index and repeated implantation failure?

Summary answer: In non-selected patients and patients with teratozoospermia, high sperm DNA fragmentation index and repeated implantation failure, the normal fertilization rates by IMSI were significantly higher than those by ICSI. However, there were no differences of the blastocyst formation rate, the implantation rate and the delivery rate between the two groups.

What is known already: Morphological selection of fertilizing spermatozoa under a high magnification has been introduced to provide a better clinical outcome than the conventional ICSI. However, it is still a controversial issue whether human sperm head vacuoles affect ICSI outcome or not. Most of the earlier studies showed no effectiveness of IMSI in non-selected patients.

Study design, size, duration: This is a retrospective analysis of outcome in 11,215 with IMSI and 8,963 oocytes with ICSI conducted for women with all age from 2008 to 2012. In this study, we compared results of the two procedures on sibling oocytes. Embryo transfers were performed with only IMSI or ICSI derived embryos.

Participants/materials, setting, methods: Firstly, the comparison of outcomes between IMSI and ICSI in non-selected patients was performed. Among them, patients with teratozoospermia (Kruger test <4%), high sperm DNA fragmentation index (DFI ≥ 30%) and 2 times more than implantation failure were selected and the comparison of outcomes between IMSI and ICSI was performed.

Main results and the role of chance: In non-selected patients, the normal fertilization rate by IMSI was significantly higher than that by ICSI (71.1% vs 68.8%, $P < 0.01$). However, there was no difference of the good-quality embryo rate on day 3, the blastocyst formation rate, the implantation rate and the delivery rate between the two groups (good-quality embryo rate on the day 3: 45.5 vs 45.2%, blastocyst formation rate: 61.7 vs 61.9%, implantation rate: 38.0 vs 41.6%, the delivery rate: 26.4 vs 29.3%). In patients with teratozoospermia, high sperm DNA fragmentation index and repeated implantation failure, the normal fertilization rates by IMSI were significantly higher than those by ICSI. However, there were no differences of the good-quality embryo rate on day 3, the blastocyst formation rate, the implantation rate and the delivery rate.

Limitations, reason for caution: One of the reasons for the almost same results between IMSI and ICSI is that we performed IMSI and ICSI of sibling oocytes at the same time by one embryologist. Another reason is that selecting good-quality sperms by embryologists is possible even if low magnification because of introducing IMSI.

Wider implications of the findings: In non-selected patients and patients with teratozoospermia, high sperm DNA fragmentation index and repeated implantation failure, the ART outcomes excluding the normal fertilization rate by IMSI were almost comparable with those by ICSI in the case of performing IMSI and ICSI of sibling oocytes at the same time.

Study funding/competing interest(s): Funding by hospital/clinic(s). This study was not funded and there are no conflicts of interest.

Trial registration number: NA.

Keywords: intracytoplasmic sperm injection, intracytoplasmic morphologically selected sperm injection, outcome

P-306 Does the position of the inner cell mass during biopsy affects implantation?

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Study question: The most common trophoectoderm biopsy technique requires assisted hatching (AH) on day 3, but this process makes the inner cell mass (ICM) can be inside, outside or in the herniation in the moment of biopsy. Therefore, the position of the inner cell mass could affect implantation and early fetal development.

Summary answer: The position of the ICM at the time of the biopsy does not affect embryo implantation, although there is a tendency to decrease implantation if it is in the herniation position.

What is known already: Trophectoderm biopsy allows more accurate genetic diagnosis without apparent embryo damage. The AH previous to the biopsy itself is performed on day 3 because it promotes embryo hatching and is easier to remove the cells. However, the ICM may have gone along with trophoectoderm cells through the hole hatching or stay in the herniation. In our knowledge there is any publication relating the position of the ICM with the implantation of euploid embryos.

Study design, size, duration: Prospective study. We include the known clinical results of 44 euploid embryos transferred coming from 39 women that underwent CCS treatments from September to December 2014.

Participants/materials, setting, methods: At least one euploid embryo was transferred to 39 patients. AH was performed on day 3 using laser pulses (Saturn Active, Research Instruments). On day 5 of development, conventional trophoectoderm biopsy was done. The position of the ICM in the moment of biopsy was recorded. Clinical outcomes were evaluated.

Main results and the role of chance: There was no statistically significant difference in the positive pregnancy test when the ICM was inside (group I), outside (group II) or in the herniation (group III) (55.6, 70.0 and 28.6%, respectively, $p = 0.24$). The position of the ICM did not affect implantation (41.7% in group I, 62.5% in group II and 28.6% in group III, $p = 0.40$). However, we can observe a tendency to decrease the clinical results when the ICM is in the herniation position.

Limitations, reason for caution: Study currently under development to increase the number of cases and test the study question.

Wider implications of the findings: The results obtained in this preliminary study confirm that the position of the ICM in the moment of trofoectoderm biopsy does not affect implantation. However, we observe a tendency to decrease implantation when the ICM is in the herniation, probably due to the manipulation during biopsy that makes the ICM would be subjected to more movement and pressure. So, we could suggest waiting longer for biopsy if we observe the ICM located in the herniation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu.
Trial registration number: NA.
Keywords: ART, CCS, BIOPSY PROCEDURE, implantation

P-307 Morphokinetic data obtained until day 2 are not sufficient to predict blastocyst formation and quality

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Study question: Are morphokinetic data obtained until the 4-cell stage enough to predict blastocyst formation and quality?

Summary answer: Kinetic expressions of day 2 embryos were retrospectively compared with their blastocyst qualities on day 5. These exact timings of usable and unusable blastocysts were found to overlap by at least 30.2%, showing that kinetics of a day 2 embryo is not sufficient to predict blastocyst formation and quality.
What is known already: Blastocyst formation and quality were predicted using early cleavage parameters (time of division to 5cells (t5), time between division from 3 to 4cells (s2) and time between division from 2 to 3cells (cc2)) (Cruz et al., 2012). Furthermore, another time-lapse system is mainly based on timings of early cleavages (until the 4-cell stage) although the full scoring algorithm of the prediction of blastocyst formation and quality is not publically available (Conaghan et al., 2013).
Study design, size, duration: This retrospective cohort study was conducted from October 2011 to November 2014. It included 3503 embryos having achieved the blastocyst stage and belonging to 706 infertile patients transferred on day 5. Incubation was performed in time-lapse incubators (EmbryoScope™) and blastocysts were scored according to Gardner's classification (114-120 h post-ICSI).
Participants/materials, setting, methods: Blastocysts were classified into two groups: usable and unusable. The first one includes 3-4-5AA blastocysts, and also those graded as 3-4-5BB, AB or BA. Embryos of inferior quality and those manifesting a developmental arrest after the 8-cell stage were noted as unusable. Differences were analyzed by Mann Whitney test.
Main results and the role of chance: The studied kinetic parameters were: time of division to 2cells (t2), time of division to 3 cells (t3), time of division to 4 cells (t4), s2 and cc2. When usable (n = 2043) and unusable blastocysts (n = 1460) were retrospectively compared all timings apart from t3 were significantly different ($p < 0.001$). The mean usable blastocyst rate was calculated as 58.32%. The data were then divided into quartiles and the distribution of usable and unusable blastocysts was compared. For each of the five variables, two consecutive quartiles for which the usable blastocyst rates were the highest, were selected for further evaluation. The maximum increase in usable blastocyst rate was found for the second and third quartiles of cc2 and determined as only 11.48%. The gain in prediction was mediocre.
Limitations, reason for caution: The cohort studied involved only infertile patients (female, male or combined) and is in this respect a heterogeneous population.
Wider implications of the findings: The data gathered from cleavage timings until day 2 and the quartile approach have been described as the basis of some morphokinetic models published so far. However, the predictive power of these information is limited. Thus, data from late embryonic development phases are required for creating more robust inter-laboratory reproducible models.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital.
Trial registration number: Approved by the ethical committee of Memorial Sisli Hospital Istanbul Turkey.
Keywords: time lapse, cell division, morphokinetic assessment

P-308 Sperm Long noncoding RNA and mRNA profiling in poor embryo development patients identify central role of long noncoding RNAs in embryo development

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Study question: Do the long noncoding RNAs (LncRNAs) in sperm play roles in embryo development and could the expression of LncRNA be used as biomarker for poor embryo development?
Summary answer: Above four hundred of lncRNAs were up-regulated and more than six hundred of lncRNAs were down-regulated in sperm samples of poor embryo development. The expression of the most lncs were conformed with real-time PCR. Pathway analysis showed an upregulation of genes related to cell metabolism and genes related to stress response were overrepresented in the GO analysis.
What is known already: Recent evidence has shown that long noncoding RNA play critical roles in spermatogenesis. Long noncoding RNA could also be used as disease biomarker.
Study design, size, duration: The sperm samples of Three pairs of poor embryo development were chosen and sperm RNAs were extract and analyzed by microarray.
Participants/materials, setting, methods: Sperm were purified from the IVF failure patients and then human LncRNA Array v2.0 (8x60 K, Array star) was used to analysis the the expression of Lncs and mRNA in the RNA of the sperm sample. Real-time quantitative PCR was used to confirm the result. Bioinformatics analysis was used to check the possible function of the most significantly differentiated expressed LncRNA and mRNA.
Main results and the role of chance: Of the hundreds of differentiated LncRNAs identified in the microarray, two LncRNA, one is Lnc-CMPK1 and another one, Lnc, LA-16C390E6.5 was confirmed in the real time PCR with larger sample size. LA-16c390E6.5 as a human specific long noncoding RNA, has the best predictor value in the ROC analysis, with Cut-off value of 3.73 has the 100% sensitivity and specificity. Therefore further functional study could reveal whether could be used to further predict the embryo development.

GO analysis indicate that stress response represent the most significant part of the lnc-RNA in embryo loss of IVF patients. The down-regulated GO analysis shows that cell metabolism represent the major process related to the embryo development.
Limitations, reason for caution: The present method was validated only in small sample. Large samples with male infertility should be used and further confirm whether the LncRNA and mRNA could be a reliable biomarker for embryo development. Functional study could reveal the detailed mechanism of how the LncRNA regulate the function of embryo development.
Wider implications of the findings: Sperm lncRNA play important role in embryo development. The aberrant expression of LncRNA could impact on the embryo development. Lnc such as LA-16c390E6.5 could be a useful biomarker for the prediction of embryo development.
Study funding/competing interest(s): Funding by national/international organization(s) – National Basic Research Program of China (2012CB944903).
Trial registration number: NA.
Keywords: long noncoding RNA, sperm

P-309 Parameters associated with survival and implantation following vitrification(vit) of blastocysts (Blasts)

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Study question: It remains unclear whether Blast stages, grades and osmotic responses during vitrification (Vit) or rewarming (Wrm) are predictors of blastocyst survival or implantation.
Summary answer: Parameters associated with implantation (Im) were patient age (age), day of vitrification (day) and trophectoderm grade (TE). There was no significant association between Im and blast stage (S), inner cell mass grade (ICM), collapse, re-expansion (R), oocyte source, or uterus used.
What is known already: It was reported that there are significant associations between Im and S, ICM and TE when blastocysts are transferred fresh. For vitrified warmed blastocyst in frozen embryo transfer (FET) cycles it is remains controversial which parameters are most closely related to the implantation or survival.
Study design, size, duration: We have retrospectively applied multiple logistic regression (MLR) to Blast stages, grades and collapse during blast Vit and re-expansion after Wrm for FET at our facility from 2012 – February, 2014, to determine if they are associated with Blast survival or Im. We do not perform artificial collapse.
Participants/materials, setting, methods: Blasts were Vit on days 5 or 6 in Cryotips using Irvine Scientific solutions and protocol. MLR considered whether survival or Im were associated with age, day, S, ICM, TE, collapse of the Blast in Equilibration (C1) and Vitrification Media (C2), R, oocyte source or and uterus used.
Main results and the role of chance: 169 Blasts (2BB or better) from 97 patients were Wrm'd and transferred to patients or gestational carriers. Every Wrm'd embryo survived Vit and Wrm (100% survival) only 5 Blasts where lost in 4 cryotips that exploded. Transfer of Wrm'd Blasts yielded a clinical pregnancy rate of 64.5% (63/97) with an Im rate of 40.8% (69/169). None of the parameters was associated with Blast survival since all Blasts survived. Parameters associated with Im (via the Akaike Information Criterion) were age, day and TE:

$$\ln(ORIm) = -0.0237 \times age - 0.857 \times day - 0.534 \times TE + 5.67$$

where TE was made numeric (A = 1, B = 2, C = 3). There was no significant association between Im and C1, R, C2, oocyte source, or uterus used.
Limitations, reason for caution: The lack of association between Im and C1, R, C2 suggests that osmotic events are not required for survival or Im. We hope to compare results with other groups who benefit from artificial collapse in order to understand when artificial collapse provides a benefit.
Wider implications of the findings: Associations between Im and younger age, earlier day, better TE following Vit and Wrm revealed virtually no dramatic effect of Vit since the same parameters are associated with Im following fresh transfer of Blasts. Blast Vit without artificial collapse is capable of exceptional survival and adequate Im with no dependence on osmotic collapse.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Georgian-American Center For Reproductive Medicine ReproART.
Trial registration number: NA.
Keywords: embryo, blastocyst, trophectoderm, vitrification

P-310 Effect of embryo freezing process on birth weight

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Study question: Are there differences between birth weights of children born after frozen embryo transfer (FET) and those coming from fresh embryos?
Summary answer: Singletons from FET lead to higher birth weights than embryos from fresh cycles.
What is known already: Recent investigations have showed that freeze-thawed embryos are related with a high incidence of macrosomia suggesting late consequences in the metabolic status of the offspring.
Study design, size, duration: In order to ascertain those finding we performed this transversal study including 121 births at term of single pregnancies from 60 FET and 61 fresh cycles carried out during 2013.
Participants/materials, setting, methods: One hundred and twenty one patients who gave birth at term, coming from single pregnancies after 61 IVF cycles and 60 FET cycles. Univariate analysis was performed to compare FET group versus fresh group including Fisher's exact test for quantitative variables and Student's *t*-test for categorical variables.
Main results and the role of chance: Birth weight of children coming from FET were higher than those coming from fresh embryos (p.0.034) being 3331 ± 416 g and 3125 ± 571 g respectively. Both groups had same age, parity, gestational age at birth and had no differences between own and donated oocyte proportion.
Limitations, reason for caution: On account of the small number of cases in this study it would be necessary perform a more extensive study.
Wider implications of the findings: The long term consequences of freezing should not be discarded. On the other hand women with known risk of low birth weight may benefit from postponing the transfer making a subsequent FET.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu.
Trial registration number: NA.
Keywords: outcome, weight, FET, fresh, transfer

ENDOMETRIOSIS/ENDOMETRIUM

P-311 Predictability of frequency of uterine contractions in the early luteal phase after controlled ovarian stimulation in non-progesterone supplemented oocyte donors

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Study question: To identify demographics, treatment cycle characteristics and endocrine profile predictors of the frequency of uterine contractions in the early luteal phase in non-progesterone supplemented subjects undergoing controlled ovarian stimulation.
Summary answer: Demographic variables or treatment cycle characteristics do not predict the frequency of uterine contractions in the luteal phase of controlled ovarian stimulation cycles. Endogenous progesterone levels are correlated with the frequency of uterine contractions in the luteal phase of controlled ovarian stimulation cycles, when following the long GnRH agonist protocol and no exogenous supplementation of progesterone is provided.
What is known already: Improving implantation of the transferred embryos/blastocysts is one of the major challenges in ART. It has been hypothesised that elevated uterine contractility at the time of transfer in women undergoing IVF/ICSI procedures could have a detrimental effect on implantation rates, and several compounds with anticipated impact on uterine contractility are currently in clinical development. However, the pathophysiology of the elevated uterine contractility during the luteal phase of controlled ovarian stimulation is not well understood.
Study design, size, duration: Retrospective investigation of baseline data from a randomised, double-blind, placebo-controlled trial in 84 oocyte donors who underwent controlled ovarian stimulation in the long GnRH agonist or antagonist protocol, triggering of final follicular maturation with hCG, and received no supplementation with progesterone after oocyte retrieval (OR).
Participants/materials, setting, methods: Demographic variables, type of protocol and total gonadotropin dose were recorded. Serum values for estradiol, LH and progesterone were obtained 2 days after OR (OR + 2). Transvaginal ultrasound recordings (Voluson i, GE Healthcare) of a continuous cine-loop image of at least 5 min were done on day OR + 2 in a standardised format. The frequency of uterine contractions was determined by a central independent assessor blinded to treatment allocation, using computer-assisted time series motion analysis software.
Main results and the role of chance: The median frequency of uterine contractions on day OR + 2 was 2.4/min (interquartile range: 2.1; 3.1). Correlations between the frequency of uterine contractions and age, body mass index, body weight and total gonadotropin dose were low and none were significant. The median frequency of uterine contractions was not significantly different between the two protocols. In subjects following the long GnRH agonist protocol, a significant negative correlation was observed between progesterone levels and the frequency of uterine contractions (r = -0.43, p = 0.002), but not in subjects following the GnRH antagonist protocol.
Limitations, reason for caution: Multicentre trial with multiple ultrasonographers.
Wider implications of the findings: Further research is needed to evaluate the pathophysiology of the elevated uterine contractility during the luteal phase of controlled ovarian stimulation cycles.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Ferring Pharmaceuticals.
Trial registration number: NCT01043120.
Keywords: uterine contractility, implantation, embryo transfer

P-312 Predictability of frequency of uterine contractions in the early luteal phase after controlled ovarian stimulation in progesterone-supplemented oocyte donors

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Study question: To identify demographics, treatment cycle characteristics and endocrine profile predictors of the frequency of uterine contractions in the early luteal phase in subjects undergoing controlled ovarian stimulation and supplemented with progesterone.
Summary answer: Demographic variables or treatment cycle characteristics do not predict the frequency of uterine contractions in the luteal phase of controlled ovarian stimulation cycles. The endocrine profile associated with the type of protocol may influence the frequency of uterine contractions in the luteal phase of controlled ovarian stimulation cycles supplemented with vaginal progesterone.
What is known already: Improving implantation of the transferred embryos/blastocysts is one of the major challenges in ART. It has been hypothesised that elevated uterine contractility at the time of transfer in women undergoing IVF/ICSI procedures could have a detrimental effect on implantation rates, and several

compounds with anticipated impact on uterine contractility are currently in clinical development. However, the pathophysiology of the elevated uterine contractility during the luteal phase of controlled ovarian stimulation is not well understood.
Study design, size, duration: Retrospective investigation of baseline data from a randomised, double-blind, placebo-controlled trial in 105 oocyte donors who underwent controlled ovarian stimulation in the long GnRH agonist or antagonist protocol, triggering of final follicular maturation with hCG, and were supplemented with vaginal progesterone 600 mg/day from day 1 to day 5 after oocyte retrieval (OR) (OR + 1 to OR + 5).
Participants/materials, setting, methods: Demographic variables, type of protocol and total gonadotropin dose were recorded. Serum values for estradiol, LH and progesterone were obtained 2 and 5 days after OR (OR + 2 and OR + 5, respectively). Transvaginal ultrasound recordings (Voluson i, GE Healthcare) of a continuous cine-loop image of at least 5 min were done on days OR + 1, OR + 2 and OR + 5 in a standardised format. The frequency of uterine contractions was determined by a central independent assessor blinded to treatment allocation, using computer-assisted time series motion analysis software.
Main results and the role of chance: A significant decrease in the median frequency of uterine contractions was observed in the early luteal phase: from 3.0/min on OR + 1 to 2.7/min on OR + 2 and to 2.0/min on OR + 5 ($p < 0.001$). Correlations between the frequency of uterine contractions and age, body mass index, body weight and total gonadotropin dose were low and none were significant. The median frequency of uterine contractions was not significantly different between the two protocols. However, in subjects following the GnRH antagonist protocol, a significant negative correlation was observed between LH levels and the frequency of uterine contractions on OR + 2 ($r = -0.46$, $p = 0.009$), and a significant positive correlation between estradiol levels and the frequency of uterine contractions on OR + 5 ($r = 0.36$, $p = 0.049$). No correlations were observed for the long GnRH agonist protocol.
Limitations, reason for caution: Multicentre trial with multiple ultrasonographers.
Wider implications of the findings: Further research is needed to evaluate the pathophysiology of the elevated uterine contractility during the luteal phase of controlled ovarian stimulation cycles.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Ferring Pharmaceuticals.
Trial registration number: NCT00587327.
Keywords: uterine contractility, implantation, embryo transfer

P-313 Endometrial growth inhibition by natural compounds evaluated as new therapeutic agents for endometriosis

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Study question: The objective of our study was to evaluate the effect on the development of experimental endometriosis both *in-vitro* and *in-vivo* of Wogonin (WG), an active constituent of Chinese Herbal Medicine, and two of the main antioxidant compounds found in rosemary leaves: Carnosic Acid (CA) and Rosmarinic Acid (RA).
Summary answer: All natural compounds evaluated exerted an inhibitory effect on endometrial cultured cells growth or endometriosis development. CA, RA and WG significantly inhibited cell proliferation of the human endometrial stromal cell line, T-HESC, and of primary cell cultures. In a murine model, these compounds diminished significantly the size of endometriotic-like lesions.
What is known already: Endometriosis is a chronic disease, with high levels of recurrence after standard medical therapy. The past few years have seen a renewed interest in the use of natural compounds due to their advantages: long term administration with minimal side effects. WG is known to inhibit proliferation and induce apoptosis in many kinds of cancerous cells. Similarly, antioxidant and antitumor activities have been reported after treatment with CA and RA.
Study design, size, duration: Primary cultures of stromal cells from endometrial biopsies of patients with endometriosis and controls and the T-HESC cell line were incubated with WG, CA or RA for 24-h. Endometriosis was surgically induced in BALB/c mice. Animals received WG, CA or RA daily from post-surgical day 14 until day 28.
Participants/materials, setting, methods: Cell proliferation was evaluated by MTS assay in primary and T-HESC cell cultures. Mice were randomly assigned to different treatment groups: RA-1mg/kg ($n = 11$); RA-3mg/kg ($n = 10$); CA-2mg/kg ($n = 11$); CA-20mg/kg ($n = 10$); WG-20mg/kg ($n = 12$) and Control ($n = 8$). Animals received CA or RA daily by intraperitoneal injection or WG daily by gastric gavage.
Main results and the role of chance: WG 40, 80 and 160 μM; CA 10, 12.5 and 25 μg/ml and RA 25, 50 and 100 μg/ml significantly inhibited cell proliferation in T-HESC cell line ($p < 0.01$, $p < 0.05$ and $p < 0.001$ vs. basal, respectively). All these compounds also inhibited cell proliferation of human endometrial stromal primary cultures ($p < 0.05$, $p < 0.01$ and $p < 0.05$ vs. basal for WG, CA and RA respectively). Wogonin induced an increase in the G2/M phase of the cell cycle in T-HESC, indicating an arrest at this stage. Furthermore, WG, CA and RA reduced the mean volume of established lesions in surgically induced endometriosis in mice ($p < 0.05$ vs. Control). The number of established lesions did not differ from those of the Control group. No evidence of toxicity was observed in mice.
Limitations, reason for caution: More studies should be addressed to fully understand the mechanistic behind the results obtained and to evaluate the safety of this type of compounds for endometriosis patients.
Wider implications of the findings: Nowadays, natural compounds are being considered for the treatment of diverse diseases. Amongst these is cancer which shares important similarities with endometriosis pathogenesis. All natural compounds evaluated in this study, WG, RA and CA, exerted an inhibitory effect on *in vitro* stromal endometrial growth and *in vivo* endometriosis-like lesions development. Our studies support the further investigation of novel, potentially safe and well-tolerated botanical products as future endometriosis treatments.
Study funding/competing interest(s): Funding by national/international organization(s) – ANPCyT, CONICET and Fundación Roemmers, Buenos Aires, Argentina.
Trial registration number: NA.
Keywords: endometriosis, natural compounds

P-314 Imbalance Progesterone Receptor-A/-B ratio via +331G/A polymorphism enhance MMP-2, -9 expression in endometriosis

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Study question: Is there any significant association between the level of *MMP-2* and *MMP-9* gene expressions and altered ratio of *Progesterone Receptor-A/-B* (*PR-A/PR-B*) via +331G/A polymorphism in endometriosis?
Summary answer: Imbalance ratio of *PR-A/PR-B* via +331G/A polymorphism in endometriotic tissue may be able to consequence Matrix MetalloProteinases 2 and 9 (*MMP-2, MMP-9)* overexpression, which can be important in etiology and pathogenesis of endometriosis.
What is known already: MMPs degrade extracellular matrix components to provide normal remodeling and contribute to pathological tissue destruction in endometriosis. It is accepted that MMPs are resistant to suppression by progesterone in endometriosis. The capacity of progesterone affect to gene expression is dependent on PR-A/PR-B. Imbalanced ratio of PR isoforms may cause progesterone resistance in endometriosis. Many studies have investigated the role of +331G/A in the etiology of various cancers, which can elevate the expression of PR-B isoform.
Study design, size, duration: Blood samples were recruited from 98 women undergoing laparoscopy for endometriosis and 102 healthy fertile women at Royan Institute, Iran in 2013-2014. Ectopic and eutopic tissue samples were prepared from twenty endometriosis (stages III and IV) and endometrial tissue from 20 non-endometriosis women at Royan Institute.
Participants/materials, setting, methods: After DNA extraction from blood samples, allele and genotype frequencies were determined by PCR-RFLP. Then, RNA was extracted from tissue samples to analysis of *PR-A, PR-B*, *MMP-2* and *MMP-9* mRNA expression by Real-time PCR.

Main results and the role of chance: We were able to demonstrate significantly low expression level of *PR-B* isoform in ectopic tissues ($p = 0.002$). Although, *PR-A* expression was higher in the same ectopic samples compared to control group ($p > 0.05$). This method permitted us to demonstrate significant overexpression of *MMP-2* and *MMP-9* in ectopic samples compared to control endometrial tissues, as well ($p > 0.05$ and $p = 0.014$, respectively). Our data showed the frequency distributions 98.04%, 1.96% for GG, G/A genotypes in +331G/A polymorphism and 97.96%, 2.04% in patients and control groups respectively ($p = 0.968$). Although our data didn't show any significant association with +331G/A in our groups, however, we were able to demonstrate higher expression level of *PR-B* in patients with G/A compared to patients with GG genotypes.
Limitations, reason for caution: The frequency of A allele in +331G/A polymorphism is very low between different population. Therefore, we need higher sample size to study the association of this polymorphism with levels of PR-B isoform expression in Iranian women with endometriosis.
Wider implications of the findings: Many studies have been shown that the expression level of *PR-B* reduced during endometriosis, which can affect the function of progesterone. Patients with G/A had high expression level of *PR-B* compared to GG genotypes. Therefore, +331G/A have been found to lead increased transcriptional activity of *PR-B* by favoring G/A or AA. Our findings support this observation; *PR-A/PR-B* ratio was altered in ectopic tissues, which may cause *MMP-2,-9* overexpression that can be important in pathogenesis of disease.
Study funding/competing interest(s): Funding by hospital/clinic(s). This work was supported by a grant from the Royan institute, Reproductive biomedicine group, Tehran, Iran [91000358].
Trial registration number: There is no trial work.
Keywords: endometriosis, MMPs, PR-A/PR-B ratio, +331G/A polymorphism

P-315 Endometrioma and Artificial Reproductive Techniques (ART) outcomes: Results from Meta-Analysis and Systematic review

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Study question: Does endometrioma influence ART outcomes and is surgical treatment to endometrioma prior to ART beneficial?
Summary answer: Women with endometrioma undergoing ART have similar reproductive outcomes compared to those without the disease, although the cycle cancellation rate is significantly higher. Given that the reduced ovarian reserve may be attributed to the presence of endometrioma per se, with additive detrimental impact from surgical intervention, individualisation of care in women with endometrioma prior to ART may help optimise their ART results.
What is known already: Endometriosis is a disease known to be detrimental to fertility. Women with endometriosis and the presence of endometrioma may require ART to achieve a pregnancy. Surgical treatment of endometriosis and endometrioma prior to ART is widely practiced even though very little evidence exists to provide robust guidance to clinicians. The specific impact of endometrioma alone and the impact of surgical intervention of endometriosis on the reproductive outcome of women undergoing ART are areas that require further clarification.
Study design, size, duration: We performed systematic review and meta-analysis. We searched studies published between 1980 and 2014 on endometrioma and ART outcomes. We performed electronic and manual search. Published cohort or case controlled studies (retrospective or prospective), and randomized controlled trials were eligible for inclusion.
Participants/materials, setting, methods: We included studies that had women who underwent ART with the presence of endometrioma in one of the comparison groups and at least a control group. Main outcome measures are Live birth rate (LBR), Clinical pregnancy rate (CPR), mean number of oocyte retrieved (MNOR) and adverse effects of treatment.
Main results and the role of chance: 33 studies were included for the meta-analysis. The majority of the included studies were retrospective studies (30/33) and 3 were RCTs. Compared to women with no endometrioma undergoing ART, women with endometrioma had a similar LBR (OR 0.98 95% CI [0.71, 1.36], 5 studies, 928 women, $I^2 = 0\%$), CPR (OR 1.17 95% CI [0.87, 1.58], 5 studies, 928 women, $I^2 = 0\%$), a lower MNOR (SMD -0.23 95% CI [-0.37, -0.10] 5 studies, 941 cycles, $I^2 = 37\%$) and a higher cycle cancellation rate compared to those without the disease (OR 2.83 95% CI [1.32, 6.06], 3 studies, 491 women, $I^2 = 0\%$). Compared to women with no surgical treatment, women who had their endometrioma surgically treated before ART had similar LBR, CPR and MNOR.
Limitations, reason for caution: Our study may be confounded by the high clinical heterogeneity of the included studies. The majority, with the exception of 3 studies, were all non-randomised controlled trials. We have however assessed and included only studies with either high or moderate quality according to Ottawa Scoring Systems.
Wider implications of the findings: Given that the reduced ovarian reserve may be attributed to the presence of endometrioma per se, with additive detrimental impact from surgical intervention, individualisation of care in women with endometrioma prior to ART may help optimise their ART results.
Study funding/competing interest(s): Funding by hospital/clinic(s) – funding by national/international organization(s) – Ministry of Education, Malaysia; Complete Fertility Centre Southampton.
Trial registration number: Nil.
Keywords: endometrioma, artificial reproductive technology, surgery, pregnancy, outcomes

P-316 A New Innovative Method to Increase Live Birth Rate in Case of Previous Unexplained Repeated Embryo Implantation Failure after intra-conjugal IVF/ICSI or oocyte donation

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Study question: Is a preconceptional endometrial immune evaluation able to identify the uterine immune mechanisms generating repeated embryo implantation failures? Can we, based on the documented immune endometrial profiles, correct the deleterious mechanism to promote subsequent effective embryo implantation ?
Summary answer: From a cohort fertile control, we defined an immune endometrial 'equilibrated profile' and documented deregulations in patients with repeated embryo implantation failures (RIF) after IVF/ICSI or oocyte donation (IVF-OD). We personalized accordingly to the identified mechanism their treatments before the following IVF/ICSI or IVF-OD and assessed the clinical pregnancy rate at the subsequent embryo transfer.
What is known already: The embryo implantation remains the main limiting factor during in vitro fertilization (20% of success). Uterine remodeling events are required before implantation for a successful pregnancy. During the implantation window, uterine NK cells (uNK) and Th1/Th2 cytokines balance are crucial for implantation. This unique immune reaction is essential to promote embryo adhesion and regulate the invasion phase. Disequilibrium of such a vital reaction may impede implantation.
Study design, size, duration: The study design was a prospective cohort study including 411 RIF patients (311 after IVF/ICSI and 100 after IVF-OD). Patient were included between 2012 and 2014 with a prospective follow-up during 1 year.
Participants/materials, setting, methods: An endometrial biopsy was performed classically with a cormier pipelle in the luteal phase. After confirmation by a datation of the luteal phase, we quantified uNK by immunohistochemistry using anti-CD56 $^{+}$IgG and mRNA expression 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. Based on these biomarkers, we identify if a deregulation (low or over-immune activation) was present and personalized further treatments either to promote or control the immune activation before the next subsequent embryo transfer.
Main results and the role of chance: Endometrial immune profiles appeared to be deregulated in 85% of the RIF IVF/ICSI patients and in 60% of the RIF IVF-OD patients. In RIF-IVF/ICSI patients, an over activation was diagnosed in 60% whereas a low activation was diagnosed in 25%. In RIF IVF-OD patients, an over activation was diagnosed in 51% whereas a low activation was

diagnosed in 9%. Accordingly, we advised personalized cares and assessed their effects by the ongoing pregnancy rate (PR) and live birth rate (LBR) occurring after the first following embryo transfer. We report a LBR of 40% in deregulated-treated IVF/ICSI patients while LBR stagnate at 15% if no deregulation was diagnosed. The PR in RIF-IVF/OD patients was 50% with no difference between deregulated or no deregulated patients (ongoing results).
Limitations, reason for caution: RIF and early miscarriages have been suggested to be inducible by stress, so we cannot exclude that the 'new scientific evaluation' proposed had a positive influence for some of these patients. Scientifically, only a randomized controlled trial may prove that a preventive uterine immune profiling may increase subsequent LBR.
Wider implications of the findings: Approximately 80% of the Human embryo implantations fail to implant. Multiple factors may contribute to this failure, but the majority of the implantation failure cases are attributed to a poor uterine receptivity conjugated with a poor oocyte quality. Before conception, endometrial immune profiling appears as a real and affordable solution able to explain a RIF history but also to correct the deleterious mechanisms and hence may increase significantly the chance of a subsequent live birth.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – MatriceLab Innove.
Trial registration number: NA.
Keywords: embryos implantation failures, uterine receptivity, endometrium, immune profiling, birth rates

P-317 Transvaginal Sonographic diagnosis and grading of endometriosis: A three year study

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Study question: Is transvaginal sonography (TVS) accurate for diagnosis and grading of endometriosis in comparison with laparoscopy?
Summary answer: Our study demonstrated that TVS has a reliable accuracy and sensitivity in diagnosis and grading of endometriosis.
What is known already: Sonographic depiction of Endometrioma.
Study design, size, duration: Prospective study on 101 infertile women during 3 years.
Participants/materials, setting, methods: This was a prospective study at Royan Institute, Iran, from April 2010 till March 2013. All infertile women who showed at least one of the endometriosis symptoms or detected signs via clinical examination (TV) were recruited in the study. Patients underwent preoperative TVS by a single radiologist and all sonograms and videos were captured in a DVD for each sample. Patient's documents, TVS findings and laparoscopy results (which were video captured) were assessed to collect data. Grading of endometriosis based on TVS findings was as follow: mild (superficial: echogenic calcified points on serosal surface of ovaries), moderate (endometrioma: low level hemogenic cystic lesion) and sever (DIE: by detection of fixation and deviation of ovaries and distance between them). Agreement between sonography findings & laparoscopy results, sensitivity, specificity, positive & negative predictive values, and accuracy of TVS in diagnosis of endometriosis, regarding laparoscopy as gold standard, were calculated by SPSS16 software using McNemar's & Kappa tests, logistic regression model and other statistic tests.
Main results and the role of chance: Totally, 101women were recruited in the investigation. Patients aged 30.78 ± 4.79 years in average and the duration of infertility was 4.2 ± 2.91 years among them. Endometriosis was detected in 78 cases by laparoscopy. Overall accuracy, sensitivity, specificity, PPV and NPV of the TVS in diagnosis of endometriosis were calculated 72.3%, 67.2%, 79.1%, 81.3 and 64.2% respectively. Sensitivity and PPV of TVS in grading of endometriosis were as follow: 43.2% and 57.6% in mild, 77.4% and 82.8% in moderate, and 85.8% and 94.7% in severe endometriosis.
Limitations, reason for caution: none.
Wider implications of the findings: Our study demonstrated that TVS has a reliable accuracy and sensitivity in diagnosis and grading of endometriosis. Furthermore, "ovarian position" and "fixation of ovaries", particularly when they are detected both together, strongly suggest severe endometriosis (odds ratio of 14.78 and 46.89 respectively). Therefore, they can be used as important criteria for radiologists & gynecologists.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Royan Institute for reproductive biomedicine research center.
Trial registration number: NA.
Keywords: transvaginal sonography (TVS), laparoscopy, endometriosis, infertility

P-318 Extinction of S100A protein affects apoptosis of endometrial epithelial cells, a mechanism probably involved in patients with multiple implantation failures

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Study question: We investigated function(s) of one endometrial receptivity biomarker, a S100A family member expressed in epithelial cells, using shRNAs. We analyzed the S100A protein extinction impact in relation to apoptosis. We also assessed mRNA expression level of S100A during the implantation window of patients with multiple implantation failures (MIF) after IVF.
Summary answer: Serum withdrawal in S100A protein knockdown-primary endometrial epithelial cells induced apoptosis compared with scrambled shRNA cells. This candidate was down-regulated during the implantation window in patients with MIF.
What is known already: Many biomarkers of human endometrial receptivity have been previously reported. However, few studies i) investigated the relevance of their biomarkers in patients with MIF, and ii) performed functional analyses to identify their role(s) and function(s) during the implantation window of fertile patients.
Study design, size, duration: The epithelial endometrial cells were isolated. An approach by shRNAs was used for stable gene silencing of the candidate. Serum withdrawal-induced apoptosis was investigated in shRNA S100A epithelial cells compared with scrambled shRNA cells. Endometrial biopsies from 27 patients with MIF were used for mRNA S100A expression quantification.
Participants/materials, setting, methods: ShRNA S100A and scrambled shRNA epithelial cells were culture in Dulbecco's modified Eagle medium supplemented with 1 or 10% heat-inactivated fetal bovine serum. After three days, apoptotic cells were detected by both immunofluorescence staining of activated caspase 3 and DAPI nuclear staining. S100A was quantified by qRT-PCR in endometrial biopsies.
Main results and the role of chance: In epithelial cells, serum withdrawal induced apoptosis only in shRNA S100A epithelial cells compared with scrambled shRNA cells. 50% of cells were positive for activated caspase 3 and fragmented nuclei according to the DAPI staining in shRNA S100A epithelial cells compared with scrambled shRNA cells. In addition, patients with MIF were characterized by a down-regulation of S100A mRNA expression level compared with fertile patients (p-value = 0.0012). As apoptosis plays a central role in the endometrium during the period of establishment of the implantation window, a deregulation of this signaling pathway due to the down-expression of the S100A mRNA can explain implantation failures in MIF.
Limitations, reason for caution: The mechanism by which the under-expression of the S100A mRNA affects apoptosis, and consequently implantation, must be investigated.
Wider implications of the findings: This study should open new perspectives in the understanding of mechanisms regulating human endometrial receptivity of infertile patients.
Study funding/competing interest(s): Funding by commercial/corporate company(ies). This work was partially supported by a grant from the Ferring Pharmaceutical Company.
Trial registration number: NA.
Keywords: endometrial receptivity, biomarker, MIF, primary endometrial cells, shRNAs

P-319 Hypoxia involves in endometriosis by regulating microRNAs expression

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Study question: Does hypoxia regulated microRNAs (miRs) involve in endometriosis development?
Summary answer: Hypoxia treatment in endometrial stromal cells dramatically changed several miRs expression, including miR-29c-5p, miR-200b, miR-199a and miR-126.
What is known already: Dysregulated miRs expressions in endometriosis tissues were well documented. And hypoxia was recently reported involved in the pathogenesis of endometriosis. But whether hypoxia regulated miRs participate endometriosis development is widely unknown.
Study design, size, duration: Endometrial stromal cells were isolated from normal endometrial tissues. Desferroxamine (DFO), CoCl2, 1% O2 were used to mimic hypoxia cell culture condition.
Participants/materials, setting, methods: Hypoxia induce factor 1a (HIF-1α) and VEGF expression were tested by immunofluorescence (IF) and western blot (WB) respectively to verify hypoxia effect. Quantitative real-time PCR (qRT-PCR) was applied to determine the expression changes of miRs in endometrial stromal cells with or without hypoxia treatment.
Main results and the role of chance: HIF-1α protein was accumulated in nucleus and VEGF protein expression was increased in endometrial stromal cells treated by hypoxia (1% O2, DFO and CoCl2) for 4, 8, 12 and 24 h, compared to the untreated cells. To determine whether miRs expression may be changed by hypoxia in endometriosis, miRs reported to be aberrantly expressed in endometriosis tissues were determined by qRT-PCR. Our results demonstrated that miR-29c-5p, miR-200b, miR-199a and miR-126 were all significantly upregulated in hypoxia-treated endometriosis stromal cells compared to those untreated cells.
Limitations, reason for caution: Only 4 miRs were tested in this study. MiRs expression profile should be determined by miR-array or miR-sequencing in endometrial stromal cells treated with or without hypoxia. Moreover, hypoxia regulated miRs' function should be further validated in vitro and in vivo.
Wider implications of the findings: The present findings demonstrated that hypoxia regulated several miRs expression in endometrial stromal cells, suggesting that those miRs may be involved in the pathogenesis of endometriosis.
Study funding/competing interest(s): Funding by national/international organization(s) – National Science Foundation of China (No.81402131 and No.81270657). Zhejiang Provincial Natural Science Foudation of China (No. LY14H040006).
Trial registration number: NA.
Keywords: endometriosis, hypoxia, HIF-1α, microRNAs

P-320 Tracking the implantation window: synchronizing endometrial preparedness for implantation with stage of blastocyst to be transferred in antagonist IVF cycles involving single blastocyst transfers

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Study question: Is it absolutely irrelevant to take into account the day of menstrual period in IVF cycles? We sought to track the relevance of 'implantation window' by contemplating a correlation between stage of blastocyst transferred and endometrial preparedness for implantation with respect to day of menstrual period in antagonist IVF cycles.
Summary answer: Synchrony between stage of blastocyst transferred and endometrial preparedness for implantation with respect to day of menstrual cycle has a definitive influence on clinical pregnancy rates (CPR) in IVF cycles. Asynchrony and out of phase blastocyst transfer may lead to missing out the implantation window and unnecessarily hamper CPR.
What is known already: Debating the existence of the mythical implantation window (~day 18-22 of natural cycle) or considering day of menstrual period may not hold much significance in IVF cycles involving day 3 embryo transfers. However, with an excellent blastocyst gradation system available and a rising trend towards day 5/6 blastocyst transfers, it seems pertinent to investigate whether stage of blastocyst transferred has any influence on clinical pregnancy rates if this transfer is synchronized with day of menstrual period.
Study design, size, duration: Retrospective analysis of 443 cycles (February 2012 to September 2014) in women undergoing antagonist treatment protocol followed by oocyte retrieval approximately on day 14 ± 2 of their menstrual period. All cycles involved day 5/6 single blastocyst transfer (sBT) of top (AA) or good (AB/BA) quality blastocysts (BC) of various stages.
Participants/materials, setting, methods: Slightly modified Gardiner's system for Blastocyst stage grading was followed. Stage 1: early BC; 2: Full Blastocyst; 3: Expanded BC; 4: BC with point of hatching; 5: Hatching BC; 6: Hatched BC. Inner cell mass, Trophectoderm were graded A, B, C as per Gardiner's system. Clinical Pregnancy rate was main outcome measure.
Main results and the role of chance: Overall CPR = 26.64% (118/443). CPR was heavily influenced by transfer of various stages (1 to 6) of BCs on different days of menstrual period (days 18-23 ± 1, covering the implantation window) as follows:

Day 17: 1 = 0%; 2 = 20%; 3 = 22.22%; 4 = 6.66%; 5 = 0%, 6 = 0%
Day 18: 1 = 0%; 2 = 0%; 3 = 37.14%; 4 = 27.03%; 5 = 16.66%; 6 = 0%
Day 19: No stage 1 BT; 2 = 0%; 3 = 30.77%; 4 = 31.03%; 5 = 22.22%; 6 = 0%
Day 20: 1 = 0%; 2 = 0%; 3 = 30.44%; 4 = 30%; 5 = 35.71%; 6 = 42.86%
Day 21: 1 = 0%; 2 = 0%; 3 = 16.67%; 4 = 36.67%; 5 = 44.44%; 6 = 40%
Day 22: No stage 1 BT; 2 = 0%; 3 = 18.75%; 4 = 50%; 5 = 50%; 6 = 50%
Day 23: No stage 1 BT; 2 = 0%; 3 = 21.05%; 4 = 25%; 5 = 66.67%; 6 = 66.67%
Day 24: No stage 1, 2 BTs; 3 = 0%; 4 = 0%; No stage 5 BT; 6 = 100%

Evidently, as the day of menstrual period advances, higher stage blastocyst Transfer gives better pregnancy rates.
Limitations, reason for caution: This study took into account transfer of only top and good quality blastocysts in an effort to minimize the factor of embryo quality on CPR. Also, it would be better to segregate and compare cycles where all women have undergone oocyte retrieval on similar days of menstrual period.
Wider implications of the findings: This study provides a non-invasive insight into the so called 'mythical' implantation window and endometrial preparedness for implantation. Accordingly, if embryo development leads or lags w.r.t endometrial receptivity, blastocysts may be frozen and transferred in the next conducive coordinated natural cycle. The results may help establish a new paradigm for synchronized development of embryo and endometrium for better pregnancy rates in IVF cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Self funded: by our own IVF centre – Vaunshdhara Clinic and Assisted Conception Centre, Nagpur, India.
Trial registration number: NA.
Keywords: blastocyst, implantation window, antagonist cycles, clinical pregnancy, endometrial receptivity

P-321 Use of an endometriosis model in rats to reveal new non invasive biomarkers

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Study question: Biomarker research for early diagnostic of endometriosis is emerging but so far a non-invasive test is not yet available, therefore we decided to use an experimental endometriosis model in rats combined to metabolomics and miRNA analysis in body fluids to reveal new biomarkers.
Summary answer: Metabolomics profiles and miRNAs expression are differentially expressed in rats with endometriosis as compared to normal rats.
What is known already: Endometriosis is believed to be multifactorial and emerging data provide evidence that dysregulation of miRNA expression could be involved in the development of the pathology.

Study design, size, duration: Endometriosis was surgically-induced in twenty female Sprague–Dawley rats as described by Sharpe-Timms (*N Y Acad Sci,* 2002, *955*(1), 318-327).
Participants/materials, setting, methods: Endometriosis was surgically induced in 20 female Sprague–Dawley (Endo) rats by implantation of six autologuous pieces (5 mm^2 byopsy punches) from the distal two thirds of the left uterine horn to the arterial blood supply of the small intestine. Twenty female Sprague–Dawley rats underwent a control surgery with removal of the uterine horn and sutures, without tissues, were placed around arteries in the arterial cascade of the intestinal mesentery (Sham). During the next four weeks blood samples were collected weekly and submitted to nuclear magnetic resonance analysis (NMR). After post-treatment of NMR data, orthogonal partial least squares discriminant analysis (OPLS-DA) were performed. After 4 weeks all rats were sacrificed. Circulating miRNAs were firstly analyzed after four weeks.
Main results and the role of chance: In the course of the induction and development of the pathology, metabolics analysis displayed a significant discrimination between rats with endometriosis and Sham operated as early as after one week. This continues throughout the 3 other time points analyzed. Discriminant pics revealed that lipoprotein, LDL and VLDL, and lactate are increased in endo rats whereas glucose is decreased. Several miRNAs are also found differentially expressed between Endo and Sham rats (miR 22, miR145* and miR195).
Limitations, reason for caution: Experimental results in rat model with implantation of autologuous endometrial tissue need to be confirmed in human samples.
Wider implications of the findings: The present preliminary results will be useful to test those potential non invasive biomarkers in women.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – University of Liège and F.R.S.-FNRS.
Trial registration number: NA.
Keywords: biomarkers, diagnosis, miRNAs, metabolomics, rat model

P-322 miR-133b reverses hydrosalpinx-induced impairment of endometrial receptivity and embryo implantation through down-regulating serum- and glucocorticoid-inducible kinase 1(SGK1)

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Study question: To explore the mechanism of microRNA-133b (miR-133b) involved in regulation of endometrial receptivity and embryo implantation.
Summary answer: Our results suggest that miR-133b up-regulates HOXA10 expression through down-regulating SGK1 expression via directly targeting SGK1 3'UTR, and reverses hydrosalpinx-induced impairment of BeWo spheroid attachment in Ishikawa cells.
What is known already: Previous studies have demonstrated that hydrosalpinx interferes with endometrial receptivity and embryo implantation by decreasing the expression of HOXA10, a transcription factor which has been demonstrated to be essential for endometrial receptivity and embryo implantation. Our previous study has demonstrated that Ishikawa cells treated with hydrosalpinx alters the expression of miR-133b, a member of microRNAs (miRNAs) which play crucial roles in endometrial receptivity and embryo implantation.
Study design, size, duration: Mid-secretory endometrial tissue samples were collected from 11 subjects with hydrosalpinx and 11 disease-free women as control. Ishikawa cells treated with or without hydrosalpinx, then infected with Ad-LacZ, Ad-miR-133b and Ad-flag-SGK1 adenovirus or transfected with CTL inhibitors and miR-133b inhibitors at the indicated dose for 48 h.
Participants/materials, setting, methods: Luciferase reporter assay was performed to identify SGK1 as the target gene for miR-133b. The expression of SGK1, HOXA10 mRNA and miR-133b were determined by qRT-PCR. Western blot was used to measure SGK1 and HOXA10 levels. Moreover, attachment assay of BeWo spheroids to Ishikawa cells was performed to measure embryo implantation.
Main results and the role of chance: miR-133b and HOXA10 were down-regulated whereas SGK1 was up-regulated in a concentration-dependent manner in Ishikawa cells treated with hydrosalpinx at concentrations of 0, 25 and 50% for 48 h. As expected, the mid-secretory endometrium from subjects with hydrosalpinx had lower expression of miR-133b and HOXA10 and higher expression of SGK1. Moreover, Luciferase assay results showed that miR-133b directly targeted SGK1 3'UTR and down-regulated SGK1 protein expression, subsequently increased HOXA10 expression. Furthermore, BeWo spheroid adhesiveness was inhibited by SGK1 over-expression in Ishikawa cells (27.56 ± 4.94 vs $37.56 \pm 3.63\%$, $p < 0.05$, $n = 3$), whereas over-expression of miR-133b or HOXA10 could reverse the impairment of embryo adhesiveness mediated by hydrosalpinx (41.88 ± 3.93 vs $24.35\% \pm 2.72\%$, $p < 0.05$, $n = 3$ and $45.64 \pm 3.13\%$ vs $22.94 \pm 2.81\%$, $p < 0.05$, $n = 3$, respectively).
Limitations, reason for caution: Our study is performed mainly in vitro for now, the effect of miR-133b on endometrial receptivity and embryo implantation in vivo and the regulation between miR-133b and SGK1 in mouse model is to be further investigated.
Wider implications of the findings: Our present findings provide a novel mechanism that miR-133b improves endometrial receptivity marker genes expression and embryo implantation through reducing SGK1 expression, leading to reverse hydrosalpinx-induced impairment of endometrial receptivity and embryo implantation. These data suggest miR-133b might be a potential target for treatment of infertility subjects with hydrosalpinx.
Study funding/competing interest(s): Funding by national/international organization(s). This work was supported by The National Natural Science Foundation of China Grant 81170570 and 81370683.
Trial registration number: NA.
Keywords: miR-133b, SGK1, hydrosalpinx, endometrial receptivity, embryo implantation

P-323 Expression of endometrial vascular endothelial growth factor-A, -C and placental growth factor in women with elevated progesterone on the day of and after hCG administration

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Study question: Is the expression of endometrial angiogenesis related factors affected by progesterone level on the day of and the day after hCG administration in controlled ovarian stimulation cycles?
Summary answer: Endometrial expression levels of VEGF-A, VEGF-C and PLGF were significantly altered in the women with elevated progesterone level on the day of and the day after hCG administration.
What is known already: Elevated serum progesterone on the day of hCG administration in controlled ovarian stimulation is associated with reduced endometrial receptivity. Endometrial angiogenesis activity plays a critical role around the time of embryo implantation.
Study design, size, duration: We investigated VEGF-A, VEGF-C and PLGF in precisely timed endometrial biopsies (hCG + 7, where the day of hCG administration is hCG + 0) obtained from 20 women with elevated progesterone (P4 ≥ 1.7 ng/ml on hCG + 0 and ≥9.5 ng/ml on hCG + 1) and compared the results with 20 women with normal progesterone in cancelled fresh ET cycles.
Participants/materials, setting, methods: The protein expression levels of VEGF-A, VEGF-C and PLGF were examined by immunohistochemistry. A semi-quantitative analysis was performed by H-score analysis of staining intensity in the luminal epithelium, glandular epithelium and stroma, separately. We also correlated expression of VEGF-A, VEGF-C and PLGF with progesterone level on hCG + 0 and hCG + 1.
Main results and the role of chance: VEGF-A expression in glandular epithelium in women with elevated progesterone level was higher ($P = 0.001$) than in women with normal progesterone level. A significantly higher stromal expression of VEGF-A ($P = 0.017$), VEGF-C ($P = 0.023$) and PLGF ($P = 0.033$) was found in women with elevated progesterone level than in women with normal progesterone level. There was a significant correlation between the serum progesterone level on the day of hCG administration and endometrial glandular epithelial expression of VEGF-A ($r = 0.537$, $P = 0.010$) and PLGF ($r = 0.411$, $P = 0.044$). No correlation was seen between the serum progesterone level and VEGF-C expression in any compartment.
Limitations, reason for caution: Immunohistochemistry and H-score analysis for staining intensity are semi-quantitative methods to determine the amount of protein expression. The study could potentially be strengthened by the quantitative RT-PCR measurement.

Wider implications of the findings: The significantly altered expression of endometrial VEGF-A, VEGF-C and PLGF around the time of embryo implantation provides a molecular explanation for the observed reduction of endometrial receptivity in the women with high progesterone level around the time of hCG administration.
Study funding/competing interest(s): Funding by national/international organization(s) – CHEN X. is a recipient of Hong Kong PhD fellowship from Hong Kong Research Grants Council.
Trial registration number: NA.
Keywords: elevated progesterone level, endometrium, VEGF-A, VEGF-C, PLGF

P-324 Fertility in patients with untreated colorectal endometriosis

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Study question: Which is the pregnancy rate in patients with colorectal endometriosis who did not undergo surgery?
Summary answer: Patients with colorectal endometriosis have a spontaneous pregnancy rate of about 30% and a total pregnancy rate of about 50%.
What is known already: Reproductive outcomes after surgical treatment of colorectal endometriosis are well documented both in infertile women and in those with unknown fertility status. However, the pregnancy rate of patients with untreated bowel endometriosis is unknown.
Study design, size, duration: This prospective single-centre cohort study was performed between May 2009 and December 2014. It included 55 patients with colorectal endometriosis wishing to conceive.
Participants/materials, setting, methods: Colorectal endometriosis was diagnosed by magnetic resonance imaging enema. Criteria for exclusion from the study were: bowel stenosis > 60%, previous surgery for endometriosis or adnexal diseases, infertility, ureteral stricture, doubtful ovarian cysts, abnormal semen parameters of male partners. Data were presented according to intention to treat.
Main results and the role of chance: The median age of the patients was 33 years (range, 24-41 years). 78.2% of the patients were under hormonal therapy that was discontinued. Five patients interrupted the research of pregnancy because of change of partner, health problems or change of plans. 17 patients (30.9%) conceived spontaneously; the median time required to conceive was 9 months (range, 2-32 months). 12 patients (21.8%) conceived by intrauterine insemination or by in vitro fertilisation; the median time required to conceive was 21 months (range, 9-46 months). The total pregnancy rate in the study population was 52.7% (29/55; 95% C.I., 38.8-66.3%) after a median follow-up of 21.5 months (range, 2-54 months). After the first pregnancy, there were 3 conceptions among the 7 patients who tried to conceive again.
Limitations, reason for caution: The major limitation of the study is the small sample size. Patients wishing to conceive must interrupt hormonal therapies that are contraceptive and may have to tolerate severe pain and intestinal symptoms.
Wider implications of the findings: Patients with colorectal endometriosis should be informed on the possibility to conceive either spontaneously or by assisted reproductive technologies without undergoing surgical excision of endometriosis.
Study funding/competing interest(s): Funding by University(ies) – University of Genoa, Italy.
Trial registration number: NA.
Keywords: fertility, endometriosis, bowel endometriosis

P-325 A comparison of pelvic magnetic resonance imaging, trans-vaginal and trans-rectal sonography with laparoscopic findings in diagnosis of deep infiltrating endometriosis

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Study question: To compare the sensitivity, specificity and accuracy of pelvic magnetic resonance imaging (MRI), trans-vaginal and trans-rectal sonography (TVS and TRS) with laparoscopic findings in diagnosis of deep infiltrating endometriosis (DIE).
Summary answer: The advantages of TRS, which makes it a reasonable substitute for TVS as a first-line imaging modality, include its supremacy in diagnosis of DIE lesions based on its more favorable sensitivity, negative predictive values, accuracy and likelihood ratios. TVS was found to be superior to pelvic MRI for sensitivity, while pelvic MRI turned to be the most specific technique in diagnosis of pelvic DIE.
What is known already: As far as we know, previous similar investigations have not sufficiently compared the performance and diagnostic value of TVS, TRS and MRI in endometriosis in a large population scale. Comprehensive studies which assess the diagnostic significance of such modalities and classify the results for each anatomical location of DIE, would possibly encourage gynecologists to consider revision in previous results.
Study design, size, duration: A cross-sectional prospective study conducted from March 2010 to late December 2014.
Participants/materials, setting, methods: A total of 317 patients with endometriosis undergoing operative laparoscopy were enrolled. Pelvic MRI, trans-vaginal and trans-rectal sonography were performed prior to operation. Sensitivity, specificity and accuracy of pelvic MRI, TRS and TVS were measured with regard to the diagnosis of DIE lesions.
Main results and the role of chance: Regardless of anatomical location, TRS possessed a better sensitivity for diagnosis of DIE lesions as compared to TVS and MRI (81.12 vs. 80.14 and 77.87, respectively). Meanwhile, the specificity was higher for MRI as compared to TVS and TRS (97.14 vs. 96.65 and 95.77, respectively). The accuracy was marginally higher in TRS as compared to TVS and MRI (93.28 vs. 93.14 and 92.79, respectively).
Limitations, reason for caution: Although over 500 cases underwent laparascopic surgery for endometrosis in the setting of this study, only non-virgin subjects could be enrolled since TVS evaluation was not possible in virgins.
Wider implications of the findings: Providing evidence to suggest the best imaging modality (TVS, TRS or MRI) in the diagnosis of deep infiltrating endometriosis.
Study funding/competing interest(s): Funding by University(ies) – Laparascopy Research Center, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
Trial registration number: CT-P-92-4095-SUMS.
Keywords: deep infiltrating endometriosis, MRI, transrectal sonography, trans-vaginal sonography

P-326 Aquaporin-2 Expression as predictor of endometrial receptivity in ICSI cycles

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Study question: Can endometrial expression of Aquaporin-2 (AQP-2) be used as predictor of endometrial receptivity in patients undergoing ICSI cycles?
Summary answer: This study have demonstrated that endometrial AQP-2 expression during the window of implantation (WOI) is strongly correlated to endometrial receptivity.

Using a semi-quantitative scoring system, an AQP-2 expression score of 2 was proved to have a validated predictive value for implantation, as well as biochemical and clinical pregnancy.
What is known already: Aquaporin-2, a small trans-membrane water channel, is expressed in the epithelium of human endometrium in a cycle-dependent manner, peaking in the mid-secretory phase, coinciding with the 'window of implantation'. The few available studies, mainly on animals, have indicated that this redistribution of AQP-2 might influence the endometrial receptivity.
Study design, size, duration: A pilot study investigating the expression and predictive value of endometrial AQP-2 expression. Fifty patients were recruited over one year, at a tertiary hospital. No reference-test for endometrial receptivity is available for comparison.

The assessors of AQP-2 expression and the data analysts were blinded to the patients' outcomes.

Participants/materials, setting, methods: Fifty couples with unexplained or male infertility planned for ICSI were included. Those with endometrial pathology were excluded.

Endometrial biopsies, by suction catheters, were obtained on day (LH + 7) prior to ICSI. Immuno-histochemical staining for AQP-2, using semi-quantitative scoring (0 to 4) was used. Patients were followed up for pregnancy.

Main results and the role of chance: Our results showed that endometrial AQP-2 expression was positively correlated to both the biochemical and clinical pregnancy rates. With positive AQP-2 expression, the OR for biochemical pregnancy was 7.03 (95% CI 1.91-25.91), and for clinical pregnancy 10.73 (95% CI 2.23-51.58).

The best predictive parameters were achieved with the AQP-2 expression cut-off score of 2. The sensitivity for predicting biochemical and clinical pregnancy rates were 58.06% (95% CI 39.1-75.5) and 92.86% (95% CI 66.1–99.8), the specificity 84.21% (95% CI 60.4–96.6) and 77.78% (95% CI 60.8–89.9), the positive predictive value 85.7% (95% CI 63.7–97.0) and 61.9% (95% CI 29.0–96.3), and the negative predictive value 55.2% (95% CI 35.3–73.9) and 96.6% (95% CI 81.8–99.9), respectively.

Limitations, reason for caution: This study was a pilot study. No previous studies on humans were available for sample size calculation or for comparing the results. Also, the endometrial expression of AQP-2 might be altered by the hormonal treatments of the subsequent ICSI cycles.

Wider implications of the findings: We suggest that testing the implantation window endometrial AQP-2 expression can help predict the success of subsequent ICSI cycles, and hence, directs clinical decision into either performing fresh embryo transfers, or, alternatively, vitrification of the embryos, and subsequent transfers when endometrial receptivity becomes more favourable.

We also hope that with deeper understanding of molecular biology and genetics of the implanting endometrium, medications could be developed that enable enhancing its receptivity through upregulating AQP-2 expression.

Study funding/competing interest(s): Funding by University(ies) – Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Trial registration number: NA.

Keywords: aquaporin-2, implantation, endometrial receptivity, prediction of IVF success

P-327 Jagged-1 protein related signaling pathway effects on embryo implantation

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Study question: To investigate the relationship between the Jagged-1 protein and the embryo implantation rate, mouse embryos were cultured and implantation competency was quantified.

Summary answer: Generally, an in vitro model should show a time delay in implantation compared with the timing of embryo implantation in vivo. The addition of rJagged-1 seemed to partially decrease the time gap between the *in vitro* and the *in vivo* model.

What is known already: Jagged-1 protein triggered Notch 1 signaling pathway has been studied for decades and its important role on the reproductive tract had been proved.

Study design, size, duration: For the collection of embryos we raised female ICR mice aged 4 to 5 weeks and male ICR mice aged 3 to 12 months for use in these experiments. Ovulation are induced in female mice by an intraperitoneal injection of pregnant mare serum gonadotropin (PMSG) followed by human chorionic gonadotropin (hCG) 48 h later. The following morning, the females exhibiting vaginal copulatory plugs are separated for the proposed experiments. The day of appearance of a vaginal plug are set as 0.5 dpc and 8-cell embryos were collected on 2.5.dpc The embryos are cultured in HTF-BSA (4mg/ml) or HTF-BSA with recombinant Jagged-1 protein. By matrigel assay we observed the growth competency and implantation ability.

Participants/materials, setting, methods: Western blotting analysis of N_1ICD of embryos cultured in HTF, HTF + rJagged-1(rJag-1). Fifty embryos per group were analyzed. The embryo spreading and invaded areas were observed and compared between different culture medium (HTF vs HTF + rJagged-1) on 5.5 dpc and 6.5 dpc. On 6.5 dpc, the invaded areas were stained with hematoxylin for further quantification. Quantification of the spreading and invaded areas of embryos cultured in different culture medium (HTF vs HTF + rJagged-1). Finally, the spreading and invaded areas were quantified using Motic Images Plus 2.0.

Main results and the role of chance: Generally, an in vitro model should show a time delay in implantation compared with the timing of embryo implantation in vivo. The addition of rJagged-1 seemed to partially decrease the time gap between the *in vitro* and the *in vivo* model.

Limitations, reason for caution: animal model may not reflect human embryo implantation completely.

Wider implications of the findings: This finding may be further applied on conception and even contraception.

Study funding/competing interest(s): Funding by national/international organization(s) – National Defense Medical Center Medical Research.

Trial registration number: NA.

Keywords: jagged-1 protein, embryo implantation

P-328 Measurement of oxidative stress in the follicular fluid of infertility patients with an endometrioma

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Study question: In order to measure the oxidative stress in the follicular fluid (FF) from a single follicle of patients with unilateral endometrioma (EM) in assisted reproductive technology (ART) treatment, we evaluated whether an EM might affect the environment of follicular growth.

Summary answer: The oxidative stress and antioxidant potential in the FF of the patients with unilateral EM showed values similar to those without an EM. Therefore, we concluded that EMs do not affect the environment for follicle growth during ART treatment.

What is known already: FF reflects its environment during oocyte growth, and, the evaluation of oxidative stress in the FF could be used to predict oocyte quality. Endometriosis is believed to affect the environment during follicular growth in ART, because endometriosis produces several cytokines that interfere with reproduction. EMs are located in the ovaries; hence, both follicular growth and oocytes could be directly affected by this condition. However, no report has evaluated the environment of a single follicle.

Study design, size, duration: Between December 2011 and July 2013, 26 patients with a unilateral EM (EM group) and 29 without EM (control group) were enrolled in this study. The FF was obtained during the first puncture of follicular aspiration, and was stored at -30? until it could be assayed.

Participants/materials, setting, methods: Oxidative stress was measured using a Free Radical Elective Evaluator (WISMERLL, USA). The d-ROM and BAP tests were used to measure oxidative stress (U.CARR) and anti-oxidant power (μmol/L), respectively.

Main results and the role of chance: The d-ROM values in the EM and control groups were 328.7 ± 97.8 and 414.9 ± 84.2, respectively, and the BAP values in the EMC and control groups were 2,474.3 ± 432.0 and 2,552.8 ± 435.58, respectively, and these two values were similar in both groups. In the EM group, there were no significant differences in the d-ROM and BAP values obtained from the right and left measurement of FF for any single patient. The number of patients whose modified BAP/d-ROM ratio < 1.0 in the EM and control groups were 16 and 15, and the percentage was similar (61.5 and 51.7%).

	EM group	Control group
d-ROM (U.CARR)[#]	328.7 ± 97.8*	414.9 ± 84.2
BAP (mmol/L)[#]	2,474.3 ± 432.0	2,552.8 ± 435.58

[#]mean ± SD.

Limitations, reason for caution: It is difficult to prevent the mixing of blood with the FF after a second puncture during follicle aspiration, and, therefore, we had only the first puncture on which to base our evaluation of the oxidative stress and the anti-oxidant power of the follicle.

Wider implications of the findings: There were no differences in the values (d-ROM and BAP) of the FF taken from the ovaries with or without an EM in the same women who had a unilateral intraovarian EM. The measurements of oxidative stress in the FF for a single follicle might reflect the complete amount

of oxidative stress in an ovary. Therefore, an EM might not affect the environment of follicle growth.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The authors have received no funding for this study, and they have no financial interest in any companies. There are no competing interests.
Trial registration number: This study had no RCT status, and, therefore, it received no trial registration number.
Keywords: oxidative stress, anti-oxidant power, follicular fluid, endometriosis, ART

P-329 JNK and p38 inhibition suppresses PGE2-stimulated aromatase and estrogen receptor levels in human endometriosis

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Study question: The aim of this study was to investigate the molecular and cellular mechanism by which PGE2 regulates aromatase and ER β ((ERβ) expression in endometriosis. A better understanding of the common mechanism responsible for regulation of estrogen biosynthesis may lead to the discovery of new therapeutic targets for endometriosis.
Summary answer: We indicates that PGE2 activates p38 and JNK signaling pathways, further stimulating c-Jun and ATF2 binding to aromatase and ERb promoter regions with elevated estradiol production. Inhibition of p38 and/or JNK could impede or halt the growth of endometriomas and limit estrogen biosynthesis.
What is known already: Endometriosis is an estrogen-dependent disease. Aromatase is the key enzyme for estrogen biosynthesis, and estrogen's action is mediated primarily by nuclear estrogen receptors (ERs). Elevated levels of aromatase mRNA have been found in ovarian endometriomas. A strong association exists between prostaglandin E2 (PGE2) levels and development/severity of endometriosis. Mitogen-activated protein kinase (MAPK) pathways are implicated in the regulation of cellular proliferation and inflammation in endometriosis.
Study design, size, duration: Aromatase and ERb mRNA were examined by real-time RT-PCR. Aromatase activity was measured by Radioimmunoassay (RIA).ERβ, p38, JNK, ERK1/2, ERK5, ATF2 and c-Jun protein expression were detected by western blot assay. Estrogen production was measured by ELISA kits. The association between ATF2 and c-Jun were assessed by coimmunoprecipitation assay.
Participants/materials, setting, methods: Chromatin Immunoprecipitation assay was performed to investigate the effect of PGE2 on the interaction of ATF2 and c-Jun with aromatase and ERb promoter region. The effects of inhibiting p38 or JNK in the presence of PGE2 on estradiol synthesis and xenograft growth were investigated in endometriosis xenografts.
Main results and the role of chance: Aromatase and ERb mRNA levels in ESCs were 2240 fold and 23 fold higher than in the same endometrial cells. PGE2 treatment resulted in a significantly increase in aromatase mRNA (5.9-fold) and activity (11.3-fold). PGE2 also significantly stimulated ERβ expressions and estradiol levels. Bt2cAMP treatment resulted increased aromatase and ERβ mRNA. The P38, JNK, ERK1/2, ERK5 pathways were rapidly phosphorylated following PGE2 stimulation. Addition of p38 or JNK inhibitor/siRNA markedly reduced PGE2 induced aromatase and ERβ expression. PGE2 induced ATF2 translocation and enhances its association with c-Jun. Further, PGE2 enhances binding of ATF2 and c-Jun protein to the aromatase and ERβ promoter region. P38 and JNK inhibition in vivo abrogates PGE2-induced aromatase and ERb expression, estradiol production ($p < 0.05$) and growth of endometriosis grafts.
Limitations, reason for caution: none.
Wider implications of the findings: Inhibition of p38 and/or JNK could impede or halt the growth of endometriomas and limit estrogen biosynthesis. Thus, p38, JNK and their downstream transcription factors, c-Jun and ATF2, may be potential new drug targets for tissue-specific ablation of aromatase and *ERB* expression in endometriosis. Further studies about p38 and JNK inhibitors in the treatment of human endometriosis are still needed.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by the National Natural Science Foundation of China (Grant 81270674) and the Natural Science Foundation of Beijing, China (Grant 7132204).
Trial registration number: NA.
Keywords: endometriosis, aromatase, estrogen receptor

P-330 Free circulating levels of the endogenous immunoregulatory cytokine granulocyte colony stimulating factor (GCSF) correlate with implantation and clinical pregnancy in IVF cycles

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Study question: We addressed the question whether serum levels of the unbound, free GCSF on day of blastocyst transfer (BT) and on day 7post BT correlate with implantation and clinical pregnancy in conventional IVF cycles.
Summary answer: Free G-CSF levels in serum, unbound to its receptor, on day of blastocyst transfer (BT) and day 7 post BT are reliable non-invasive prognostic factors for implantation and clinical pregnancy respectively in young women undergoing conventional IVF involving day 5 expanded blastocyst transfer.
What is known already: GCSF is endogenously produced in different tissues/cells including endothelium and decidual cells of endometrium. GCSF may be involved in immunoregulation by promoting T-cell tolerance towards the embryonicallograft. GCSF is produced by endometrial cells only when epithelial and stromal cellular elements are mutually interactive i.e. during the implantation window. It mediates embryo-endometrium dialogue and has direct trophic effect on the trophoblast cells by binding to its natural receptor, c-fms, expressed on the trophoblast.
Study design, size, duration: Prospective pilot study of fresh non-donor conventional IVF cycles ($n = 44$, mean age = 31.71 ± 3.96 years) carried out from June 2013 to December 2013. Standard controlled ovarian stimulation with r-FSH and antagonist protocol was followed. All cycles involved expanded blastocyst transfer (BT) (mean: 1.9 ± 0.4) on day 5 after oocyte retrieval.
Participants/materials, setting, methods: Serum levels of GCSF were measured in equal number of pregnant and non-pregnant cycles on day of blastocyst transfer (BT) and day 7 post BT. Serum β-hCG > 25mIU/ml on day 7 of BT indicated implantation whereas appearance of gestational sac in sixth week with cardiac activity established clinical pregnancy.
Main results and the role of chance: Serum GCSF levels differed significantly between pregnant ($n = 22$) and non-pregnant ($n = 22$) groups on day of BT (69.50 ± 6.7 vs. 127.8 ± 31.0 pg/ml, $p = 0.04$) and day 7 BT (46.92 ± 4.9 vs. 95.22 ± 14.00 pg/ml, $p < 0.0008$).A significant inverse correlation was observed between day of BT serum GCSF level and implantation (Pearson $r = -0.31$) and of day 7 BT serum GCSF with clinical pregnancy (Pearson $r = -0.5$). Interestingly, serum GCSF levels showed a significant decline from day of BT to day 7 of BT in the pregnant group (69.5 ± 6.7 vs. 46.92 ± 4.9, $p = 0.0093$) whereas the decline in levels was statistically non-significant in non-pregnant group (127.8 ± 31.0 vs. 95.22 ± 14.00 pg/ml, $p = 0.36$).
Limitations, reason for caution: This pilot study is limited by a small sample size. Moreover, since it is widely known that there is a redundancy in the roles of various cytokines; an intricate understanding of specific functions of the plethora of cytokines involved during the embryo-endometrial cross-talk is essential before carefully interpreting the results.
Wider implications of the findings: Endogenously produced GCSF facilitates implantation by binding to its receptor on trophoblast cells. Since it is impossible to analyze the activity of bound GCSF, it will be worthwhile to estimate circulating levels of the free unbound GCSF. Moreover, since this binding specifically occurs during the 'window' period, the estimation of free GCSF may give us more insight and understanding of the as yet enigmatic 'implantation window'.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The study was self funded i.e by our own IVF centre.
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Trial registration number: NA.
Keywords: implantation, cytokines, GCSF, IVF, clinical pregnancy

P-331 Ovarian tissue surrounding endometrioma function: vascular inmunohistochemichal analysis

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Study question: To assess ovarian tissue surrounding endometrioma function by evaluating vascularization, follicle count and proliferation index.
Summary answer: Endometrioma affects surrounding ovarian tissue function as primordial follicle count, vascularization and proliferation cell index are diminished compared with contralateral non-endometrioma ovaries. These changes are not associated with endometrioma size.
What is known already: There are historical controversies about the influence of endometrioma surgery on subsequent ovarian function, but little research about the influence of endometrioma on the ovarian tissue that surrounds it prior to the actions of surgery. The hypothesis that there may be an alteration of ovarian endometriotic cyst adjacent histology with compromised blood supply and irrigation of follicular apparatus arises.
Study design, size, duration: Prospective study. 32 patients less than 40 years with clinical ovarian unilateral > 3 cm endometrioma diagnosis were operated by laparoscopy. After ovarian stripping cystectomy, an ovarian surrounding endometrioma tissue sample was taken (study group); another ovarian tissue sample from contralateral ovary without endometrioma was taken as control group.
Participants/materials, setting, methods: Samples were processed for histological primordial follicle count, inmunohistochemical vascular assessment with monoclonal CD34 antibody specific for endothelial cells and proliferation cellular index with PCNA. Both groups main outcome measures were compared by x^2 and non-parametric tests. Statistical correlation between ovarian endometrioma size and inmunohistochemical parameters was also performed.
Main results and the role of chance: Patients average age was 30. 5 ± 4.1years. 7 endometriomas were from right ovary and 25 from left ovary. Endometrioma average size was 5 ± 1.5 cm (3.5 a 8 cm rank). There was significantly less primordial follicular count (2.18 ± 2.7 vs 6.21 ± 6.2, $p < 0.05$), less vascular inmunostained area (2.87 ± 1.65 vs 4.46 ± 2.4, $p < 0.05$) and less proliferation cell index (5.2 ± 1.8 vs 7.4 ± 4.2, $p < 0.05$) in ovarian parenchyma surrounding endometriomas compared with ovarian contralateral tissue without endometrioma. No correlation was found between endometrioma size and primordial follicular count, vascular inmunostained area, nor proliferation cell index of the endometrioma surrounding tissue [Pearson coefficient: -0.24 (IC 95%: -0.44 to 0.11, NS); -0.054 (IC 95%: -0.39 to 0.30, NS) and 0.129 (IC 95%: -0.45 to 0.22, NS) respectively].
Limitations, reason for caution: None.
Wider implications of the findings: These results reopen the debate about whether patients with endometrioma to be submitted to assisted reproduction should be previously operated with the risk of generating even greater damage over the ovarian reserve; or stimulate better without surgery. While there is wide clinical experience and literature on the subject, has not yet reached an uniform consensus.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: endometrioma, CD34, immunohistochemical, ovarian-function, ART

P-332 Myofibroblasts polarised arrangement and TGFß receptors spatial expression in the peritoneal endometriosis – reappraisal of the microenvironment of the superficial peritoneal endometriosis

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Study question: Do the myofibroblasts and TGFß receptors follow a spatial arrangement in the superficial peritoneal endometriosis (EM)? Do the peritoneal fluid (PF), oestrogen (E_2) and TGFß1 affect cellular proliferation of fibroblasts and epithelial cells? Does TGFß1 level differ in PF in EM patients than control?
Summary answer: The myofibroblasts and TGFß receptors are spatially arranged in the peritoneal endometriosis microenvironment. The centre exhibited more contractile phenotype of the myofibroblasts and higher TGFßR1 and 3. PF and E_2 favour the profibrotic evolution of the lesions while TGFß1 inhibits the epithelial cell proliferation. TGFß1 did not differ in both.
What is known already: The shedded endometrium is affected by the peritoneal fluid in endometriosis patients in its retro-grade journey to the abdominal cavity. It stimulates the stromal cell proliferation. The shedded endometrium implants itself on the peritoneum where it develops through an EMT process into EM. TGFß1 is usually involved and induces the transformation of fibroblast into myofibroblasts. Myofibroblasts have been seen before in different EM lesions.
Study design, size, duration: In a pro- and retrospective study eighty-six premenopausal patients (EM patients = 55 and non-EM patients = 31) were included. PF, superficial peritoneal EM lesions, healthy peritoneum and unaffected peritoneum from EM patients were collected during laparoscopy at Charite University of Medicine between 2012 and 2014.
Participants/materials, setting, methods: Immunohistochemistry staining for TGFß receptor 1, 2, 3, collagen I and calponin was done. The proliferation rate of L-929 and 12Z cell lines incubated with PF, E_2 and TGFß1 was studied. ELISA kit for measuring TGFß1 level in PF in EM and control patients was used.
Main results and the role of chance: The collagen I immunoexpression was higher in the peripheral peritoneum and tend to decrease towards the centre of the lesion. Contrarily, calponin was higher expressed on the endometriotic stromal cells and tend to decrease towards the peripheral peritoneum. Both were higher expressed in the peritoneum of EM than non-EM. The unaffected peritoneum from EM patients and the healthy peritoneum did not show any difference. TGFßR1 and 3 were higher in the centre than in the peripheral compartment. TGFßR2 was always higher expressed than the other two receptors. PF from EM patients as well as E_2 stimulated the proliferation rate of L-929 cell line, while TGFß1 inhibited that of 12Z cell line. TGFß1 level did not show any difference between EM and non-EM patients.
Limitations, reason for caution: It is a descriptive study and further in-vitro modulation of the different TGFß receptor subunits and its possible linkage to calponin and collagen I expression is recommended. Isolation of the cells from the different compartments of this polarised microenvironment could be of value for individual in-vitro studies.
Wider implications of the findings: Our data could support the presumption of the polarisation of the microenvironment of the peritoneal endometriosis. The higher TGFßR1 and 3 in the centre might be linked to the more calponin expression. This could open question for further targeting the receptor subunit to control the myofibroblastic activity and hence fibrosis and pain.
Study funding/competing interest(s): Funding by University(ies) – funding by commercial/corporate company(ies) – the first author was granted a promotion-scholarship from the Ernst Schering Foundation and Humboldt University in Berlin, Germany.
Trial registration number: NA.
Keywords: peritoneal endometriosis, TGFß1, collagen I, myofibroblasts, peritoneal fluid

P-333 Metabolome of recurrent implantation failure versus recurrent implantation success: exploring the feasibility of implantation marker(s) identification in serum

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Study question: Is there any difference in serum metabolic expression of women with repeated implantation success (RIS) compared with repeated implantation failure (RIF)? If any, whether these metabolites hold merit to be considered as pre-determined set of marker(s) and can help in predicting the chances of a successful pregnancy in women undergoing IVF?
Summary answer: Several metabolites were found to be significantly altered on comparing women with RIS and RIF. Among these metabolites, adipic acid, urea and D-glucose showed highest predictive values in discriminating the two groups.
What is known already: RIF, commonly encountered during IVF-ET, is defined as the failure to implant in at least three consecutive IVF attempts, despite transfer of good quality embryos at each cycle. In contrast, RIS refers to the group of women who conceive each time over multiple IVF-ET cycles, irrespective of whether average quality, fresh, or frozen embryos are transferred. Nuclear magnetic resonance based metabolomics involves reproducible quantification of intracellular metabolites and is helpful in identifying promising predictive biomarkers.
Study design, size, duration: Age, BMI and indication matched 24 women with RIS, who conceived each time during their multiple IVF-ET cycles and 28 women with RIF (≥ 3 IVF-ET attempts) were recruited from January 2004 to October 2014.

Participants/materials, setting, methods: Serum samples were collected during implantation window from RIS and RIF patients undergoing IVF-ET. Carr-Purcell-Meiboom-Gill spin-echo spectra were obtained and processed data was subjected to statistical analysis. Several multivariate and univariate statistical analyses were performed for group discrimination and marker(s) identification, respectively.
Main results and the role of chance: Principal component analysis, partial least squares discriminant analysis (PLS-DA) and orthogonal-PLS-DA show distinct classification between RIS and RIF based on the differently expressed metabolites. The metabolites, adipic acid, urea and D-glucose contributed most towards this differentiation and were significantly up-regulated in RIF as compared to RIS ($p < 0.05$). Oxidative stress is known to be associated with poor endometrial receptivity. Increased adipic acid levels, a byproduct of lipid peroxidation, is likely due to elevated ROS in RIF. Higher levels of urea in RIF indicate imbalance in nitrogen metabolism which may interfere with the normal inductive effects of progesterone on the uterine microenvironment. Up-regulated D-glucose in RIF is not surprising since it is well established that high glucose environment is not conducive to embryo implantation.
Limitations, reason for caution: This study needs to be extended to a larger sample size for data reproducibility and accurate predictability. Alterations in expression of these metabolites need further validation in a larger cohort of samples using biochemical tests.
Wider implications of the findings: Implantation marker(s) identification in serum of women during the IVF treatment cycle will be a major breakthrough in predicting the possibility of success in IVF cycles. Women unlikely to have successful implantation may be identified early and treatment protocol planned accordingly.
Study funding/competing interest(s): Funding by hospital/clinic(s) – the study was funded by our own private infertility centre: Institute of Reproductive Medicine. The authors have no conflict of interest.
Trial registration number: NA.
Keywords: metabolome, recurrent implantation failure, recurrent implantation success, implantation marker

P-334 Serum nitric oxide on day 7 post transfer may indicate a receptive endometrium and is a non-invasive marker in ongoing IVF cycles

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Study question: To determine if serum nitric oxide (NO) level on day of embryo transfer (ET) and day 7 post ET (d7ET) reflects optimum endometrial vascularization and whether it can be interpreted as a non-invasive marker; if so does it correlate with *in vitro* fertilization (IVF) outcome?
Summary answer: Serum NO level with a threshold value of > 4.2 nm/ml on d7ET correlates well with clinical pregnancy and may be considered as a non-invasive marker for embryo implantation in ongoing IVF cycles.
What is known already: It is known that controlled ovarian stimulation adversely affects receptivity in assisted reproductive techniques (ART) cycles. It causes suboptimal synchrony between endometrium and transferred embryos and therefore can be responsible for many IVF cycle failures. As many aspects of IVF-ET have already become optimized, other aspects, such as possible alterations in endometrial development and receptivity, implantation and/or early placentation have become the focus of modifiable factors that may further enhance success and safety.
Study design, size, duration: A prospective cohort study was conducted from October 2013 to November 2014, including 227 patients stratified on the basis of favorable/unfavorable issues of IVF (Fertility & Sterility 19: 2011). All patients underwent IVF using standard downregulation with GnRH agonist and gonadotropins. 8 cycles were cancelled due to inadequate endometrial thickness.
Participants/materials, setting, methods: Serum NO was measured on day of ET and d7ET using spectrophotometric methods. Endometrial flow velocimetry was assessed on day of ET. Cycles were initially divided on the basis of pregnancy while subsequent stratification was based on d7ET NO levels. Clinical pregnancy and implantation rate were the main outcome measures.
Main results and the role of chance: Overall clinical pregnancy and implantation rate was 36.98% and 31% respectively. NO level on dET correlated strongly with endometrial vascularity (Spearman $r = 0.5709$, 95% CI: 0.3920–0.5011; $p < 0.001$) and clinical pregnancy (Spearman $r = 0.6655$, 95% CI: 0.4732–0.7972; $p < 0.0001$). Serum NO level on d7ET (5.11 ± 2.19 *vs*. 0.82 ± 0.21 nm/ml; $p < 0.001$) was significantly higher in pregnant ($n = 81$) than in non-pregnant ($n = 138$) group. High (> 4.2) d7ET NO group ($n = 108$) illustrated significantly much higher clinical pregnancy rate (43.51% vs. 32.43) compared to low d7ET NO population ($n = 111$). Threshold value of serum d7ET NO to achieve clinical pregnancy was found to be > 4.2 nm/ml (Sensitivity 91.89%, Specificity 77.53%, Likelihood ratio 3.51, ROCAUC 81.35%).
Limitations, reason for caution: The predictability of serum NO levels on d7ET as a biomarker in envisaging live birth outcome are limited to patients with favorable criteria for IVF. Moreover keeping an eye over the sample size it warrants the results to be proved in a randomized controlled trial.
Wider implications of the findings: d7ET serum NO level stands as a novel non-invasive marker for clinical pregnancy and implantation rates in ongoing IVF cycles as against human endometrial study which constitutes an invasive method for embryo implantation assessment and cannot be applied to an ongoing cycle. It has wide implication in clinical practice and has a good prognostic accuracy for predicting clinical pregnancy in the index cycle.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The study was funded by our own private infertility centre; Institute of Reproductive Medicine. The authors have no conflict of interest.
Trial registration number: NA.
Keywords: nitric oxide, non-invasive marker, endometrial flow velocimetry, embryo transfer

P-335 A novel role for microRNA miR-218 in regulating invasiveness of endometriotic cells

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Study question: What is the functional impact of miR-218 dysregulation in endometriosis?
Summary answer: miR-218 regulates endometriotic cell invasiveness, possibly by targeting the EGFR and the TGFbeta-modulating proteoglycan decorin.
What is known already: microRNAs are small noncoding RNAs implicated in the posttranscriptional regulation of gene expression. miR-218 is downregulated in endometriosis (Wang WT, JCEM 2013;98:281), suggesting a potential involvement in the pathogenetic process.
Study design, size, duration: *In vitro* study on the endometriotic cell line 12Z, the endometrial stroma cell line ST-T1b, and on primary stroma cells derived from the eutopic endometrium of 3 patients with laparoscopically confirmed endometriosis.
Participants/materials, setting, methods: Cells were transiently transfected with miR-218 precursors to study the effects of miRNA upregulation in vitro. The impact of miR-218 on cell viability was studied by MTT assay, whereas invasiveness was assayed in matrigel invasion chambers. Predicted targets of miR-218 were identified by microRNA.org database analysis, and target regulation was confirmed by quantitative real-time PCR and Western blotting.
Main results and the role of chance: Matrigel invasiveness was reduced by 62% ($n = 6$, $p = 0.01$) in miR-218 transfected 12Z cells. miR-218 transfection moderately reduced ST-T1b cell viability by 10% ($p < 0.05$, N = 6). qPCR revealed significant downregulation of EGFR and decorin mRNA expression by > 50% in the two cells lines and in patient-derived primary cells ($p < 0.01$ for 12Z, $p < 0.05$ for stroma cells, $n = 6$). Decorin downregulation by miR-218 was confirmed by Western blotting in St-T1b cells.
Limitations, reason for caution: This is an in vitro study based on a transient transfection approach. Additional targets may be involved in the phenotypic changes.
Wider implications of the findings: This study demonstrates a role for miR-218 in regulating endometriotic cell invasiveness, which may be mechanistically linked to downregulation of the EGFR and the TGFbeta binding proteoglycan decorin. Upregulation of miR-218 may be a possible therapeutic approach worth exploring in additional preclinical models of endometriosis.
Study funding/competing interest(s): Funding by University(ies) – Münster University Hospital.
Trial registration number: NA.
Keywords: endometriosis, microRNA, invasive growth, EGFR

P-336 Oxidative stress biomarkers in follicular fluid of women with endometriosis and tubal factor infertility- Is there a correlation with in-vitro-fertilization (IVF) outcome?

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Study question: Does oxidative stress (OS) affect outcome during in-vitro-fertilization cycles (IVF)? The objective was to compare oxidative stress (OS) biomarkers in follicular fluid of women with endometriosis and tubal factor infertility undergoing in-vitro- fertilization (IVF) and correlate them with assisted reproductive technique (ART) outcome including oocyte fertilization, embryo cleavage and quality.
Summary answer: Women with endometriosis have lower pregnancy outcome during IVF cycles. There are altered levels of OS bio-markers in the follicular fluid of women with endometriosis undergoing IVF cycles. This may be responsible for lower pregnancy rate in women with endometriosis undergoing IVF.
What is known already: Oxidative stress may play a role in oocyte development and maturation which gets disturbed in endometriosis. The oocyte competence is lowered when levels of Reactive oxygen species (ROS) exceed the ability of the follicular fluid and the oocyte to counterfeit them with insufficient anti-oxidants, leading to lower oocyte potential and embryogenesis and consequently lower pregnancy rates. Levels of various OS bio-markers explored in follicular fluid during IVF cycles, but results do not convincingly suggest role of OS. A novel marker 8-Isoprostane has not been studied as it has better potential to define OS.
Study design, size, duration: Cross-sectional study. One hundred and seven women with endometriosis and tubal factor infertility undergoing IVF cycles at a tertiary fertility center. Sample size was calculated to 45 women in each group based on literature review. The study was conducted from July 2012 to June 2014.
Participants/materials, setting, methods: Follicular fluid from women with endometriosis (n = 50, group I) and tubal factor infertility (n = 57, group II) were evaluated for OS bio-markers including Reactive oxygen species (ROS), Total anti-oxidant capacity (TAC), eight-hydroxy-2-deoxyguanosine (8-OHdG) and 8-Isoprostane (8-IP) levels. ROS levels were detected by chemiluminesence, TAC, 8-OHdG and 8-IP by enzyme immunoassay.
Main results and the role of chance: Levels of ROS [(39.6cpm vs 54.8 cpm) (p = 0.2] and 8-OHdG [(6157.9 pg/mll vs 5393.0 pg/ml) (p = 0.05)] were comparable in both groups. Levels of 8-IP were significantly higher in group I as compared to group II [85.2 pg/ul vs 39.2 pg/ul) (p = 0.01)]. Levels of TAC were significantly higher in women with endometriosis as compared to tubal factor [(5.4 vs 4.1 mM of trolox) (p = 0.005) and could explain comparable ongoing clinical pregnancy rate in both groups (24.0 vs 24.5%, p = 0.34). There was no correlation of OS biomarkers with ART outcome in tubal factor. Follicular fluid from endometriosis revealed a negative correlation of ROS (-0.42; p = 0.002)with cleavage rate and positive correlation of TAC with fertilization rate (0.44; p = 0.001, cleavage rate (0.42; p = 0.03) and good grade embryos (0.4; p = 0.001).
Limitations, reason for caution: The study needs to be done on a larger sample size to validate the results.
Wider implications of the findings: OS may have a role in endometriosis related IVF outcome as revealed by higher levels of 8-IP in follicular fluid. OS bio-markers could be useful in prognosticating IVF outcome in women with endometriosis. Presence of adequate anti-oxidants in follicular fluid is required to counterfeit the effects of OS on oocyte competence. This could have wider implications to add anti-oxidants as supplements in women with endometriosis undergoing IVF cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – All India Institute of Medical Sciences, New Delhi.
Trial registration number: NA.
Keywords: endometriosis, oxidative stress, reactive oxygen species, follicular fluid, IVF

P-337 Induction of post-menstrual regeneration by ovarian steroid withdrawal in the functionalis of xenografted human endometrium

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Study question: Does the endometrial functionalis have the potential to undergo self-renewal after menstruation and how is this process controlled by ovarian steroids?
Summary answer: Endometrial xenografts subjected to withdrawal of estradiol and progesterone shrink but also show signs of proliferation and tissue repair. New estradiol supply blocks apoptosis, prevents atrophy but is not sufficient to increase volume graft.
What is known already: Menstruation, i.e. cyclic proteolysis of the extracellular matrix of endometrial functionalis, is induced by a fall in estrogen and progesterone concentration and is followed by tissue regeneration. However, there is debate about whether regenerating cells must originate from the basalis or from stem cells and whether new estrogen supply is required for the early repair concomitant with menstruation.
Study design, size, duration: Fragments from human endometrial functionalis (from 24 hysterectomy specimens) were xenografted in ovariectomized SCID mice and submitted to a 4-day estradiol and progesterone withdrawal (to mimic menstruation) followed by reexposure to estradiol (to mimic the proliferative phase). We measured signs of proliferation and changes in graft volume.
Participants/materials, setting, methods: Endometrium was collected from spontaneously cycling women. Cell proliferation was examined by immunolabeling Ki-67, cyclin D1 and phosphorylated-histone H3. Xenograft volume was measured by magnetic resonance imaging. Xenograft histomorphometry was performed to determine how the different tissue compartments contributed to volume change.
Main results and the role of chance: Hormone withdrawal induced a rapid decrease in graft volume mainly attributable to stroma condensation and breakdown, concomitant with an increase of proliferation markers. Reinsertion of E pellets after induced menstruation blocked volume decrease and apoptosis and stimulated epithelial and stromal growth, but, surprisingly, did not induce graft enlargement.
Limitations, reason for caution: Mechanisms of endometrial remodeling are different in women and mice and the contribution of circulating inflammatory cells in both species remains to be clarified. Moreover, during human menstruation, endometrial fragments resulting from tissue proteolysis can be expelled by the menstrual flow, unlike in this model.
Wider implications of the findings: Menstruation is a multifocal event within the functionalis. This is the first evidence that endometrial fragments that are not shed after menstrual tissue breakdown can support endometrial regeneration. Endometriosis is commonly thought to result from the retrograde migration of menstrual fragments of the degraded functionalis into the peritoneal cavity. Our study supports their potential to regenerate as ectopic endometrium.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – This work was supported by the Fonds de la Recherche Scientifique Médicale, Concerted Research Actions, Communauté Française de Belgique, Région wallonne, Région bruxelloise and Loterie nationale. P.H. and B.F. are research associates of the Belgian Fonds de la Recherche Scientifique (F.R.S.-F.N.R.S.).
Trial registration number: There is no trial registration number for this study.
Keywords: mouse model, Ki-67, repair

P-338 An in vitro implantation model reveals morphologic and functional details of human blastocyst attachment to uterine epithelium

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Study question: What are the morphological details of early human blastocyst implantation events, specifically pertaining to trophectoderm breaching of the endometrial epithelium and trophectoderm outgrowth?
Summary answer: Human blastocysts of varying grades attach readily to the endometrial Ishikawa cell line in an in vitro model of implantation. Subsequent fluorescence microscopy revealed apoptosis in Ishikawa cells interacting with the blastocyst. Trophectoderm syncytialisation was also seen upon outgrowth on top of, and into, the Ishikawa cell layer.
What is known already: Apoptosis in the endometrial epithelium has been suggested as a mechanism for breaching this barrier during human embryo implantation. Human trophectoderm syncytialisation has also been observed upon blastocyst invasion beyond the endometrial epithelium and into the underlying stroma.
Study design, size, duration: We used an in vitro model of the initial stages of human embryo implantation, which allowed detailed fluorescent microscopic interrogation of morphology. Fifty-nine human blastocysts consented for research (with ethical approval and a licence from the HFEA) from fresh IVF/ICSI cycles and long term-storage were used.
Participants/materials, setting, methods: Day 6 human blastocysts were co-cultured with the endometrial Ishikawa cell line for 48 h. Blastocyst attachment was monitored at 24 and 48 h by gentle agitation, and attached blastocyst sites were fixed at 48 h, processed for immunofluorescence and imaged using an Apoptome-equipped Zeiss Axiophot microscope.
Main results and the role of chance: Attached blastocysts that exhibited fully expanded blastocoels were considered to be at an early attachment stage with limited trophectoderm outgrowth. Five of these attachment sites were labelled with the apoptosis marker anti-cleaved caspase3, with four of these revealing multiple apoptotic Ishikawa cells directly underneath the attached blastocyst. Co-labelling with phalloidin and DAPI also revealed morphological details at these sites, such as potential trophectoderm syncytialisation in contact with Ishikawa cell apical surfaces. Attached blastocysts which had collapsed after 48 h co-culture were considered to be at a later stage of attachment, with outgrowing trophectoderm. Nine of these attachment sites were labelled with the cell-cell adhesion marker anti-E-cadherin, alongside phalloidin and DAPI, which demonstrated syncytialisation in outgrowing trophectoderm in contact with Ishikawa cells.
Limitations, reason for caution: In vitro models are the only method for studying human embryo implantation. Our model was designed for microscopy and for screening interventions into implantation, and is highly simplified. The use of the Ishikawa cell line as a surrogate endometrial epithelium may not reflect dynamic receptivity in the in vivo endometrium.
Wider implications of the findings: We provide the first direct evidence for human blastocyst-induced endometrial epithelium apoptosis as a mechanism for breaching the epithelial barrier in early implantation. Furthermore, we document the formation of the initial syncytiotrophoblasts in human placental development. We show that these can form during early outgrowth into, and on top of, endometrial epithelium in the Ishikawa model. Importantly, this study highlights the potential of Ishikawa cells for further mechanistic investigation of the earliest stages of implantation.
Study funding/competing interest(s): Funding by national/international organization(s) – The study was funded by Wellbeing of Women grant RG1442 and a postgraduate studentship from the Anatomical Society of Great Britain and Ireland. The authors declare no competing interests.
Trial registration number: NA.
Keywords: human blastocyst, implantation, in vitro model, fluorescence microscopy

P-339 Granulocyte colony stimulating factor(G-CSF) : Does it really improve the endometrial thickness in women with persistent thin endometrial thickness undergoing frozen endometrial transfer?

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Study question: Does a persistently unresponsive endometrium improves in thickness with administration of granulocyte colony-stimulating factor (G-CSF) in frozen embryo transfer cycle?
Summary answer: The study failed to show a significant improvement in the endometrial thickness after administration of G-CSF.
What is known already: Endometrial thickness less than 7 mm has been shown in studies to hamper pregnancy rates. Many women continue to have unresponsive thin endometrium despite using all available modalities of treatment. Few small studies have reported the use of G-CSF to be efficacious in increasing the endometrial thickness in women with thin endometrium. None of the reported studies are randomized controlled trials (RCT) though.
Study design, size, duration: Prospective observational cohort study of 250 women with persistent unresponsive endometrium < 7 mm was conducted in craft hospital from 2012-2014.These women have tried and failed high dose estradiol therapy, long duration estradiol therapy, different routes of estradiol, natural cycle endometrial preparation, sildenafil citrate and vitamin E before they were offered off label use of G-CSF.
Participants/materials, setting, methods: Endometrial preparation was done with estradiol valerate 2 mg three times a day and escalated in phased manner. On day 20th of estradiol, if the endometrium was less than 7 mm, they were recruited for the study. Intrauterine G-CSF infusion was done and endometrial thickness was assessed 2 days later.
Main results and the role of chance: Overall, there was no significant increase in the endometrial thickness at 48 h post infusion.(p = 0.09).73/220 women (33.18%) had their endometrium improved to above 7 mm. They underwent embryo transfer and resulted in clinical pregnancy rate of 34.2% (25/73) per embryo transfer and implantation rate of 18%.
Limitations, reason for caution: Despite the fact that it is one of the largest reported study till now, it was not a randomized controlled trial. Also, since pregnancies have been reported with endometrium as thin as 3.7 mm,7 mm cut off for thin endometrium is at best arbitrary.
Wider implications of the findings: The persistently thin endometrium can be improved by G-CSF in only approximately 1/3rd of the women. This raises questions over the previous studies showing significant benefit of the intervention. The solution for the ongoing controversy can be achieved only by a large preferably multicentric RCT.
Study funding/competing interest(s): Funding by hospital/clinic(s) – craft hospital.
Trial registration number: NA.
Keywords: thin endometrium, IVF, granulocyte colony stimulating factor

P-340 Transcriptome pattern comparison between endometrium and peripheral blood

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Study question: Can the transcriptome in peripheral blood reflect gene expression pattern in the endometrium around the time of implantation?
Summary answer: Preliminary results based on clustering analysis and gene pathway analysis suggest it is possible that the transcriptome in peripheral blood may be used to study changing gene expression in the endometrium around the time of implantation.
What is known already: Endometrium becomes receptive 6-9 days after luteinizing hormone surge, which is considered as the window of implantation. Transcriptome studies showed distinctive gene expression profiling of endometrium in the window of implantation. However, it is unclear if transcriptome in the peripheral blood may reflect the changes in the endometrium.
Study design, size, duration: This is a prospective, cross-sectional study with 8 subjects. Endometrium biopsy and blood collection were both timed precisely on day LH+ 7, following written consent.
Participants/materials, setting, methods: Subjects were recruited from the IVF clinic in Prince of Wales Hospital. RNA was extracted from endometrium and blood, investigated with gene expression array (Agilent Sureprint G3 8 × 60 k), and analyzed with GeneSpring 13.0. Differentially expressed genes were identified by using moderate T-test.
Main results and the role of chance: Clustering analysis of four pairs (endometrium and peripheral blood) of samples showed similarity in clustering pattern. Venn diagram and pathway analysis showed similar regulation pattern with 11 genes in 6 pathways, suggesting that peripheral blood may reflect endometrium gene expression in the peri-implantation period.
Limitations, reason for caution: Additional samples will be performed to confirm the preliminary findings.

Wider implications of the findings: The above findings suggest that it may be possible to utilize the transcriptome pattern in peripheral blood to reflect the gene expression profile in the endometrium around the time of implantation.
Study funding/competing interest(s): Funding by University(ies) – The Chinese University of Hong Kong.
Trial registration number: CRE Ref. No. 2014.637.
Keywords: transcriptome, endometrium receptivity, peripheral blood

P-341 Role of 3D Ultrasound and Power Doppler in the prediction of IVF/ICSI outcome

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Study question: Do endometrial parameters like endometrial volume & vascularity indices by 3D US & Power Doppler have any predictive value in IVF/ICSI outcome?
Summary answer: Miscarriage rate was higher when endometrial volume was <2 ml. Vascularity indices by 3D US& Power Doppler showed no significant difference in pregnant and non-pregnant group.
What is known already: 3D US volume calculation of the endometrium helps to correlate the cycle outcome with quantitative parameter than endometrial thickness. Pregnancy and implantation rates were previously demonstrated to be significantly lower when endometrial volume was < 2 ml and no pregnancy was achieved when endometrial volume was < 1 ml.
Study design, size, duration: Retrospective study of 161 ART cycles with 88 stimulated IVF/ICSI cycles & 73 recipient cycles over a period of 12 months.3D Ultrasound &Power Doppler done on the day of HCG in stimulated IVF/ICSI cycles & on the progesterone supplementation day in recipient cycles to assess the endometrial volume and endometrial perfusion.
Participants/materials, setting, methods: Endometrial volume assessment done on VOLUSON 730 (WIPRO GE) using transvaginal 3D probe& VOCAL software..Patients were divided according to endometrial volume into 3 subgroups; < 2 ml, 2–4 ml and > 4 ml..3DPowerDoppler & volume histogram done to obtain vascularity index (VI), Flow index (FI),& Vascularity flow index (VFI). Pregnancy rates were compared between all groups.
Main results and the role of chance: Pregnancy rates between 3 groups of endometrial volume in stimulated cycles; < 2 ml, 2-4 ml and > 4 ml was 55, 44.1, 40% respectively.& miscarriage rate was 36.6, 10.5,10.0%, respectively. In recipient cycles pregnancy rates in; < 2 ml,2-4 ml, > 4 ml.
Groups were; 55, 61.5 and 85.7%, respectively&miscarriage rate was 13.3, 12.5 and 0%. No significant difference in pregnancy rates between the three groups in stimulated & recipient cycles. Endometrial volume failed to predict outcome of IVF/ICSI. 2 pregnancies seen with < 1 ml endometrial volume. Increased miscarriage rate seen in stimulated & recipient cycles with < 2 ml endometrial volume. In stimulated IVF/ICSI cycles, VI/FI/VFI mean values were 4.6/26.9/1.4 respectively in pregnant group & 4.3 /26.7/1.6 in non-pregnant group. In recipient cycles, VI/FI/VFI mean values were 3.6/25.9/1.0.respectively in pregnant group & 1.7/24.2/0.6 in non- pregnant group. No significant difference in endometrial vascularity between pregnant &nonpregnant groups.
Limitations, reason for caution: Three Dimensional ultrasound has been proposed as a promising tool for evaluating endometrium accurately.3D Power Doppler indices seem to reflect endometrial perfusion. But its exact relationship with real blood flow is not fully understood. More studies using larger groups are necessary to draw firm conclusions.
Wider implications of the findings: Present study shows that there is no correlation between endometrial volume and pregnancy rates in stimulated cycles & recipient cycles. Contrary to the fact that no pregnancy was achieved If endometrial volume is < 1 ml, 2 pregnancies we had in < 1 ml endometrial volume., gives hope of conception in women having poor endometrium.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Rao Hospital, 120, West Periyasamy Road, R. S Puram, Coimbatore – 641002, Tamil Nadu, India.
Trial registration number: NA.
Keywords: endometrium, ultrasound implantation markers, three dimensional ultrasound and power Doppler, endometrial receptivity

P-342 Vitamin D and endometriosis: the serum level and its anti-inflammatory and anti-proliferative effects

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Study question: How much proportion of vitamin D (VD) deficiency among patients with endoetriosis? Does VD have the anti-inflammatory and anti-proliferative effects on endometriosis *in vitro*?
Summary answer: VD deficiency was associated with the progression of endometriosis. On endometriotic stromal cells (ESC), VD had an anti-inflammatory effect: repressing the production of cytokine, PGE_2, and the expression of its synthesis/degradative enzymes, and VD also showed an anti-proliferative effect. These effects were suggested to be mediated through NFkB activation pathway.
What is known already: Endometriosis is a chronic inflammatory disease and controlling its inflammation and immune responses can be a therapeutic strategy. VD is known to modulate the immune system and associations between VD deficiency and disease risks have been reported in many chronic inflammatory diseases. Regarding endometriosis, the circulating level of VD is controversial, and only one *in vivo* mouse study demonstrated that the VD receptor agonist reduced endometriosis by inhibiting peritoneal inflammation.
Study design, size, duration: Under informed consents, sera were taken from control ($n = 37$), patients with stage 1-2 ($n = 17$) and stage 3-4 ($n = 22$) endometriosis. All samples were collected from October to March, from 1998 to 2014. Endometriosis tissues were obtained from ovarian endometriomas ($n = 35$) for ESC isolation.
Participants/materials, setting, methods: Serum 25-(OH)VD, 1,25-$(OH)_2VD_3$ were measured by RIA. ESC were stimulated with IL-1β and VD (1,25$(OH)_2D_3$). RT-PCR, ELISA and Western blotting were used to measure IL-8 and PGE_2 synthesis/degradative enzyme mRNA expression, IL-8 and PGE_2 productions and IkBα expression respectively. Cell proliferation was analyzed by BrdU incorporation and Cell Counting Kit-8.
Main results and the role of chance: Serum 25-(OH) VD level in stage 3-4 endometriosis (17.2 ± 1.1 ng/ml) was significantly lower than control (21.8 ± 1.3 ng/ml, $p < 0.05$) and stage 1-2 endometriosis (21.5 ± 1.4 ng/ml, $p < 0.05$). Serum 1,25-$(OH)_2$ VD_3 levels were not different among groups. VD (10^{-7} M) reduced IL-1b (5 ng/mL) -induced IL-8 mRNA expression at 6 h ($p < 0.05$), and IL-8 production ($p < 0.05$). VD reduced IL-1β-induced PGE_2 secretion ($p < 0.05$), and IL-1β-induced PGE2 synthesis (COX-2, mPGES-1 and mPGES-2) and degradative (15-PGDH) enzyme mRNA expression ($p < 0.05$). VD decreased the number of ESC to 84.0% ($p < 0.05$) and BrdU incorporation to 73.5% ($p < 0.05$), but VD did not affect apoptosis. VD significantly decreased TNF-α increased IkBα in endometriosis ($p < 0.05$).
Limitations, reason for caution: Although the number of patients is not large enough to obtain conclusive results, the data showed striking evidences that VD deficiency may cause endometriosis. Serum VD level may be influenced by races and the duration of sunshine, and our results can not be generalized to other population than Japanese women.
Wider implications of the findings: Our results showed that VD deficiency is associated with the progression of endometriosis and VD has anti-inflammatory and anti-proliferative effects on endometriosis, which may be mediated through NFkB activation pathway. Current medical treatment for endometriosis is limited to hormonal agents and cannot be used for women who wish to conceive. Our study suggest that VD: a natural substance in the body that do not affect female hormonal milieu, could be an alternative medicine for preventing or controlling endometriosis.
Study funding/competing interest(s): Funding by national/international organization(s) – Ministry of Education, Culture, Sports, Science and Technology.
Trial registration number: NA.
Keywords: endometriosis, vitamin D, inflammation, proliferation

P-343 Endometrial volume measurement in prediction of IVF/ICSI outcome

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Study question: To investigate the role of estimating endometrial volume, on the day of hCG, in prediction of IVF outcome.
Summary answer: The findings of the present study suggest that, analysis of endometrial volume on the day of the hCG administration had no predictive value for conception in IVF cycles.
What is known already: Successful implantation in ART cycles depends on multiple factors among which is endometrial receptivity, one of them is endometrial volume. Some studies reported relationship between endometrial volume on the day of hcg administration and positive pregnancy rate and some others say not.
Study design, size, duration: In this prospective study endometrial volume were measured in 167women undergoing an IVF/ICSI cycle, on the day of HCG,(between 2009 and 2011).
Participants/materials, setting, methods: In this prospective study endometrial volume were measured in 167women undergoing an IVF/ICSI cycle, on the day of HCG,(between 2009 and 2011). Using eXtended Imaging Virtual Organ Computer-aided Analysis (3D XI VOCAL) method volume was digitally manipulated to display a multiplanar view in longitudinal view of the endometrium in order to obtained the images manually in 10 sections, which showing simultaneously three orthogonal planes (axial, longitudinal, and coronal).Patients were divided into 3 groups, according to endometrial volume calculated. Group 1endometrial volume < 2cc, Group 2 with endometrial volume of 2-4.5cc, and Group 3 with endometrial volume > 4.5cc.
Main results and the role of chance: The study included 167 women with no significant difference in background characteristics between all subgroups. The overall pregnancy rate was 39.8%. Participant's age ranged from 20-38 year old and the mean age was 29.9 ± 4.23. Pregnancy rates between the three groups of endometrial volume was 26.7%,37.8% and 50% in group 1,2 and 3 respectively. Although there is no statistically significant difference between positive pregnancy rate and endometrial volume, likelihood ratio of pregnancy in group 3 is higher than another two groups.
Limitations, reason for caution: More sample size with balanced size in three groups is more valuable for analysing the data.
Wider implications of the findings: Further study with larger sample size is needed.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Imaging department of Royan institute.
Trial registration number: NA.
Keywords: pregnancy rate, IVF, endometrial volume

P-344 In silico, in vitro and in vivo analysis identifies a potential role for steroid hormone regulation of FOXD3 in endometriosis associated genes

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Study question: Can bioinformatics analysis of publically available microarray datasets be utilised in identifying potentially important transcription factors (TF) in the hormonal regulation of the endometrium?
Summary answer: We have predicted a central role for the novel transcription factor, FOXD3 in secretory phase/endometriosis by bioinformatics analysis of endometrial microarray datasets, and confirmed its presence for the first time in healthy human endometrium; demonstrated that FOXD3 is differentially expressed in endometriosis and regulated by progesterone *in-vivo and in-vitro*.
What is known already: The reported endometriosis-associated endometrial aberrations are confined to the progesterone-dominant secretory phase and progesterone-resistance is a proposed causative factor. Bioinformatics is used to collate gene expression data to identify the key players in a disease process.
Study design, size, duration: The study was initially an '*in-silico*' study, with confirmatory 'wet lab' data. Endometrial samples from women with (n = 30) or without (n = 30) endometriosis not on hormonal therapy, 20 women with endometriosis treated with Mirena™-IUS and ectopic endometriosis lesions from 9 women were also analysed. Ishikawa endometrial cell line was also used.
Participants/materials, setting, methods: Initial mining and Bioinformatic analysis of microarray datasets identified key transcription factors, and FOXD3 expression levels were examined in human endometrial samples by western blotting (WB), immunohistochemistry (IHC), and PCR. The progesterone regulation of endometrial FOXD3 levels was examined *in vivo* and in a cultured human endometrial cell-line *in vitro*.
Main results and the role of chance: Initial mining and subsequent bioinformatics analysis of human endometrial microarray datasets identified FOXD3 to be a key regulator of gene expression specific to secretory phase/endometriosis. FOXD3 was dynamically expressed in healthy endometrium and differentially expressed in endometriosis. Progestagen (Levonorgestrel) treatment reduced the high endometrial FOXD3 protein ($p < 0.01$) and mRNA levels seen in untreated women with endometriosis, with a shift of epithelial FOXD3 from the nucleus to the cytoplasm. Treatment of Ishikawa cell-line with Medroxyprogesterone acetate for 72 h also down-regulated FOXD3 mRNA and protein.
Limitations, reason for caution: The quality of Bioinformatics analysis and results depends on the published micro-array data. The wet laboratory experiments are only descriptive without functional analysis of FOXD3 in the establishment of endometriosis.
Wider implications of the findings: FOXD3 in endometrium is regulated by progesterone and an in depth analysis of FOXD3 might provide insights into proliferative disorders of the endometrium.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Funding by national/international organization(s).

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Trial registration number: NA.
Keywords: FOXD3, endometriosis, bioinformatics, Mirena IUS, endometrium

P-345 Analysis of transcriptional expression of extracellular proteins in the endometrial stromal or epithelial cells during estrus

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Study question: To date, development of non-cellular niche has been not reported in endometrial stromal (ES) and epithelial (EE) cells. Therefore, as a basic study for constructing endometrial non-cellular niche, we analyzed transcriptional expression of extracellular proteins in the ES and EE cells, respectively.
Summary answer: We could identify type of extracellular proteins showing significant transcriptional up-regulation in ES and EE cells.
What is known already: ES and EE cells consisting of endometrium experience a variety of alterations during the estrus cycle by lots of hormones secreted in the sexual organs. Simultaneously, this hormonal regulation makes it difficult to investigate precisely effects of a specific treatment on ES and EE cells. Accordingly, construction of artificial endometrium using non-cellular niche of ES and EE cells is requested for eliminating chaotic treatment effects detected in the implantation-related studies.
Study design, size, duration: This study was designed prospectively. Post-isolation of ES and EE cells from mouse uterus, expression of extracellular proteins, including extracellular matrix-, cell-to-cell interaction- and integrin-related proteins, were investigated transcriptionally. All experiments were independently replicated 5 times.
Participants/materials, setting, methods: ES and EE cells were isolated enzymatically from 6-weeks-old ICR female mouse uterus in estrus phase and transcriptional level of extracellular matrix-, cell-to-cell interaction- and integrin-related proteins was measured by real-time PCR.
Main results and the role of chance: In case of extracellular matrix-related proteins, ES and EE cells showed significant increase in the expression of 4 (*FN*, *NID*, *ColI*, and *ColIII*) and 7 (*FN*, *NID*, *LN*, *TN*, *ColI*, *ColIII*, and *ColV*) genes, respectively. Moreover, in the cell-to-cell interaction-related proteins, significant increase of 4 (*Cdh2*, *Cdh3*, *Icam1* and *Pecam1*) or 3 (*Cdh1*, *Icam1* and *Pecam1*) genes transcription were observed in ES or EE cells. Finally, transcriptional level of 5 integrin alpha (*Itga2*, *Itga5*, *Itga6*, *Itga9* and *ItgaV*) and 3 integrin beta subunit genes (*Itgb1*, *Itgb3* and *Itgb5*) were significantly increased in ES cells, whereas EE cells showed significant transcriptional up-regulation of 6 integrin alpha (*Itga2*, *Itga5*, *Itga6*, *Itga7*, *Itga9* and *ItgaV*) and 5 integrin beta subunit genes (*Itgb1*, *Itgb3*, *Itgb4*, *Itgb5* and *Itgb6*).

Limitations, reason for caution: Based on these transcriptional expressions, studies on translational expression of extracellular proteins should be progressed further in order to confirm precisely type of extracellular proteins expressed in ES and EE cells.
Wider implications of the findings: These results will contribute greatly to develop non-cellular niche mimicking microenvironment of endometrium.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by a grant of the Korea Health Technology R&D Project (A121515-1201-0000100), Ministry of Health and Welfare, Republic of Korea.
Trial registration number: NA.
Keywords: extracellular proteins, transcriptional level, endometrial stromal cells, endometrial epithelial cells

P-346 Personolized embryo transfer (pET) after Endometrial Receptivity Array (ERA) in patients with recurrent implantation failure –an observational study

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Study question: This observational study has been performed in order to determine the state and the impact of window of implantation (WOI) determined by endometrial receptivity array (ERA) on clinical outcome in patients with recurrent implantation failure (RIF).
Summary answer: Our results indicate that 1/3rd of the RIF patients (34%) in our study has displaced WOI which can be attributed for their reproductive failure. Assessing WOI before an ART cycle could help the clinician to address the nature of the problem as well as possible treatment strategies in such patients.
What is known already: Recurrent implantation failure is a poorly characterized and defined outcome with a wide spectrum of pathologies. While many of these pathologies can be corrected, the underlying implantation problem due to a possible molecular asynchrony of embryo-endometrium interactions can still remain. A microarray-based molecular diagnostic test, called as Endometrial Receptivity Array has been developed to detect such synchronies and, based on ERA results to determine personalized embryo transfer (pET) day in order to maximize the clinical outcome.
Study design, size, duration: This observational study was performed between November 2013 – December 2014. ERA test was offered to candidate patients with ≥ 2 previous IVF failures with no observed endocrinological or uterine pathologies.
Participants/materials, setting, methods: For those who accepted the test (n = 78), an endometrial tissue sampling has been taken during natural cycle (7 days after LH surge) or 5 days after progesterone replacement has commenced during hormone replacement therapy cycles. Biopsied tissue samples were stored, shipped and processed for RNA expression analysis according to the manufacturer's recommendations. According to the ERA test results, blastocyst-stage frozen embryo transfers were performed in 40 patients.
Main results and the role of chance: A total of 90 ERA tests were performed in 78 patients. In 83 of 90 samples (90%), valid RNA was obtained for analysis. There were no intra or port-operative complications. Twenty eight patients has a non-receptive (pre- or post-receptive) endometrium at the day of endometrial biopsy (34%). In 40 frozen embryo transfers that were performed according to ERA results, 20 gestations were achieved (50%). The rate of gestation remained the same in the subgroup of patients who had pET due to displaced WOI.
Limitations, reason for caution: The main limitation is the number of patients that were included in the study. As the number of cases included in this study as well as other studies are increased, the significance and the possible impact of ERA test-based pET programs can be assessed and validated in more realistic clinical scenarios.
Wider implications of the findings: Our results, being in parallel with the limited number of reported cases in the current literature, can indicate that assessment of endometrial receptivity prior to a frozen embryo transfer cycle can be beneficial for clinical outcome in cases with RIF.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study received no funding and there are no conflicts of interests to be declared.
Trial registration number: NA.
Keywords: endometrial receptivity, personalized embryo transfer, recurrent implantation failure

P-347 Cytokines related with implantation window can be increased with Metformin treatment in endometrial stromal cells on hyperinsulinemic and hyperandrogenic environment

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Study question: Evaluate the effects of Metformin on IL-8 and IL-1β gene expression in a model of hyperinsulinemia and hyperandrogenism in endometrial stromal cells *in vitro*.
Summary answer: Metformin increases IL8 and IL1b gene expression levels in hyperinsulinemia and hyperandrogenism in endometrial stromal cells.
What is known already: The preparation of the endometrium for implantation involves a complex sequence of events and a variety of signaling molecules. The concentrations of IL-8 and IL-1β are correlated to the implantation process. The failure rate of this process is high in PCOS patients that affects from 6 to 8% of women in reproductive age. Despite the uncertainty about the primary cause of PCOS, there are reports about the importance of hyperinsulinemia and hyperandrogenism in infertility promoting.
Study design, size, duration: The present study was an experimental study in vitro. The endometrial cells were obtained from patients undergoing hysterectomy. We analyzed seven (N = 7) primary cultures of endometrial stromal cells with hyperinsulinemic and hyperandrogenic environment during 14 days in 24 and 48 h of Metformin treatment.
Participants/materials, setting, methods: The stromal cells were cultivated: (EP) estradiol, progesterone; (EPM) estradiol, progesterone, 10^{-3} M metformin; (EPI) estradiol, progesterone, 100 ng/mL insulin; (EPD) estradiol, progesterone, 10^{-6} mol/L dihydrotestosterone; (DI) estradiol, progesterone, insulin, dihydrotestosterone; (DIM) estradiol, progesterone, insulin, dihydrotestosterone, metformin. The IL-8 and IL1b gene expression was evaluated by real time PCR.
Main results and the role of chance: Increased gene expression of IL-8 was observed in EPM group treated for 48 h compared to the same group treated for 24 h (P < 0,05). Similarly, the EPM group showed higher IL-1b gene expression compared to all other groups treated with metformin for 48 h (P < 0.05). However, the DIM group, also metformin treated, did not show statistically significant difference in treatment time of any studied genes. It suggests an inhibitory action of insulin on these genes expression in the hyperinsulinemic and hyperandrogenic groups.
Limitations, reason for caution: These results are regarding to cell cultures, therefore physiologic influences were not measured.
Wider implications of the findings: Similarly to other results we have demonstrated higher interleukins genes expression when cells were treated with Metformin, not only in an hyperinsulinemic environment, but also in an hyperandrogenic environment. Additionally, our study demonstrated that even if acute treatment were carried out (24 h), there was higher IL8 gene expression.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Universitätsklinikum Heidelberg, Universidade Federal do Rio Grande do Sul, Hospital de Clínicas de Porto Alegre.
Trial registration number: NA.
Keywords: metformin, IL8, IL1beta, endometrium

P-348 Intrafollicular iron and ferritin in women with ovarian endometriomas

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Study question: to evaluate whether iron contained in ovarian endometriomas may diffuse through the cyst wall to follicular fluids and affect ovarian function.
Summary answer: Iron content did not differ in follicular fluids belonging to ovaries with and without endometriomas, while ferritin concentration resulted significantly higher in affected gonads compared to controls.
What is known already: Endometriotic cysts contain huge amount of free iron that can mediate the production of Reactive Oxygen Species (ROS) potentially harmful to the surrounding cells. The amount of oxidative stress in the ovarian cortex surrounding an endometrioma and in granulosa cells from patients with endometriosis was shown to be higher compared to controls. It has been hypothesized that factors present in the endometriomas, and iron in particular, may diffuse in the surrounding tissue causing ROS generation.
Study design, size, duration: A prospective case series, between January 2012 and January 2013. Sample size was decided considering as biologically relevant an Odds Ratio of having levels of iron/ferritin in the affected gonad above the 90th percentile of the distribution in the intact gonad ≥ 3. On these bases, thirty-nine women were recruited.
Participants/materials, setting, methods: Women undergoing IVF/ICSI with unilateral ovarian endometriomas (≥ 10 mm) at transvaginal ultrasound the month preceding the stimulation; age 18-42 years; absence of non-endometriotic ovarian cysts. Iron and ferritin were measured on an automatic platform in pools of follicular fluids obtained from affected and contralateral intact gonads.
Main results and the role of chance: The median (IQR) concentration of iron in the affected and unaffected ovaries was 59 (44-74) and 59 (47-73) µg/dL, respectively ($p = 0.77$). The median (IQR) concentration of ferritin was 57 (31-146) and 33 (23-67) µg/mL, respectively ($p = 0.026$). Ferritin concentration was above the 90th percentile of the distribution in unaffected ovaries (132 µg/mL) in 3 (8%) intact and 11 (28%) affected gonads ($p = 0.021$). No differences emerged when considering iron. Follicular concentration of iron correlated between the two ovaries (Rho = 0.76, $p < 0.001$). A similar figure emerged for follicular ferritin (Rho = 0.55, $p < 0.001$). A significant correlation was documented when correlating ferritin and iron in the 78 available gonads (Rho = 0.42, $p < 0.001$). No statistically significant correlations emerged between follicular iron and ferritin and variables reflecting ovarian responsiveness and oocyte developmental competence.
Limitations, reason for caution: We lack an histological diagnosis of endometriosis but his limitation is of scanty relevance given the high accuracy of transvaginal ultrasound. We exclusively recruited women with indication to IVF. Inferences of our findings to the whole population of women with ovarian endometriomas should therefore be made with caution.
Wider implications of the findings: Iron may diffuse from ovarian endometriomas into the adjacent ovarian tissue. However, this phenomenon does not markedly affect ovarian function because of some effective biological mechanisms such as ferritin storage that properly counterbalance the potentially highly detrimental effects of free iron.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fondazione IRCCS Ca' Granda.
Trial registration number: NA.
Keywords: endometrioma, endometriosis, follicular fluid, iron, ferritin

P-349 sperm oviduct interaction in the human – new insights using live cell imaging

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Study question: How do spermatozoa interact with the oviductal epithelium under near in vivo conditions?
Summary answer: Only spermatozoa with high membrane integrity bind to the cilia of the oviductal epithelium. As soon as sperm quality is impaired by a disruption in general health, sperm binding is also impaired. Inflammation of the fallopian tube also results in decreased sperm binding.
What is known already: In animals spermatozoa form a sperm reservoir in the isthmus by binding with their head to the cilia of the uterine tube. Thus they maintain their capacity to fertilize for days (most mammals), months (birds) or even years (reptiles). Due to the lack of imaging technologies for investigating the human fallopian tube under *in vivo* conditions, the sperm oviduct interaction has not been investigated in the human fallopian tube up to now.
Study design, size, duration: Using a digital video microscopic system, this pre-clinical randomized study was designed to characterize the human sperm-oviduct interaction under near *in vivo* conditions. Experiments were performed, over 9 months, on the ampulla and isthmus of 5 premenopausal women undergoing hysterectomy and in one pregnant woman undergoing caesarean section.
Participants/materials, setting, methods: The fallopian tubes of the premenopausal women and the pregnant woman were investigated immediately after surgery. Ampulla and isthmus with and without co-incubation with a) fresh and b) frozen thawed spermatozoa were examined qualitatively and quantitatively using a digital video microscopic analysis system and scanning electron microscopy (SEM).
Main results and the role of chance: Human spermatozoa bind to the epithelial cells of the fallopian tube as soon as they enter the oviduct. This binding capacity is not confined to the isthmus – it also takes place in the ampulla. Where there is a disruption in general health, e.g., inflammatory joint disease, sperm binding is impaired. Similarly inflammations of the fallopian tube, which in most cases are only seen microscopically, reduce sperm binding and sperm survival time. Accumulations of mucus in the fallopian tube, which may be drug related, result in the 'sticking' of sperm and thus decreased sperm vitality.
Limitations, reason for caution: Medications and in particular hormones influence the sperm oviduct interaction and have to be taken into account.
Wider implications of the findings: Our studies show movies of the human sperm oviduct interaction for the first time, under near *in vivo* conditions. The fact that only spermatozoa of high quality are able to bind to the oviduct point to it playing a pivotal role in sperm selection. Impairments in general health, both in male and in female result in reduced sperm binding and survival time- a major cause of decreased fertility.
Study funding/competing interest(s): Funding by University(ies) – School of Medicine, University College Dublin, Ireland.
Trial registration number: NA.
Keywords: human, sperm, fallopian tube

P-350 Review of performance of patients with endometriosis in in-vitro-fertilisation (IVF) as compared to tubal factor infertility-Retrospective Cohort study

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Study question: To compare the performance of patients with endometriosis related infertility in IVF to patients with tubal factor infertility.
Summary answer: In comparison to tubal factor infertility, patients with endometriosis perform well in IVF with a reasonable clinical pregnancy rate, comparable implantation rate and number of good quality embryos generated.
What is known already: There is debate in the studies about IVF performance in endometriosis patients. Some studies are quoting reduced pregnancy rate and poor quality embryo in patients with endometriosis. However, data emerging from larger database such as human fertilisation and embryology authority (HFEA) suggests better performance.
Study design, size, duration: Retrospective review of the women undergoing IVF treatment between January 2012 to December 2013 in the assisted reproductive unit (ARU) at Hartlepool university Hospital. 41 patients with endometriosis were identified and compared to 321 patient with tubal factor infertility matched for age and duration of infertility.
Participants/materials, setting, methods: Endometriosis was confirmed by laparoscopy and staging following the American Society for Reproductive Medicine (ASRM), 73% minimal to mild endometriosis and 27% moderate to severe. The outcome measures were clinical pregnancy, implantation, embryo quality, on going pregnancy and multiple pregnancy rates. Statistical analysis was completed using GraphPad Prism ® 2015.
Main results and the role of chance: There was no statistically significant difference between the two groups regarding the clinical pregnancy rate per cycle started (*P*: 0.53, Relative Risk (RR): 0.83, 95% Confidence Interval (CI): 0.52–1.3), per cycle completed (*P*: 0.63, RR 0.87, 95% CI: 0.55–1.4), Implantation rate (*P*: 0.10, RR: 1.4, 95% CI: 0.96–2.1), miscarriage rate (*P*: 1.00, RR: 0.74, 95% CI: 0.10–5.2), ongoing pregnancy rate (*P*: 1.00, RR: 0.97, 95% CI: 0.61–1.5), and Twin pregnancy rate (*P*: 0.64, RR: 1.4, 95% CI: 0.37–5.5).

Endometriosis patients had a comparable percentage of good quality embryos (Grade 1 and 2) generated (83% versus 80%).

Limitations, reason for caution: The study is retrospective in nature. The sample size of endometriosis group is small as compared to the tubal factor group; however, all the patients were diagnosed with Laparoscopy. We also identified an unequal distribution of the severity of endometriosis in the study group.

Wider implications of the findings: The findings from our study suggest that women with endometriosis perform fairly well in IVF in term of embryo quality, implantation, and multiple pregnancy rates as compared to women with tubal factor infertility. Clinical pregnancy and ongoing pregnancy rates in endometriosis patients are lower but not statistically different from patients with tubal factor infertility.

Study funding/competing interest(s): Funding by hospital/clinic(s) – North Tees and Hartlepool NHS Foundation Trust.

Trial registration number: This study was registered with clinical audit department (CG 156).

Keywords: IVF, endometriosis, fallopian tubes, implantation, embryo quality

P-351 miRNA expression of the endocervix as a biomarker for endometrium receptivity in patients undergoing assisted reproductive technologies

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Study question: To compare the miRNA expression in the endocervix and the endometrium during implantation window in patients undergoing *in vitro* fertilization (IVF) treatment? Could endocervix miRNA expression be a new less-invasive method to assess the implantation window of infertile patients undergoing *in vitro* fertilization (IVF) treatments?

Summary answer: The miRNA expression in endocervix was not totally concordant with the endometrium in infertile patients during the implantation window. However, we identified five microRNAs differentially expressed in both the endometrium and in the endocervix in patients who became pregnant and may be considered potential candidates for biomarkers of endometrium receptivity.

What is known already: Despite of high technology used to IVF treatments, the function of endometrium remains little explored in the assisted reproductive field. The endometrial gene expression has been recently used to identify the implantation window. miRNA are small molecules and may act as a post-transcriptional gene expression regulator. Endometrial biopsy constitutes an invasive method and cannot be applied to an ongoing cycle. Biomarkers for endometrial receptivity in the endocervix could represent a less-invasive approach and may be performed during the IVF treatment.

Study design, size, duration: This prospective cohort study included 32 good prognosis infertile women undergoing fresh IVF treatment cycles using standard conventional protocol, at an University Center from July 2012 to December 2013.

Participants/materials, setting, methods: Women candidates to IVF treatment underwent an endometrial biopsy and endocervical brush during the luteal phase (implantation window) in the menstrual cycle prior to IVF treatment. The endometrial and endocervix samples were submitted to RNA purification and were analyzed by miRNA PCR-array (miScript miRNA PCR Array, Qiagen).

Main results and the role of chance: It is interesting to note that among 86 miRNA evaluated, 14 miRNA were significantly downregulated in the endocervix in relation to endometrium, 11 miRNA were significantly upregulated in the endocervix in relation to endometrium, and 61 miRNA were similar between two kind of samples. We can suppose that about one-third of miRNA evaluated were differentially expressed between endometrium and endocervix, and hence, endocervix miRNA expression does not accurately represent the endometrium. On the other hand, when comparing pregnant and non-pregnant patients, pregnant patients revealed five miRNA downregulated in endometrium and upregulated in endocervix. So, those miRNA in the endocervix, could represent, in an inverse way, a possible new biomarker profile of implantation window, since the endocervical cells are not suitable for invasion. If the efficiency of those markers would be proved, it is also possible to perform the exam during the cycle.

Limitations, reason for caution: This study is a screening of miRNA expression in endometrium and endocervix of infertile patients undergoing IVF cycles in a limited sample size. The concordant miRNA expressed can represent a biomarker profile in the endocervix, and must be validated in a higher number of samples in an ongoing cycle.

Wider implications of the findings: Considering that human endometrial study constitutes an invasive method for embryo implantation assessment and cannot be applied to an ongoing cycle, this study offers endocervix miRNA expression as a less-invasive possible marker for implantation window. It may have wide implication in clinical practice and could be a decisive factor for either transferring embryos in same cycle and cryopreserving them and postponing transfer to subsequent cycle if endometrium is not well prepared.

Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by "Fundação de Amparo à Pesquisa do Estado de São Paulo", Brazil (FAPESP) Proc. number 2012/16911-0. There is no interest conflict related to this study.

Trial registration number: NA.

Keywords: endometrium, endocervix, microRNA, implantation window, IVF

P-352 Ethanol sclerotherapy of ovarian endometriomas before IVF: long term data on safety and efficacy

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Study question: To evaluate long-term safety and efficacy of ethanol sclerotherapy (EST) in the treatment of ovarian endometriosis before IVF.

Summary answer: EST of endometriomas appears to be a safe procedure associated with a low recurrence rate and a good fertility outcome.

What is known already: Conventional surgical treatment of endometriomas may decrease ovarian reserve and response to subsequent fertility treatments. This is particularly true in patients with advanced stage endometriosis, who have had multiple previous ovarian surgeries. In recent years, minimal invasive techniques like ethanol sclerotherapy were developed to minimize the effect of surgery on ovarian tissue. However, reports on safety and efficacy are still lacking.

Study design, size, duration: A prospective cohort study was conducted from October 2004 to December 2014, including a total number of 107 patients undergoing 129 ethanol sclerotherapy procedures. The mean follow-up period was about two years (range from 0.5 to 7.5 years). Six patients were lost to follow-up.

Participants/materials, setting, methods: Candidates for IVF presenting with severe endometriosis and one to four endometriomas with a large diameter of 25 to 65 mm, were included in the study. After 12 days of pituitary desensitization by GnRH agonists, EST was performed in an outpatient basis and ovarian stimulation was started 15 days later.

Main results and the role of chance: The mean patients' age was 33.2 years. The mean diameter of endometrioma was 44.6 mm. The procedure was successful in 95.4% of cases, and was globally well tolerated (visual analog scale of 2.9) under local anesthesia. We did not observe any major complication (infection, hemorrhage, ...), and all cysts' fluid cytologies were benign.

Recurrence rate, defined on ultrasound as a cystic image of more than 20 mm on the previously treated ovary, was estimated at 7.0% (8/115) on the 3-months' visit and at 13.8% (16/116) at the end of follow-up. Risk factors for recurrence were analysed by a Cox proportional hazards model. Pregnancy rate was 45.6% (36/79) after the first cycle of IVF and cumulative pregnancy rate at 1 year was 64.4% (47/73), including nine spontaneous pregnancies.

Limitations, reason for caution: Follow-up period which varied between patients and cases lost to follow up might underestimate recurrence rate. Analysis of survival data accounting for censored observations was consequently done.

Wider implications of the findings: This is one of the biggest series of EST in women undergoing fertility treatments. Results on safety and efficacy encourage us to consider this treatment as an alternative option to conventional surgery prior to IVF in future randomized trials.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Authors declare no competing interests. Data collection and analysis was funded by the obstetrics and gynecology department of Bichat Hospital.
Trial registration number: NA.
Keywords: ethanol sclerotherapy, endometrioma, IVF, endometriosis, pregnancy

P-353 Correlation between the ultrasound elastographic features of endometriotic rectovaginal nodules infiltrating the rectum and symptoms

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Study question: Do the elastographic features of endometriotic rectovaginal nodules infiltrating the rectum correlate with symptoms?
Summary answer: The stiffness of endometriotic rectovaginal nodules infiltrating the rectum assessed by elastography correlates with the intensity of dyschezia and the severity of intestinal symptoms.
What is known already: Transvaginal ultrasonography has high specificity and sensitivity in diagnosing rectovaginal nodules infiltrating the rectum. No data is available on the elastographic features of these nodules.
Study design, size, duration: This prospective study was performed between June 2014 and December 2014 and included 16 patients with rectovaginal nodules infiltrating the rectum diagnosed by transvaginal ultrasonography. A standard ultrasonography machine (Voluson E6, GE Healthcare) equipped with transvaginal probe and software for elastography was used.
Participants/materials, setting, methods: Patients with previous surgery for bowel endometriosis and those under hormonal therapies were excluded from the study. The elastograms were classified into 5 patterns according to the distribution of the blue area in the nodule (from score 1, low stiffness to score 5, high stiffness).
Main results and the role of chance: The mean (±SD) age of the study population was 33.3 (±6.2) years. The mean largest diameter of the endometriotic nodule was 27.8 (±7.8 mm); the mean volume of the nodule was 6.6 (±5.1) cm^3. The mean intensity of dyschezia (measured on a VAS scale) was 4.2 (±2.4) cm. The mean total gastrointestinal symptoms score was 28.6 (±15.7) cm. The elasticity score was ≤3 in 5 patients, 4 in 4 patients and 5 in 7 patients. The elasticity score was significantly correlated with the intensity of dyschezia (Spearman's correlation coefficient = 0.839; $p = 0.01$) and with the gastrointestinal symptoms score (Spearman's correlation coefficient = 0.774; $p = 0.01$).
Limitations, reason for caution: Only patients with rectal nodules were included in the study; therefore, the findings of this study cannot be applied to patients with endometriotic nodules located in other bowel segments. Another limitation of this study is the small sample size.
Wider implications of the findings: If the findings of this study will be confirmed by larger investigations, elastography should be introduced in the ultrasonographic evaluation of rectal endometriotic nodules. Future studies should assess the changes in the elastographic features of these endometriotic nodules during hormonal therapies.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Piazza della Vittoria 14 S.r.l.
Trial registration number: NA.
Keywords: rectal endometriosis, transvaginal ultrasonography, elastography, symptoms

P-354 Old and new "protagonists" on the ultrasound "scene" for the diagnosis of deep pelvic endometriosis: evidence-based algorithm based on systematic literature review and meta-analysis

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Study question: In the suspicion of deep-pelvic-endometriosis (DPE), which is the most appropriate ultrasound technique for diagnosis in each specific anatomical location? Beginning from evidences in literature, is it possible to establish a diagnostic algorithm with relation to the accuracy, reproducibility and required skill/learning-curve of each of the proposed techniques?
Summary answer: Transvaginal sonography (TVS) is an easily reproducible and well tolerated and is widely considered as first line diagnostic technique to evaluate patients with a suspicion of DPE. When TVS is inadequate for diagnosis, second-line "modified techniques" are recommended. However, these techniques, though fascinating, require further validation especially with regards to reproducibility.
What is known already: Evidences have suggested TVS as a good method to identify DPE as it is well accepted and widely available. However, its accuracy is generally lower for an accurate assessment of DPE severity when compared to MRI (magnetic-resonance-imaging). Considering the high prevalence of DPE, a routine MRI to evaluate DPE may not be cost-effective. For this reason, new proposals regarding "modified-technique" rather than "standard-TVS" are emerging to the reach MRI accuracy using ultrasound imaging.
Study design, size, duration: We have performed a Systematic literature review and meta-analysis (interval-time 1998–2004) of all prospective, observational and retrospective studies that provided clear and complete data regarding the sensitivity, specificity, positive/negative predictive value (PPV, NPV), accuracy and likelihood ratio of all ultrasound imaging techniques for the diagnosis and localization of DPE.
Participants/materials, setting, methods: The ultrasound techniques described in this systematic review are TVS, RES (rectal ultrasonography)and "TVS-modified technique" such as SCSV (saline-contrast-sonovaginography), TG-TVS (tenderness-guided-TVS), RWC-TVS (rectal-water-contrast TVS), TVS with bowel preparation, TVS sliding sign. The methodological quality of each study was evaluated with QUADAS-2. The odds-ratio was reported as 95% CI using both random-effect and fixed-model.
Main results and the role of chance: Using our key search strategy 35 manuscripts resulted eligible for systematic review and, of these, 32 were eligible for statistical evaluation. Specificity of TVS technique was greater than 85% for all DPE-sites while sensitivity ranged between 50% (bladder, vaginal-wall and recto-vaginal-septum) and 84% (rectum-sigmoid). Regarding the modified-TVS-techniques, the TG-TVS demonstrated the best accuracy for the diagnosis of bladder lesions. RES and RWC-TVS seems superior to TVS in detecting Recto-sigmoid-endometriosis with a sensitivity and specificity of 92.6 and 95%. Furthermore promising data was reported in the use of RCW-TVS (97.1% sensitivity) and SCVS (84.5% sensitivity) in the assessment of recto-vaginal septum endometriosis While SCVS seems to be the most accurate method for the diagnosis of USL, VW and VF endometriosis, the RES and TVS-sliding sign showed a sensitivity twice higher than TVS in evaluating POD endometriosis.
Limitations, reason for caution: The main limitation of this study is the small sample size of available studies and the lack of confirmed reproducibility of the technique itself. Furthermore, a great number of the "modified techniques" show excellent accuracy in detecting DPE in certain anatomical locations as opposed to others.
Wider implications of the findings: Our data allows us to suggest that TVS-modified techniques and RES should be performed by highly skilled and trained sonographers in a dedicated setting for diagnosis and management of endometriosis. These techniques should be offered when standard TVS is "non-conclusive" in the evaluation and specification of DPE localization. In adequate settings, trained clinicians may offer second level ultrasound-scans with a similar or better accuracy than MRI.
Study funding/competing interest(s): Funding by University(ies) – Authors declare no funding. Authors declare no competing of interest.
Trial registration number: Not required.
Keywords: deep pelvic endometriosis, ultrasound imaging, evidence based medicine, diagnostic accuracy, patient satisfaction

P-355 Concomitant endometriosis in malignant and borderline ovarian tumours

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Study question: What is the prevalence of concomitant endometriosis in malignant and borderline ovarian tumours?
Summary answer: The prevalence of endometriosis coexisting in women with malignant and borderline ovarian tumours is 7.3%; concurrent endometriosis is seen in 12% of women with borderline ovarian tumours and 6% of those with malignant ones.
What is known already: Clear evidence exists in the literature revealing that endometriosis is strongly linked to ovarian cancer. However, risk increase varies widely among studies. Clinical characteristics of the patients included are different. Moreover, risk factors and clinical utility of this association are ill-defined. On the other hand, counselling and treating women with endometriosis who are at high risk for cancer coexistence or conversion is encouraged.
Study design, size, duration: Retrospective cohort study. A total of 661 patients with malignant and borderline ovarian tumours were recruited between 1995 and 2011.
Participants/materials, setting, methods: A total of 661 patients with borderline and malignant ovarian tumours were evaluated and endometriosis association was sought. Clinical characteristics of patients with endometriosis-associated borderline ovarian tumours were compared to those of endometriosis-associated malignant ones. Clinical characteristics of patients with endometrioid and clear cell ovarian tumours with or without endometriosis were further analysed.
Main results and the role of chance: Forty-eight (7.3%) of the 661 malignant and borderline ovarian tumours had been associated with endometriosis. The most frequently endometriosis-associated subtypes were endometrioid (33%) and clear cell (18%) histologies. Concomitant endometriosis in endometrioid/clear cell carcinoma did not evoke poor prognosis. Of endometriosis-associated endometrioid and clear cell ovarian tumours, 70% were early stage, at younger age and 60% premenopausal. The prevalence of concomitant endometriosis in borderline tumours (12%) was significantly higher than that in malignant ovarian tumours (6%; $p = 0.02$). Of 48 endometriosis cases, 73% were atypical and 27% were typical. Of 32 endometriosis cases associated with malignant ovarian tumours, 69% were FIGO stage I-II and 31% were FIGO stage III. Infertility was noted in 38% of endometriosis-associated ovarian tumours.
Limitations, reason for caution: None.
Wider implications of the findings: The results are in accordance with the literature. Endometriosis association with borderline ovarian tumours is significantly high. Future research is warranted to identify clinical or biochemical tools for borderline ovarian cancer development or association in endometriosis cases.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Istanbul University, Cerrahpasa University Hospital.
Trial registration number: NA.
Keywords: endometriosis, tumour, borderline, malignant, ovarian cancer

P-356 Proteomic analysis of human endometrium in normal and polycystic ovarian syndrome

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Study question: Are there any proteomic differences between endometrium of healthy women and PCOS patients?
Summary answer: There is a difference signature between proteome profile of endometrium obtained from normal women and PCOS patients.
What is known already: Endometrial receptivity seems to be the major limiting factor for the success of pregnancy in polycystic ovarian syndrome (PCOS).
Study design, size, duration: This study was a basic proteomic analysis of human endometrial biopsies taken from twelve PCOS patients and twelve healthy fertile women in the proliferative (on day 2 or 3 before ovulation, $n = 6$) and secretory (on day 3–5 after ovulation, $n = 6$) phases.
Participants/materials, setting, methods: In this study, for the first time, a 2-DE based proteomic approach coupled with mass spectrometry was used to identify the changes in whole proteins between PCOS and normal endometrium. We analyzed proteome of endometrium during proliferative ($n = 6$) and luteal phases ($n = 6$) from healthy women and PCOS patients ($n = 12$). To validate this investigation western blot and quantitative real time PCR were performed.
Main results and the role of chance: Out of about 802 ± 10 protein spots reproducible detected on gels, 170 proteins showed different intensities between PCOS, proliferative and luteal endometrium. Mass spectrometry analysis of differentially expressed proteins resulted in identification of 70 proteins involved in cellular metabolism, apoptosis and immunological process. Expression of annexin A5 (ANXA5), 14-3-3 protein, antitrypsina, cathepsin D proteins was validated by western blot. The gene expression profile of these proteins was confirmed by real time PCR. The results obtained in the western blot and real time PCR followed a similar regulation of proteomic analysis.
Limitations, reason for caution: The main limitation of this study is a low number of human endometrial samples for the proteomic analysis.
Wider implications of the findings: This study provide the first insight into the global protein expression in the endometrium of PCOS patients as compared to normal women which might affect endometrial receptivity in women with PCOS.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was financed by Royan Institute.
Trial registration number: NA.
Keywords: endometrium, proteomics, PCOS, proliferative phase, secretory phase

P-357 Interactive impact of metformin, dexamethasone and testosterone on endometrial tissue in vitro

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Study question: What are the effects of androgens and its treatment options (dexamethasone and metformin) on functional changes within endometrial stromal cells.
Summary answer: In our study, we demonstrate that the negative testosterone effects, like reduction of endometrial stromal cell proliferation, can be compensated by dexamethasone in vitro under certain conditions. Furthermore dexamethasone alone augmented stromal cell decidualization and proliferation, while metformin exhibits similar effects as androgens do and fortifies their action.
What is known already: Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder, concerning 5–10 % of all women in reproductive age. Its main features are hyperandrogenism, cycle abnormalities with anovulation and distinct ovarian sonographic features. It is often associated with insulin resistance. Treatment options include dexamethasone, as well as metformin to reduce ovarian hyperandrogenism and to restore ovulation. The impact of these therapeutics on the local microenvironment of endometrial cells however remains to be elucidated.
Study design, size, duration: In order to evaluate effects of various concentrations of these medications in a hyperandrogenic microenvironment we used 13 endometrial biopsies from regularly cycling women without endometrial abnormalities or endometriosis after informed consent.
Participants/materials, setting, methods: Endometrial stromal cells were isolated after enzymatic digestion and cultured with serum containing media. Cells were treated with or without testosterone (T-8, T-6), dexamethasone (D-8, D-6) and metformin (1, 0.1, 0.01 mM), either alone or combined. Cells were then decidualized over 2 weeks and thereafter assessed for proliferation, and decidualization capacity.
Main results and the role of chance: Testosterone and low dosed dexamethasone showed no impact on decidualization, determined by stromal prolactin secretion. In contrast, high doses of dexamethasone alone increase the prolactin secretion. This effect vanished under hyperandrogenic conditions. High doses of metformin alone showed a negative effect on decidualization, which intensified under hyperandrogenic conditions and could not be compensated by dexamethasone. While low dose testosterone showed a tendency of proliferation

inhibition, high doses of testosterone significantly reduced proliferation, an effect which could be compensated by addition of high doses of dexamethasone. High doses of dexamethasone alone even promoted proliferation above baseline. While low concentrations of metformin left proliferation unaffected, high doses significantly reduced proliferation. This negative metformin effect could be partially abolished by high-dosed dexamethasone.

Limitations, reason for caution: These effects resemble the conditions in an in vitro culture with a single cell type, namely endometrial stromal cells. We can therefore not give a concrete prediction of effects on the endometrial microenvironment in vivo, if the drug components are systemically applied.

Wider implications of the findings: We confirmed in our study, that a hyperandrogenic state, which is often associated with PCOS lead to a decreased proliferation and decidualization of endometrial cells. This effect however can be partially compensated by treatment with dexamethasone, particularly, when hyperandrogenism is abolished. Metformin on the other hand also decreased proliferation and reinforced the effect observed under hyperandrogenic conditions. The decreased proliferation of metformin could only be partially compensated by dexamethasone.

Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies). Department of Gynecologic Endocrinology and Fertility Disorders at Heidelberg University Women's Hospital, Germany. Merck Serono Funding supported this trial.

Trial registration number: NA.

Keywords: endometrium, PCOS, treatment, decidualization

P-358 Adverse childhood experiences (ACE) – a risk factor for the development of endometriosis?

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Study question: Childhood abuse experiences (CAE) are very common and known to be associated with different general as well as psychiatric diseases/pain symptoms in later life. We aimed to investigate whether CAE might play a role in the development of endometriosis.

Summary answer: Traumatic experiences such as childhood sexual abuse, emotional abuse/neglect and inconsistency in the family of origin were strongly associated with a diagnosis of endometriosis. No such correlation was found for physical abuse/neglect and further adverse experiences (i.e., physical abuse of the mother, drug abuse in family, etc.).

What is known already: Traumatic childhood experiences are associated with adult diseases such as asthma, diabetes, cardiac diseases and many more. Chronic pelvic pain, fatigue and depression, i.e. symptoms of endometriosis, are also correlated with CAE. A possible pathophysiological mechanism could be through immunological modifications found as a consequence of CSA and known to be involved in the development of endometriosis. Therefore, several factors give emphasis to a role of CAE in the development of endometriosis.

Study design, size, duration: The study was designed as a multicenter retrospective case control study. Each control women was matched to a woman with a diagnosis of endometriosis with regard to age and nationality. The study is still ongoing; a total of 423 matched pairs was included for the present evaluation.

Participants/materials, setting, methods: Women with endometriosis and control women (routine annual control) were recruited in university and district hospitals in Germany/Switzerland. A modified version of the childhood trauma questionnaire was used to evaluate adverse childhood experiences. Diagnosis of endometriosis was confirmed by histology and classified according to ASRM criteria.

Main results and the role of chance: Women with endometriosis experienced significantly more often sexual abuse (20/14%, $p = 0.0197$), emotional abuse (44/28%, $p < 0.0001$), emotional neglect (50/42%, $p = 0.0123$) and inconsistency in the family of origin than control women. No statistically significant differences could be demonstrated for physical abuse/neglect (31/26%, $p = 0.1738$). Also combinations of these abuse/neglect experiences were reported significantly more often in women with endometriosis. Further adverse childhood experiences i.e. physical abuse of the mother (8/7%, $p = 0.8222$), drug abuse in the family (5/3%, $p = 0.0943$), mentally handicapped family members (1/1%, $p = 0.7271$), suicidal ideation in the family of origin (6/4%, $p = 0.2879$) and family members in prison (1/1%, $p = 0.1597$) were not statistically more frequent in women with endometriosis than in control women.

Limitations, reason for caution: Recall bias might have influenced our results. Also, some of the control women might present asymptomatic endometriosis, which would likely result in underestimation of our findings. A lack of conscious memories on abuse experiences should have no serious impact on our results as it probably affects both groups.

Wider implications of the findings: On the one hand the present findings, especially when taking the prevalence of for example childhood sexual experiences into account, add an important factor to the list of risk factors of endometriosis. On the other hand they confirm once more that traumatic childhood experiences have a serious impact on adult health. As there are effective strategies to avoid long-term consequences of abuse experiences such experiences should be asked for and treated as early as possible.

Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding.

Trial registration number: USZ_11412.

Keywords: endometriosis, adverse childhood experiences, sexual abuse, emotional abuse, risk factor

P-359 Identification and mapping of somatostatin receptors (ssts) in endometriotic tissues

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Study question: Could the presence of human somatostatin receptors in endometriotic tissues be considered a pathogenetic factor leading to the colonization of several female organs by ectopic endometrium ?

Summary answer: Somatostatin receptors sst_1 and sst_5 are highly expressed in ovarian endometriomata, in peritoneal lesions, in cervical adenomyosis, in subcutaneous endometriotic nodules and in the forms of deeply infiltrating endometriosis as vaginal, intestinal or bladder nodules.

What is known already: Somatostatin is a peptide hormone with affinity for a family of G protein-coupled receptors (sst1–sst5) that acts as inhibitory regulator of cellular functions including hormone secretion, motility, and proliferation. Moreover, sst agonists exert a direct cytostatic effect on normal (i.e., endothelial cells) and tumor cells expressing (ssts) or indirectly by inhibiting the release of growth factors. At the moment there is no data available regarding the status of sst in different forms of endometriosis.

Study design, size, duration: This descriptive study encompass observations from tissues of 50 women that underwent surgery. These patients were among those referred to our Unit for possible endometriosis, chronic pelvic pain and infertility for the last 9 years.

Participants/materials, setting, methods: Immunohistochemistry of sst1, sst2, and sst5 was performed with standard protocols; two pathologists evaluated and graded immunohistochemical staining by scoring separately the percentage of positive cells (endometrial glandular and stromal) using a proportion score (PS) and an intensity score (IS).

Main results and the role of chance: A well-defined sst1, sst2, and sst5 immunoreactivity was demonstrated both in epithelial and stromal cells of endometriotic lesions in superficial layer of peritoneum, ovarian endometriomata, cervical cystic adenomyosis, subcutaneous endometriotic nodules and in all the forms of deeply infiltrating endometriosis as vaginal, intestinal or bladder nodules. All the analyzed lesions with immunohistochemistry were studied *in vivo* by nuclear magnetic resonance and photographed during surgery. Microscopic pictures show a significantly stronger sst1 and sst5 positive staining (PS + IS scores) in endometriotic lesions (sst1: 6.4; sst5: 4.2) compared with the healthy part of the tissues (sst1: 3.1; sst5: 0.8) ($P < 0.05$). The sst2 immunoreactivity appears to be non specific being present ubiquitously and with comparable PS and IS scores both in colonized and disease-free tissues.

Limitations, reason for caution: Our findings may suggest the use of molecules such as somatostatin analogues used for years in other diseases; unfortunately somatostatin plays both physiological and pathological roles in the

female reproductive system and therefore remains difficult to understand its functional relevance in endometriosis.
Wider implications of the findings: Our data show that somatostatin receptors are expressed in all forms of endometriosis and interestingly the sst1 and sst5 receptors characterize the endometriotic lesions in comparison to surrounding healthy tissue both over and under the peritoneal layer. Endometriosis still remains a great challenge for those who attempt to treat it however the presence of these receptors could lead to the use of target therapies for patients affected by the disease.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Galliera Hospital.
Trial registration number: The study was approved by the local committee on human research; no trial registration number was requested for this observational study.
Keywords: endometriosis, somatostatin

P-360 Galectin-1 is over-expressed in endometriotic lesions from patients with minimal to mild endometriosis and promotes eutopic endometrial stromal cell proliferation

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Study question: The main objectives were to evaluate the Galectin-1 protein expression in endometriotic lesions and eutopic endometrium from patients with endometriosis, and to study the potential role of this endogenous lectin to promote cellular proliferation in primary cultures of eutopic endometrial stromal cells from patients with endometriosis and controls.
Summary answer: The protein expression of Galectin-1 was significantly higher in endometriotic lesions than eutopic endometrium, this increase occurred in patients with minimal to mild degree of endometriosis. The endogenous knock-down of Galectin-1 expression diminished cellular proliferation in eutopic endometrial stromal cells from patients with endometriosis but not from control women.
What is known already: The pathophysiologic mechanisms involved in the aetiology of endometriosis are not entirely known yet. Galectin-1 is an endogenous lectin with binding-affinity for multiple *N*-acetyllactosamine disaccharides comprised on *N*- and *O*-glycans involved in several events of tumor biology. The potential pathophysiologic role of this lectin in human endometriosis has not yet been completely elucidated; however, we recently reported that Galectin-1 substantially contributes to endometriotic-like lesions growth and vascularization in an experimental endometriosis mouse model.
Study design, size, duration: All subjects were infertile women undergoing diagnostic laparoscopy, showed regular menstrual cycles and had not received any hormonal medical treatment for the last three months. Endometriotic ($n = 41$) and endometrial biopsies ($n = 27$) were taken from women with endometriosis, and control women with tubal factor infertility, unexplained infertility or leiomyomas ($n = 27$).
Participants/materials, setting, methods: Biopsies of endometriotic lesions and eutopic endometrium were mechanically homogenized and Galectin-1 protein expression was assessed by Western blot. Knock-down of endogenous Galectin-1 expression was performed by RNA interference assay in primary cultures of eutopic endometrial stromal cells, and the cellular proliferation was measured by MTS method.
Main results and the role of chance: There were no differences in the levels of Galectin-1 expression between eutopic endometrium from patients with endometriosis and controls ($p = 0.1037$), but there was a significant increase of Galectin-1 expression in endometriotic lesions compared to eutopic endometrium from patients with endometriosis ($p = 0.0320$). This increase occurs in patients with minimal to mild degree of endometriosis ($p = 0.0368$), since in patients with moderate to severe endometriosis the endometriotic expression of this lectin did not differ significantly from eutopic endometrium ($p = 0.5573$). In addition, the transient reduction of Galectin-1 expression in eutopic endometrial stromal cells has no apparent effect on cellular proliferation in primary cultures from control patients ($p = 0.2335$), but the transient decrease of Galectin-1 expression caused a significant reduction of cellular proliferation in cultures from endometriosis patients ($p = 0.0061$).
Limitations, reason for caution: More studies should be addressed to fully understand the mechanistic behind the results obtained and to evaluate the role of Galectin-1 in other events involved in the endometriosis pathophysiology.
Wider implications of the findings: It is known that at early stages of the endometriosis development the ectopic lesions are highly metabolically active. The increased expression of Galectin-1 in endometriotic lesions from patients with minimal to mild degree of the disease, besides the reduced cellular proliferation observed in Galectin-1 knock-down endometrial stromal cells from patients with endometriosis, support the potential involvement of Galectin-1 in the endometriosis pathophysiology and validate this lectin as a possible target for future therapeutic strategies.
Study funding/competing interest(s): Funding by national/international organization(s) – National Agency for Promotion of Science and Technology (ANPCYT), National Council for Scientific and Technological Research (CONICET) and Roemmers Fundation; Buenos Aires, Argentina.
Trial registration number: NA.
Keywords: Endometriosis, Galectin-1

P-361 Human embryo co-culture up to day 14 on a two-dimension in vitro model

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Study question: Does co-culture of human embryos with Ishikawa cells sustain embryo implantation and trophoblast (TB) outgrowth *in vitro* until day (D) 14 of development?
Summary answer: Human embryos were successfully co-cultured with Ishikawa cells until D14 of development *in vitro*. The embryos attached to the Ishikawa cells and showed outgrowth formation. This outgrowth displayed markers for trophoblast formation and differentiation. More experiments are ongoing to further characterise the outgrowth (specific TB markers, hCG secretion, apoptosis).
What is known already: Implantation models have been set-up, using embryos or blastocyst-like spheroids and different endometrial cells. Ishikawa cell line is a human endometrial epithelial cell line and is considered to be a good model to study normal endometrial function. As far as we know, there is a lack of knowledge in the literature on sustained early embryonic trophoblast outgrowth and the characterisation of this process.
Study design, size, duration: High quality human blastocysts ($n = 35$) were warmed after vitrification. After 2.5 h of recovery, embryos were treated with pronase in order to remove the zona pellucida. On D6, 20 embryos were put into co-culture with the Ishikawa cells to maximum D14 of development *in vitro.*
Participants/materials, setting, methods: This study was approved by the Local and Federal Ethical Committees for research on human embryos. Embryos used for the study were obtained from patients who signed an informed consent to donate their embryos for research after the legal storage period. Embryos were morphologically evaluated and analysed by immunohistochemistry(IHC).
Main results and the role of chance: Blastocysts attached firmly (80%), through polar or mural TB, to the Ishikawa cells after co-culture for 48 h (D8). Following attachment, the blastocysts collapsed and showed outgrowth formation on D9/10. This outgrowth kept on expanding and changing morphologically until D14. Co-culture of embryos on GFP^+-Ishikawa cells confirmed the embryonic origin of the outgrowth. To characterise embryo implantation and outgrowth, IHC-staining for vimentin and E-cadherin was performed. D8 embryos' TB stained positive for nuclear vimentin, except for the attachment site. E-cadherin was found in all TB cells. Co-staining of E-cadherin and nuclear vimentin is an indication for TB invasiveness. Both proteins were absent in D14 outgrowths. The lack of vimentin at this stage might indicate TB differentiation. Preliminary TUNEL-assay analysis indicates that the outgrowth isn't apoptotic.

Limitations, reason for caution: This study is limited by the use of a two-dimension *in vitro* model and the absence of stromal cells. The effect of the cryopreservation on the process of embryo implantation and/or development cannot be excluded.
Wider implications of the findings: A better understanding of the human implantation process and TB differentiation will open doors to study the biology of human reproduction, in particular implantation failure and recurrent miscarriage. It may lead to the development of embryo culture and/or transfer medium to improve implantation and to develop non-hormonal contraceptives to prevent implantation. The outgrowth centre consists of a dark core of cells (most likely post-implantation epiblast and/or hypoblast) that needs to be further characterised.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by national/international organization(s). Agentschap voor Innovatie door Wetenschap en Technologie (IWT). Wetenschappelijk Fonds Willy Gepts (WFWG) University Hospital.
Trial registration number: NA.
Keywords: Ishikawa, trophoblast, human embryo, implantation

P-362 Molecular alterations relevant to cancer stem cell markers in a subset of endometriosis patients: A potential link towards endometriosis associated ovarian cancers

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Study question: Could endometriotic stem cells under go molecular alterations leading to endometrioma associated ovarian cancer?
Summary answer: For the first time, we report increased expression of cancer stem cell (CSC) markers in endometriotic mesenchymal stem cells (MSC) in a subset of endometriotic patients. These genes are known to be involved in pre-malignant functions in ovarian cancer, cancer metabolic pathway and epithelial-mesenchymal transition.
What is known already: Endometriosis is considered as a precursor of ovarian endometrioid and clear cell ovarian carcinoma. Epidemiological studies show a 2- to 3-fold higher risk for ovarian cancer among endometriosis patients. Endometrial adult stem/progenitors have been suggested to be involved in pathogenesis of endometriosis.
Study design, size, duration: MSC from endometrium and endometrioma from patients (paired, $n = 9$; unpaired $n = 5$) and endometrium from healthy volunteers ($n = 14$) were isolated using stem cell markers CD90, CD73 and CD105 by FACS and cultured up to seven passages in monolayer or 3D spheroids to explore CSC phenotypes.
Participants/materials, setting, methods: FACS and quantitative PCR (QPCR) were used to study proliferation and cell cycle. Custom made QPCRs relevant to MSC/CSC was performed and analysed using IPA. Immunofluorescence of spheroids performed in the following combinations: OCT3/CD133, CD44/CD133, ALDH1/CD133 to co-localise MSC and CSC markers. Paired T- test/Wilcoxon test were applied.
Main results and the role of chance: Endomtrioma from 3 patients (high risk group) showed had higher expression levels of Notch3 (FC: 11.19; $P < 0.05$), TP53 (FC: 2.02: $P < 0.05$), mTOR (FC: 1.75; $P < 0.05$). FOS (FC: 1.37; $P < 0.05$), BMI1 (FC: 2.15; $P < 0.05$) and EPAS1/HIF2α (FC: 4.04; $P < 0.05$) were deregulated in cyst MSCs. Eutopic MSCs from patients showed increased cells in S phase and decreased of G0-G1 phase and higher expression ($P < 0.05$) of NOTCH3 (FC: 7.64), CTNNB1/β-catenin (FC: 1.46), TGFβ (FC: 7.41), cKIT/CD117 (FC: 9.38).
Limitations, reason for caution: This study is conducted in a small population of endometriosis patients. A follow up of these 'high risk' patients for ovarian cancer related symptoms is required, if ethics permits.
Wider implications of the findings: This study throws light on understanding the molecular mechanism behind endometriosis associated ovarian cancer. It also opens up possibility to identifying high risk endometriosis patients for ovarian cancer and gives them the opportunity to undergo prophylactic surgery and thus reduce the rate of ovarian cancer, pain and suffering.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). KID grants from Karolinska Institutet, and research grant from Swedish research council (VR) Stockholm, Sweden.
Trial registration number: NA.
Keywords: endometriosis, stem cells, ovarian cancer

P-363 The impact of presence of endometrioma and laparoscopic cystectomy on ovarian reserve tests in comparison to non-endometriotic cysts

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Study question: Are pre- and post-operative ovarian reserve tests different in women who had endometrioma from the women with non-endometriotic benign cysts after laparoscopic cystectomy?
Summary answer: Presence of endometrioma is related with lower preoperative anti-Mullerian hormone levels (AMH) and antral follicle count and the postoperative values of both tests are statistically significantly lower in these patients while this change was not observed in non-enometriotic cysts after surgery.
What is known already: The negative impact of both presence of ovarian endometrioma per se and cystectomy on ovarian reserve has been reported by various authors. These changes were not evaluated in comparison to the non-endometriotic cysts by evaluating the most widely used ovarian reserve tests and the subfactors such as age and bilaterality.
Study design, size, duration: A prospective study including women of reproductive age; 34 with endometrioma and 33 with benign non-endometriotic cysts ≥4 mm followed up for 2 months.
Participants/materials, setting, methods: The day-2 FSH, LH, E2, AMH levels, ovarian volume, antral follicule counts (AFC) of patients with endometriomas (Group 1) and with benign non-endometrotic cysts (Group 2) were determined preoperatively and 2 months after the surgery. The pre- and post-operative values were compared within the same group and between the two groups.
Main results and the role of chance: There was no statistically significant difference between two groups in terms of age (27.1 ± 5.3 vs 24.5 ± 5, $p = 0.058$) and the median cyst diameter (72.5 vs 70.0 mm $p = 0.59$). The incidence of bilaterality was 35.2 and 21.2% respectively. The pre-operative AMH level and AFC were statistically significantly lower in Group-1 in comparison to Group 2 (3.1 ± 1.9 vs 5.7 ± 3.7 ng/ml $p = 0{,}004$, 5 vs 7 $p = 0.025$). The change in pre-post operative AMH levels were similar in patients aged ≥ 35 and <35 in both groups. The pre-operative ovarian volume and AFC were statistically significantly lower in patients who had bilateral cysts in both groups ($p < 0.05$). The post-operative AMH and AFC were statistically significantly lower in Group 1 in comparison to the pre-operative values ($p = 0.004$) while no significant change was found in all ovarian reserve tests in Group 2.
Limitations, reason for caution: A larger sample size is required to generalize the results. A longer follow-up period is required in order to confirm that the decline in AMH levels is not temporary. A non-treated control group from both arms is lacking, so it is not possible to comment on the long-term progressive detrimental effect of presence of endometrioma on ovarian reserve.
Wider implications of the findings: The decrease in AMH level and AFC should be discussed with the patients with endometrioma before surgery without underestimating the negative impact of presence of ovarian endometrioma on ovarian reserve. Bilaterality of ovarian cysts is related with lower ovarian volume and AFC, and this should also be considered before surgery.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Etlik Zubeyde Hanim Maternity and Women's Health Training and Research Hospital.
Trial registration number: Not a RMC study.
Keywords: endometrioma, non-endometriotic cyst, ovarian reserve, laparoscopy

P-364 Fas and fas-ligand in eutopic and ectopic endometrium of women affected by endometriosis: the possible immuno privilege of ectopic endometrium

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Study question: In this study we analyzed the concurrent expression of Fas-Ligand and Fas antigen in the eutopic and ectopic endometrium of women affected by endometriosis throughout the menstrual cycle.

Summary answer: The reduced expression of Fas in the ectopic endometrium with the contemporary higher expression of Fas-Ligand in the corresponding cells suggests a possible immuno privilege of this tissue.
What is known already: The Fas/Fas-Ligand system is an important mediator of apoptosis and it is involved in the maintenance of immuno privilege in several tissues. Immunohistochemical studies showed a stronger staining for Fas and Fas-L in human endometrium epithelial cells during the secretory phase than in the proliferative phase. Few data have been reported in the literature about the expression of Fas and Fas-L in the ectopic endometrial tissue in endometriosis.
Study design, size, duration: This is retrospective study. Tissue specimens were obtained from 33 women who underwent laparoscopic surgery for severe endometriosis and from 18 healthy women. The surgical procedures were carried out from September 2009 through May 2012. The immunohistochemical process was performed at Tor Vergata University Hospital from 2012 to 2014.
Participants/materials, setting, methods: The eutopic endometrium, ovarian endometriomas and peritoneal implants were obtained from 33 women with severe endometriosis (stage IV). Endometrial tissues of 18 healthy women in different phases of the menstrual cycle, obtained during hysteroscopy procedures, were used as controls. Biopsy sample were immunostaining for Fas and Fas-ligand.
Main results and the role of chance: Immunostaining for Fas-Ligand in the eutopic endometrium was positive mostly in the epithelial cells throughout the menstrual cycle, with a stronger staining in the secretory phase. The epithelial cells of endometriotic lesions showed a significantly stronger staining for Fas-Ligand independently from the menstrual phase with respect to the eutopic tissue ($P < 0.01$). Immunostaining for Fas in the eutopic endometrium showed a reduced staining during the entire proliferative phase, whereas it was strong in the secretory phase. The epithelial cells of the ectopic endometrium showed a reduced staining for Fas independently from the menstrual phase with respect to the eutopic tissue that was statistically significant ($P < 0.01$).
Limitations, reason for caution: The limitation of this study is the small number of the analyzed patients. These data should be confirmed in more extensive studies.
Wider implications of the findings: These findings may explain previous reports showing elevated soluble Fas-L levels in peritoneal fluids of women with endometriosis, and suggest that the ectopic endometrium may be a tissue with immune privilege, which may induce apoptosis in Fas positive immune cells.
Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies). Praxis DS, Praxi Provita.
Trial registration number: This is not RCT.
Keywords: endometriosis, Fas-ligand, Fas, ectopic endometrium, eutopic endometrium

P-365 Fourier transform infrared (FTIR) spectroscopy as a new tool to assess molecular changes of human granulosa cells induced by endometriosis

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Study question: Could the FTIR spectroscopy represent a new, reliable and comprehensive tool to evaluate molecular, biochemical and morphological changes on human granulosa cells (HGCs) induced by endometriosis?
Summary answer: This tool applied for the first time on HGCs, provided unique biochemical and morphological information elucidating the effects of ovarian endometriotic cysts on concentration and distribution of several molecules of interest (lipids, proteins, carbohydrates and nucleic acids), on cellular metabolism and not least on epigenetic control.
What is known already: Several studies demonstrated a reduced fecundity of 2–10% among patients with endometriosis associated with a poor ovarian reserve, reduced oocyte retrieval, lower oocyte and embryo quality and impaired implantation. Although endometriosis is generally thought to be related to infertility, its actual impact on fecundity and the molecular mechanisms activated on follicle cells underlying this detrimental effect are less clear.
Study design, size, duration: The study has been conducted between July 2013 and September 2014 on women undergoing a COH for an IVF treatment at Tecnobios Procreazione Bologna, Italy. 18 women had a diagnosis of ovarian endometriosis (staged as moderate to severe (stages III–IV); 16 women referred male infertility and represented the control.
Participants/materials, setting, methods: GCs from follicular fluid aspirates were isolated by Percoll layering. FTIR analysis was performed by using a Bruker Vertex 70 Interferometer with an Hyperion 3000 Vis-IR microscope and an FPA detector. Expression of selected genes was evaluated by Q-PCR and lipids were localized by using BODIPY®FL C16.
Main results and the role of chance: Endometriosis induced changes on HGCs: lipids metabolism and storage: FTIR evidenced an increase of total lipids (25.35% ± 1.27 vs 28.38 ± 0.63%; $P < 0.01$), phospholipids (0.78 ± 0.12 vs 1.71 ± 0.11%; $P < 0.001$) and unsaturated-lipids (1.27 ± 0.14 vs 1.53 ± 0.09%; $P < 0.05$) amount, validated by the increase of Peroxisome-Proliferator-Activated-Receptorg (1.3 ± 0.1 vs 8.12 ± 0.5a.u.; $P < 0.05$), Sterol-Regulatory-Element-Binding-Protein1 (1.35 ± 0.25 vs 6.02 ± 0.35a.u.; $P < 0.001$) and Fatty-Acid-Synthase (1.29 ± 0.38 vs 4.13 ± 0.42a.u.; $P < 0.01$) expression. The different localization and distribution evidenced by FTIR was supported by BODIPY analysis. *Carbohydrates metabolism*: the decreased concentration (6.72 ± 0.32 vs 3.89 ± 0.21%; $P < 0.05$) and distribution of carbohydrates evidenced by FTIR were validated by the decrease of Glucose-Transporter-Type1 (6.23 ± 0.6 vs 1.24 ± 0.2a.u.; $P < 0.05$) gene expression. *Apoptosis/Autophagy:* the increase of cell death spectral biomarker (25.35 ± 1.27 *vs* 28.38 ± 0.63%; $P < 0.05$) evidenced by FTIR were confirmed by the modulation of molecules involved on apoptosis (Caspase3 1.92 ± 0.61 vs 6.57 ± 0.85a.u.; $P < 0,05$, Survivin 33.89 ± 3.21 vs 1.8 ± 0.58a.u.; $P < 0.01$) and on autophagy (Beclin1 1.32 ± 0.22 vs 9.90 ± 1.04a.u.; $P < 0.001$, LC3 1.45 ± 0.31 vs 11.69 ± 1.12a.u.; $P < 0.05$). *Epigenetic control*: the increase of DNA methylation (5.43 ± 0.09 vs 6.98 ± 0.11%; $P < 0.05$) evidenced by FTIR was confirmed by the increase of DNA-Methyltransferase3a (1.32 ± 0.21 vs 7.71 ± 1.76a.u.; $P < 0.01$) expression.
Limitations, reason for caution: This is a preliminary but promising study conducted on a limited number of patients but the enrollment is still on going and study is not concluded.
Wider implications of the findings: This preliminary study, carried out on HGCs, represents the first vibrational approach to evaluate the macromolecular and biochemical changes associated with endometriosis. The findings, obtained by FTIR analysis and validated by conventional molecular tools, deeper elucidated the molecular mechanisms activated by ovarian endometriotic cysts on GCs and increased scientific knowledge in follicle damage due to inflammation. This could be useful in developing new strategies to prevent detrimental effects of endometriosis on fertility.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Tecnobios Procreazione, Bologna Italy. Università Politecnica delle Marche, Ancona Italy.
Trial registration number: Not requested as basic study.
Keywords: granulosa cells, endometriosis, Fourier Transform Infrared spectroscopy

P-366 Perfusion of intralipids may be an alternative treatment to control an endometrial over-immune activation resistant to corticoids in patients with previous repeated embryo implantation failures

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Study question: Which alternative treatment can be proposed by physicians in case of endometrial over immune activation not controlled by corticoids for patients experiencing repeated implantation failures (RIF)?
Summary answer: Perfusion of Intralipids (IL) may be useful to control excess of Th-1 cytokines and hyper-activation of immune cells through a stimulation of local endometrial immunoregulators as TWEAK (TNF weak inducer of apoptosis). After observation of their impact on endometrial biomarkers used to establish the immune profile, observed pregnancy rates were significantly improved.
What is known already: Uterine NK cells (uNK) are crucial for implantation but If they are over activated, uNK cells become cytotoxic (over immune activation) and prevent the embryo implantation. Majority of RIF patients, we previously documented, shows such deregulation of over-immune local activation with some cases inefficacy of corticoids. In such context, Intralipids were

previous described as a therapy able to control the hyper-activation of circulating NK cells and to control excess of Th-1 cytokines.
Study design, size, duration: The study design was a cohort study including 34 RIF patients with a documented over-immune activation resistant to corticoids.
Participants/materials, setting, methods: An endometrial biopsy was performed in the luteal phase on two distinct cycles. The first biopsy for the basic diagnosis of over-immune activation, the second one to assess the impact of intralipids. We quantified uNK cells by immunohistochemistry and mRNA expression of IL-15 (uNK cells maturation state), IL-18 (Th-1/Th-2 cytokines balance) and TWEAK/Fn-14 (immuno-regulation) by RT-PCR. Pregnancy Outcome at the subsequent embryo transfer under IL was recorded for these patients.
Main results and the role of chance: Under intralipids, we observed a significant increase of 76% of the TWEAK mRNA expression ($p < 0.006$, paired sample *T*-test). The increase of local immunoregulator allowed a better local control of Th-1 cytokines (assessed by the IL-18 and -15 expression) and therefore a control of local cytotoxicity. 76% (28/34) were pregnant at their subsequent embryo transfer under IL despite their long story of unexplained RIF.
Limitations, reason for caution: We cannot exclude that the use of a new treatment can have a placebo effect in these patients. Only a randomized controlled trial with placebo may prove that intralipids are an effective treatment of local cytotoxicity in these specific immune profiles.
Wider implications of the findings: Developing preventive personalized strategies based on a clear understanding of each patient's endometrial immune prof is an emerging area of innovation in Reproduction. On that regards, intralipids may be very useful especially in some patients showing an over-immune activation with low expression of local immunoregulators. IL were able to control the cytotoxicity before any embryo transfer.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – MatriceLAB Innove.
Trial registration number: No trial number.
Keywords: embryos implantation failures, uterine receptivity, endometrium, over immune activation, intralipids treatment

P-367 *In vitro* differentiation of human endometrial stromal cells: effect of endocannabinoids in insulin-like growth factor-binding protein-1 and prolactin levels

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Study question: Does the major endocannabinoids (eCBs), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), interfere with human decidualization?
Summary answer: The CB1 and TRPV1 receptors are expressed in both undifferentiated and decidualized human endometrial stromal cells. We also observed that the major eCBs and also the TRPV1 agonist capsaicin, affected human decidualization. The pre-treatment with the CB1 antagonist attenuated the effects induced by AEA and 2-AG.
What is known already: The endometrial stromal cells differentiate and proliferate into morphological and functional distinct decidual cells, which produce bioactive compounds. However, little information is available concerning the critical molecular molecules involved in decidual progress. There is growing evidences that eCBs are important mediators in reproduction, namely in hormonal control and uterine tissue remodelling. Although CB1 is expressed in endometrial stromal cells, the role of endocannabinoids in decidualization process remains unknown.
Study design, size, duration: To investigate the role of the major eCBs, AEA and 2-AG, on *in vitro* decidualization, we used a human immortalized endometrial stromal cell line (St-T1b).
Participants/materials, setting, methods: Decidualizing St-T1b cells were established using medroxyprogesterone acetate and 8-Br-cAMP for 2 and 5 days. The decidual response was characterized by transcriptional activation of prolactine (PRL) and insulin like growth factor binding protein 1 (IGFBP-1). The effects of eCBS in cell viability of undifferentiated and differentiated endometrial stromal cells were analysed by MTT. The expression of cannabinoid receptors was analysed by immunoblotting and their involvement studied with specific antagonists.
Main results and the role of chance: The eCBs, AEA and 2-AG, have no significant effects on cell viability of either undifferentiated or differentiated endometrial stromal cells in the conditions used. Additionally, these eCBs induced a significant down-regulation of IGFBP1 and PRL expression in either day 2 and 5 treated cells. CB1 and TRPV1, but not CB2, are expressed in both primary human endometrial stromal cells and in St-T1b cell line. The pretreatment with CB1 antagonist attenuated the reduction in IGFBP1 and PRL expression induced by AEA and 2-AG. Furthermore, the TRPV1 agonist capsaicin also affected decidual differentiation process. These results reinforces our previous observations showing eCBs-inhibition of rat decidualization either in vitro or in vivo.
Limitations, reason for caution: Our results were obtained with *in vitro* studies performed in cultures of immortalized endometrial stromal cells (St-T1b) by the transfection of telomerase. Further studies are required to support the effects of eCBs in *in vivo* human differentiation. Furthermore, the studies of the signalling pathways affected by eCBs requires additional research.
Wider implications of the findings: Here we present evidences that both eCBs interfere with decidualization process by reducing the mRNA levels of decidual markers, such as, IGFBP1 and PRL, proteins that are relevant for decidual function and, consequently, for pregnancy outcome. Additionally, our findings identify human decidual cells as potential targets for exocannabinoids resulting from cannabis consumption, which may affect endometrial stromal cell differentiation through interference with endocannabinoid system.
Study funding/competing interest(s): Funding by national/international organization(s) – The authors thank Fundação para a Ciência e Tecnologia (FCT) for the grant attributed to Fonseca BM (SFRH/BPD/72958/2010), Costa M (SFRH/BD/70721/2010)and Almada M (SFRH/BD/81561/2011).
Trial registration number: NA.
Keywords: endocannabinoids, decidualization, endometrium, anandamide, 2-arachidonoylglycerol

P-368 A Comparison of Hysterosalpingo-foam sonography (HyFoSy) and Hysterosalpingo-contrast sonography (HyCoSy) in the assessment of tubal patency

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Study question: To compare the efficacy of ExEm foam (HyFoSy) with saline solution (HyCoSy) as a contrast medium for hysterosalpingsonography in subfertile patients.
Summary answer: HyFoSy with ExEm foam medium increased diagnostic confidence in the evaluation of tubal patency and reduced the proportion of false occlusion results in tubes examined by HyCoSy.
What is known already: HyCoSy is operator- dependent with a high false occlusion rate. It is not possible to differentiate between true tubal occlusion and occlusion due to tubal spasm or suboptimal visualisation. It is often only possible to demonstrate proximal patency with HyCoSy by visualising paracornual flow. Tracing the flow of saline through the entire tubal length is more informative but difficult. A positive contrast agent may allow better delineation of tubal anatomy and reduce false occlusion results.
Study design, size, duration: A single- blind pragmatic randomised cross- over trial was done to compare the efficacy of saline (HyCoSy) with foam medium consisting of ExEm-gel and water (HyFoSy) for hysterosalpingsonography in 40 subfertile patients from April 2014 to January 2015.
Participants/materials, setting, methods: Randomisation was done using computer- generated block randomization. Quality of visualisation was assessed as follows: peritoneal spillage of contrast, forward flow along the entire tubal length and forward flow at the paracornual region. Absent cornual flow suggested possible tubal occlusion and prompted sequential crossover testing with the other medium.
Main results and the role of chance: 36 participants were recruited. There were no significant differences in baseline characteristics between the two groups. A higher proportion of tubes in the HyFoSy group demonstrated good evidence of complete tubal patency (peritoneal spillage or forward flow along the entire tubal length) (60.0 vs 29.5%, $p = 0.045$). A higher proportion of tubes in the HyCoSy group demonstrated possible tubal occlusion (47.7 vs 25.7%, $p = 0.045$). After crossover evaluation, 43.5% of possibly occluded tubes in the HyCoSy group were re- classified as patent when examined with ExEm foam,

compared to 9.1% of possibly occluded tubes in the HyFoSy group examined with saline (p = 0.052). No significant complications were reported. Results subject to change pending recruitment of target sample size and further statistical evaluation.
Limitations, reason for caution: Evaluation of diagnostic accuracy was not possible because of small sample size and lack of comparison with gold standard tests. Selective crossover without a washout period may cause bias when evaluating the effect sizes of each study arm on results. However this is more clinically applicable and avoids additional testing when tubes are patent.
Wider implications of the findings: HyFoSy with ExEm foam medium is more efficacious as a first- line test for tubal patency and may lead to a smaller proportion of patients requiring second- line tubal evaluation by increasing diagnostic confidence and reducing the proportion of false occlusion results.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Neuvital Pte Ltd – provided HyFoSy samples.
Trial registration number: U1111-1165-7309.
Keywords: hysterosalpingosonography, tubal patency testing

P-369 VIP induces the decidualization program on human endometrial stromal cells and conditions dendritic cells profile

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Study question: To investigate VIP (vasoactive intestinal peptide) contribution into the decidualization program, from phenotypic and functional aspects and its effect on dendritic cells (DC) immune-profile. Here, we used an in vitro implantation model based in the co-culture of blastocyst-like spheroids from trophoblast cells cultured on hESC monolayer decidualized with VIP.
Summary answer: Our results suggest that VIP contributes to the decidualization process on hESC cells inducing phenotypic markers and chemokines expression. From the functional aspect, VIP-decidualizated hESC cells allow the blastocyst-like spheroides invasion and condition Dendritic cells to a tolerogenic profile preventing the increase of costimualtory molecules and inducing IL-10 secretion.
What is known already: The decidualization program involves phenotype and functional changes on endometrial cells that facilitate the attachment and invasion of the blastocyst. Although progesterone is a key hormone, the trigger of this process is an intracellular cAMP increase. The decidualization involves the modulation of different mediators such as cytokines, chemokines and growth factors. Particularly, vasoactive intestinal peptide (VIP) is a neuropeptide produced by endometrial stromal cells among others, and displays multiple target circuits that allow immunotolerance.
Study design, size, duration: We used an in vitro implantation model based in the co-culture of blastocyst-like spheroids from trophoblast cells line Swan71 cultured on hESC monolayer decidualized with VIP.
Participants/materials, setting, methods: Human endometrial stromal cell line (hESC) was cultured with/without VIP or MPA + dbcAMP. VIP/VPAC system and decidualization markers were evaluated by RTPCR and ELISA. Blastocyst-like spheroids (BLS) were obtained from Swan71cells and cultured on hESC monolayer. Monocytes were isolated from PBMCs, differentiated to DC and studied by FACS.
Main results and the role of chance: The MPA + dbcAMP decidualization increased VIP expression and secretion ($p < 0.05$). When decidualization was induced by VIP, we observed an increase of markers IGFBP1, PRL, KLF13/KLF9 ratio, IL-8 and SDF1 expression in a concentration-dependent manner ($p < 0.05$). To evaluate functional aspects of VIP-decidualization, an invasion assay was performed. BLS were able to invade hESC monolayer decidualizated with VIP or MPA + dbcAMP. When these assays were performed with conditioned media obtained from human blastocyst we found an increase in BLS invasion on VIP-decidualizated hESC ($p < 0.05$). Finally, we evaluate the immunomodulatory effects of decidualized cells on DC and monocytes. Decidualized hESC conditioned media induced a semi-mature profile on DC preventing the induction of CD83 and CD86 expression, and increasing IL-10 secretion ($p < 0.05$). Monocytes profile was not modulated by conditioned media.
Limitations, reason for caution: The present results were studied using immortalized cell lines. Further studies are necessary to elucidate the precise mechanisms involve and the role of VIP in decidualization.
Wider implications of the findings: Our results suggest that VIP may have an important role during the decidualization process as it is able to induce the differentiation on the stromal cells, not only from the phenotypic aspect but also from a functional one, by allowing blastocyst invasion while contributing to control the immune micro-environment by inducing a tolerogenic profile on DC. We propose that VIP may be a new participant in this differentiation program.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Universidad de Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Tecnológicas.
Trial registration number: NA.
Keywords: VIP, decidualization, endometrium, immunotolerance, DC

P-370 Interleukin-7 (IL-7), Interleukin-17 (IL-17) and their receptors' (IL-7R and IL-17R) endometrial expression during implantation window, in fertile women and infertile patients

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Study question: Can we identify endometrial expression differences for IL-7, IL-17 and their receptors (IL-7R and IL-17R) between fertile women and patients experiencing repeated implantation failures or repeated miscarriages?
Summary answer: We found a significant lack of endometrial expression for the receptors IL-7R and IL-17R in infertile patients, with repeated implantation failures or repeated miscarriages.
What is known already: Switch of endometrial immune actors from an adaptative to an innate type of immunity during the implantation window is essential for both Human implantation and placentation. IL-7 like cytokines are identified in maternal-foetal interface where they are expected to promote dendritic cells and a Th2 oriented environment, crucial for a successful implantation. IL-17 is also localised in this interface and is foreseen to regulate local immunological homeostasis.
Study design, size, duration: To help understanding their hypothetical implication on embryo implantation, we decided to identify endometrial mRNA expression differences for IL-7, IL-17 and their receptors between fertile women and patients experiencing repeated implantation failures or repeated miscarriages. 51 patients were included after informed consent and Institutional Review Board approval.
Participants/materials, setting, methods: We included 17 fertile women, 19 patients with repeated implantation failures and 15 patients with repeated miscarriages. Total RNA extractions were made on middle luteal phase endometrial biopsies (days 21–24 of a natural cycle). Endometrial mRNA expression for IL-7, IL-17, IL-7R and IL-17R were studied by Real Time-PCR.
Main results and the role of chance: Endometrial mRNA expressions for IL-7 and IL-17 were very low and we observed no variation between infertile patients and the control fertile group. For the receptors, IL-7R and IL-17R, we found at significantly lower mRNA levels in infertile patients, either with implantation failures or miscarriages ($p = 0.02$ for IL-7R and $p = 0.01$ for IL-17R).
Limitations, reason for caution: Pathways of endometrial actions of IL-7 and IL-17 still need to be elucidated.
Wider implications of the findings: The endometrial lack of expression observed in infertile patients suggests the involvement of the local immune regulation systems linked to IL-7 and IL-17 in a successful implantation. These pathways seem crucial for the communication between innate immune cells and endometrial stromal cells. Defining the optimal endometrial environment at the time of implantation may involve biomarkers documenting pathways related to dendritic and T regulatory cells. The observed deregulations may be informative in that regard.
Study funding/competing interest(s): Funding by national/international organization(s) – Institut national de la santé et de la recherche médicale (INSERM), Agence de la Biomédecine (ABM).
Trial registration number: 2013-A00072-43.
Keywords: endometrial receptivity, embryo implantation, reproductive immunology, interleukin-7, interleukin-17

P-371 Endometrial injury performed during the cycle preceding ovarian stimulation increases the biochemical pregnancy rate in unselected infertile women undergoing in vitro fertilization: a randomized placebo controlled trial

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Study question: Does endometrial injury has an effect on reproductive outcome, performed during the cycle preceding ovarian stimulation in unselected infertile women undergoing in vitro fertilization (IVF)?
Summary answer: Local injury to the endometrium during the cycle preceding ovarian stimulation for IVF in unselected infertile women increases the biochemical pregnancy rate.
What is known already: Endometrial injury is defined as intentional damage to the endometrium, such as biopsy or curettage, in women undergoing assisted reproduction technology (ART) to improve endometrial receptivity. Significant improvements in implantation rates, clinical pregnancy rates and/or live birth rates on women with repeated implantation failure following endometrial injury performed in the preceding cycle were reported.
Study design, size, duration: This randomized placebo controlled trial recruited 145 unselected infertile women scheduled for IVF/ICSI treatment between March 2012 and August 2014. Subjects were randomized into endometrial injury group (n = 73) and placebo group (n = 72) according to a computer-generated randomization list.
Participants/materials, setting, methods: Subjects were recruited and randomized in the Department of Reproductive Medicine at Beijing Obstetrics and Gynecology Hospital, Capital Medical University. A total of 145 patients, <40 years old, in their first *in vitro* fertilization (IVF) cycle were randomized to two groups: endometrial injury group and placebo group. In 73 patients, endometrial biopsies were performed using a Pipelle catheter on days 9–12 or 21–24 of the menstrual cycle preceding IVF treatment. In 72 control patients, a same catheter was performed as the endometrial injury group without taking biopsy. All women were treated with a cycle of IVF/ICSI. Pregnancy outcomes were compared.
Main results and the role of chance: There were no significant differences in baseline or cycle characteristics between the two groups. The biochemical pregnancy rates of endometrial injury group [53.4% (39/73)] was significantly higher than that of placebo group [33.3% (24/72)] [RR 2.294 (95% CI 1.172–4.492), P = 0.015]. The implantation rates [23.9% (38/159) versus 18.4% (28/152); RR 1.391 (95% CI 0.804–2.407), P = 0.238], clinical pregnancy rates [37.0% (27/73) versus 30.6 (22/72); RR 1.334 (95% CI 0.669–2.662), P = 0.413], miscarriage rates [15.4% (6/39) versus 16.7% (4/24), RR 0.917 (95% CI 0.142–5.925), P = 0.927], live birth rates [26.0% (19/73) versus 23.6% (17/72), RR 1.138 (95% CI 0.535–2.421), P = 0.736] and multiple pregnancy rates [29.6% (8/27) versus 22.7% (5/22), RR 1.432 (95% CI 0.392–5.226), P = 0.586] were all compared between the endometrial injury group and placebo group.
Limitations, reason for caution: All of our recruited women were having their first IVF cycles, the results may not be generalizable to all women undergoing IVF. The mechanism of endometrial injury on endometrial receptivity need to be studied further.
Wider implications of the findings: According to the results of this study, local injury to the endometrium during the cycle preceding ovarian stimulation for (IVF) in unselected infertile women increases the biochemical pregnancy rate and may have a positive impact on endometrial receptivity.
Study funding/competing interest(s): Funding by national/international organization(s) – This project was funded by National Natural Science Foundation Project (81471520), State Scholarship Fund (2011911033), Beijing Natural Science Foundation Project (5122015), and Beijing Project of Training High-level Medical Technical Personnel in Health System. The authors declare no conflict of interest.
Trial registration number: No.
Keywords: endometrial injury, endometrial receptivity, endometrium, in vitro fertilization, pregnancy rate

P-372 Soft microenvironments inactivate the fibrotic phenotype of endometriotic stromal cells

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Study question: Are deep infiltrating endometriotic fibroblasts influenced by changes in extracellular matrix (ECM) stiffness?
Summary answer: Increased matrix stiffness may promote cell proliferation, collagen synthesis and induce myofibroblasts differentiation of endometriotic stromal cells. Soft microenvironments may preserve the quiescent fibroblast phenotype of endometriotic stromal cells.
What is known already: Deep infiltrating endometriosis is histologically characterized by dense fibrous tissue. Tissue stiffening is a hallmark of fibrosis. However, previous in vitro studies typically analyzed cells grown on rigid plastic or glass substrates with stiffness in the Giga-Pascal (GPa) range, which are much stiffer than those occurring in vivo. To investigate how changes in ECM stiffness affect endometriotic fibroblasts, it is critical to model in vivo tissue compliance conditions in vitro.
Study design, size, duration: For this laboratory study, endometrial and/or endometriotic samples from 38 patients who had histological evidence of deep infiltrating endometriosis were analyzed.
Participants/materials, setting, methods: Expression of F-actin, alpha smooth muscle actin, ki67 and procollagen type I in endometriotic stromal cells on polyacrylamide gel substrates of varying stiffness (between 2 and 30-kPa) were determined by immunofluorescence confocal microscopy. mRNA expression of collagen type I, Cyclin D1, MMP-1 and MMP-14 was measured by real-time PCR.
Main results and the role of chance: Increased matrix stiffness induced F-actin stress fiber formation and expression of alpha smooth muscle actin in stress fibers in endometriotic stromal cells. Furthermore, increased stiffness promoted cell area, ki67 expression, cyclin D1 and collagen synthesis and decreased MMP-1 and MMP-14 mRNA expression in endometriotic stromal cells. Treatment with transforming growth factor-β1 increased collagen synthesis. On soft substrates, endometriotic stromal cells exhibited a small rounded morphology with diffuse labeling for F-actin. Expression of ki67, procollagen type I, collagen type I and Cyclin D1 were significantly decreased, whereas that of MMP-1 and MMP-14 was significantly increased, in endometriotic stromal cells cultured on a soft substrate (2-kPa) compared to those on a stiff substrate (30-kPa).
Limitations, reason for caution: A tremendous gap remains between the present in vitro model and in vivo endometriotic tissues. Cell cultures more closely mimics the cellular complexity typical of in vivo endometriotic tissues are required to develop novel strategies for treatment of deep infiltrating endometriosis.
Wider implications of the findings: Hormonal suppressive therapy is not usually very effective for deep infiltrating endometriosis. Interrupting mechanical interactions between endometriotic fibroblasts and aberrant ECM may be a novel strategy for treatment of deep infiltrating endometriosis.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study was supported in part by Karl Storz Endoscopy & GmbH (Tuttlingen, Germany).
Trial registration number: NA.
Keywords: endometriosis, endometrium, extracellular matrix stiffness, fibrosis

P-373 A Mock Embryo transfer is as effective as an Endometrial Scratching to aid Implantation

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Study question: To determine whether a Mock Embryo Transfer (ET) performed the cycle before an IVF/ICSI treatment cycle is as effective at aiding implantation as a formal endometrial scratching using an endometrial biopsy catheter (Pipelle de Cornier) following a Mock-ET the cycle before an IVF/ICSI treatment cycle**.**
Summary answer: A Mock-ET performed in the luteal phase the cycle prior to commencing IVF/ICSI is as effective as a formal endometrial scratching. Subgroup analysis demonstrated that endometrial scratching resulted in statistically significant lower clinical pregnancy rates in the second cycle and had no benefit in those with recurrent failed implantation.

What is known already: Endometrial scratching causes physical trauma to the endometrium using devices such as a biopsy pipelle, aiming to improve implantation rates. It is believed this trauma results in a significant release of cytokines, growth factors and interleukins inducing decidualisation and receptivity of the endometrium creating a favourable environment for implantation. This should not be performed during the same cycle as the ET, however when in the menstrual cycle and degree of injury is still being debated.
Study design, size, duration: A retrospective cohort study of all fresh IVF and ICSI cycles performed between the 1st of January 2013 and 31st of December 2014 at the Hull IVF unit. 622 fresh cycles were performed during this period and no patients were lost to follow-up.
Participants/materials, setting, methods: In 2013 all patients underwent a Mock-ET during the luteal phase of the cycle before IVF, to measure the length of the uterine cavity and to document any difficulties that maybe experienced at the time of ET. In 2014 endometrial scratches were performed after the Mock-ET the cycle before IVF.
Main results and the role of chance: 622 fresh IVF/ICSI cycles were performed. There was no significant difference in patient demographics between the 2 groups comprising age, BMI, AMH, endometrial thickness and embryo quality. There were 133 clinical pregnancies in the mock-ET group and 105 in the Mock-ET and endometrial scratching group demonstrating no significant difference $p = 0.099$.

Subgroup analysis highlighted that there was a significant difference in pregnancy rates in the 2nd cycle between the two groups $p < 0.03$, indicating that the endometrial scratching resulted in a significant fall in clinical pregnancy rates. Endometrial scratching patients with repeated failed implantation, cycles 3 to 5 demonstrated no significant increase in clinical pregnancy rates compared to the Mock-ET only group, $p = 0.414$. This was also true for patients undergoing their 1st cycle of IVF/ICSI, $p = 0.518$.
Limitations, reason for caution: This study does not take into account conflicting variables such as ease/difficulty of embryo transfer, rigidity of catheter and cervical dilatation.
Wider implications of the findings: We feel that a Mock-ET cause's sufficient injury to the endometrium to aid implantation, thus avoiding an endometrial scratching that is not without complications such as infection and uterine perforation, and in some instances may require cervical dilatation. The literature remains divided on endometrial scratching but we feel we are the first group to highlight that it is no more beneficial than a mock-ET and in sub-group analysis may in fact be detrimental.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The Hull IVF Unit.
Trial registration number: NA.
Keywords: implantation, mock embryo transfer, endometrial scratching

P-374 A prospective randomized controlled study (RCT) of Intra-uterine administration of Granulocyte Colony-Stimulating Factor (G-CSF) before embryo-transfer on resistant thin endometrium in IVF cycles

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Study question: Does intrauterine administration of G-CSF (granulocyte colony-stimulating factor) prior to Embryo Transfer in patients with resistant thin endometrium improve endometrial thickness and pregnancy rate in IVF cycles?
Summary answer: Yes, intrauterine administration of G-CSF (granulocyte colony-stimulating factor) before embryo transfer in patients with resistant thin endometrium increases endometrial thickness and improves the pregnancy rate in IVF cycles.
What is known already: Optimal endometrial thickness reflects an adequate maturation, which is a key factor for embryo implantation. Proliferative and secretory changes in the endometrial lining are the result of a complex intrauterine environment where sex steroid hormones and different local factors play an important role for endometrial thickening. Lucena and Moreno-Ortiz found that the uterine infusion of G-CSF quickly increased endometrial thickness resulting in a successful pregnancy and healthy born baby. Li *et al*. aimed to evaluate the effectiveness of G-CSF administration for infertile women with a thin endometrium in a frozen ET program. These results suggested that G-CSF is a factor that participates during endometrial remodeling enhancing the synchronization between uterine environment and embryo development.
Study design, size, duration: 48 Infertile patients with thin endometrium younger than 42 years from Jan.2014 to Dec.2014 were included in this study. All using traditional treatments with estradiol and sildenafil citrate (Viagra) had been unsuccessful. Patients were randomly divided into two groups using a computer generated list. The study group received intrauterine administration of G-CSF (300 mg/ml) and control group received placebo-saline before Embryo Transfer in IVF cycles. *Primary Outcome Measures:* Endometrial thickness, implantation rate and pregnancy rate. *Secondary Outcome Measure:* Miscarriage rate.
Participants/materials, setting, methods: The study group ($n = 24$) received intrauterine infusion of 300 µg/ml of G-CSF, and the control group ($n = 24$) underwent placebo-saline infusion before Embryo Transfer. G-CSF was administered per intrauterine catheter by slow infusion before noon on the day of hCG administration. If the endometrium had not reached at least a 7-mm within 48 h, a second infusion was given following oocyte retrieval.
Main results and the role of chance: The endometrial growth was significantly different within the two groups. An improvement was shown between the control and G-CSF infused groups. Endometrial expansion to minimal thickness occurred within approximately 48 – 72 hour from G-CSF infusion. In all the subjects at the time of infusion of G-CSF, endometrial thickness was 6.49 ± 1.65 mm, and, after infusion, it increased significantly to 8.79 ± 1.57 mm. The IR and PR were statistically significantly higher in the group that received intrauterine infusion of G-CSF (23 and 33%, respectively) as compared with the control group (12 and 16%, respectively).
Limitations, reason for caution: A relatively new concept in thin endometrium, requiring more multicentric trials worldwide. Our study is not without limitations. Firstly, we did not have a large number of patients. Secondly, we applied Aspirin and/or Sildenafil citrate which also could have a positive effect on endometrial thickness. We can only speculate that the other factors could have impact on endometrial thickness. But we do not believe that this could have essential impact on our results. Wider implications of the findings: Uterine perfusion with G-CSF represents a promising new tool for the currently mostly intractable problem of inadequate, thin endometrium. A thin endometrium is one of the most difficult problems encountered in assisted reproduction every day practice. Several methods were proposed, to increase thin endometrium in women undergoing IVF. These therapies included tocopherol, pentoxifylline, low-dose aspirin, sildenafi l citrate, estradiol administration and hCG priming. Endometrial perfusion with G-CSF may be effective in expanding chronically unresponsive thin endometrium, which was resistant to traditional remedies. This treatment also deserves further investigation for its potential to improve implantation chances in association with IVF and, therefore, pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s) – BTTB CENTRE.
Trial registration number: BTTBC/2014/12
Keywords: thin-endometrium, IVF-ICSI, intrauterine-infusion, G-CSF (granulocyte colony stimulating factor), embryo-transfer

P-375 ER map: re-defining the transcriptomic signature of endometrial receptivity. Validation of a new method for endometrial receptivity evaluation

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Study question: To re-define the human endometrial receptivity signature by gene expression analysis of a new set of genes specifically involved in the development of the endometrial receptivity and immune response. Validation of these biomarkers with a new method of gene expression analysis.
Summary answer: A new method for endometrial receptivity gene expression analysis has been generated. It combines a new list of genes involved in endometrial receptivity and immune response with the use of new platform of digital PCR.
What is known already: The endometrium reaches a receptive status for embryonic implantation around day 20–21 of the menstrual cycle. This period of time is known as the window of implantation (WOI) and it occurs 7 days after the peak of endogenous LH (LH + 7). It is known that the endometrium shows

a specific gene expression profile during the WOI and that its transcriptomic signature can be used for the evaluation of the endometrial function.
Study design, size, duration: Sixty endometrial samples at LH + 2 (*n* = 30)and LH + 7 (*n* = 30) were collected in the period of March to November, 2014. Endometrial biopsies were collected from fertile women (<37 years) by a pipelle catheter. Oligonucleotide primers for gene expression analyses of one hundred and 192 genes were designed.
Participants/materials, setting, methods: Total RNA from every sample was purified by using Trizol protocol. RNA quality was assessed by using Agilent Bioanalyzer. Gene expression was measured by quantitative real time PCR (RT-PCR) using BioMark platform from Fluidigm for the 192 endometrial receptivity and immune response genes. Oligonucleotide pairs were designed by using GeneFisher 2.0 platform.
Main results and the role of chance: A list of 185 genes with functional relevance in the process of endometrial receptivity and immunological response related with embryonic implantation has been generated. These genes have been selected doing a strict review of the literature since 2008. Another 7 housekeeping genes have been included in the list for normalization of gene expression data. Gene ontology analyses have revealed that cellular proliferation and migration, apoptosis, cellular communication and immune response are the most over-represented biological terms in this group of genes. Validation analysis by standard PCR has shown the correct of 88.5% of the oligonucleotide. The resting 11.5% showed no amplification, double amplification or amplification of incorrect sequences. Sequencing of PCR products was performed for the verification of gene identity.
Limitations, reason for caution: Further studies with more samples are needed to reduce the biological variability shown for some genes. New oligonucleotide have to be designed to replace those oligonucleotide pairs that produced non-specific amplifications.
Wider implications of the findings: A new system for human endometrial evaluation based on has been created. This new tool has been designed with 192 genes not previously used for this purpose and a new algorithm for gene expression analysis. This new molecular tool has been developed in a new system, BioMark platform from Fluidigm, a cost-effective method for gene expression analyses.
Study funding/competing interest(s): Funding by national/international organization(s) – The original study has been funded by SINAERE, Seville, Spain.
Trial registration number: NA.
Keywords: endometrial receptivity signature, gene expression, endometrium, immune response, biomarker

P-376 A prospective randomised controlled study(RCT)comparing the effect of Pre-IVF treatment with Dienogest vs GnRH-Analogue in improving pregnancy outcome in Endometriosis

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Study question: Is the IVF outcome with Dienogest Pre-IVF treatment in endometriosis comparable with GnRH analogue?
Summary answer: Yes, the IVF outcome with Dienogest pre-IVF treatment in endometriosis is comparable with GnRH analogue. What is known already: Dienogest is an oral progestin that has been investigated extensively in the treatment of endometriosis and studies have demonstrated that dienogest 2 mg daily effectively reduces endometriotic lesions, and improves quality of life. Dienogest also show a favorable safety and tolerability profile, with predictable adverse effects, better patient compliance, and low withdrawal rates. GnRH agonists are an established therapy for endometriosis. Although GnRH agonists provide effective pain relief and reduce the progression of endometriotic implants, the hypoestrogenic state that they induce is associated with several side effects. Therefore, the use of GnRH agonists requires 'add-back' therapy.
Study design, size, duration: Our study involved 46 infertile patients below 42 years old, who were treated at our hospital between Mar. 2014 to Dec.2014. After confirming the diagnosis of endometriosis, the patients selected for IVF were randomly divided into two groups, using a computer generated list.The study group of 23 patients received oral Dienogest 2mg per day for 12 weeks and the control group of 23 patients received GnRH-Analog before IVF. *Primary Outcome Measure:* Implantation rate and cumulative pregnancy rate. *Secondary Outcome Measures:* Gonadotropin usage, number of oocytes retrieved and miscarriage rate.
Participants/materials, setting, methods: This is a prospective randomised controlled study (RCT) of 46 women with endometrioss. The study group of 23 patients received oral Dienogest 2 mg/day for 12 weeks and the control group of 23 patients received GnRH Analog as pre-IVF treatment.
Main results and the role of chance: Cumulative clinical pregnancy rates were comparable in both the groups – 35% in study group (Dienogest) vs – 39% in the control group (GnRH Analogue.) Implantation rates and miscarriage rates were also comparable. Though the total Gonadotropin usage was lower in study group (Dienogest), it was not statistically significant.
Limitations, reason for caution: Adverse drug reactions with Dienogest were breast discomfort nausea and irritability. Other adverse drug reactions included fatigue, weight gain, headache, depression and breast engorgement. These side effects were those typical of progestins with no major safety concerns. A relatively new concept in IVF in endometriosis, requiring more multicentric trials worldwide.
Wider implications of the findings: Women with endometriosis treated with IVF have lower pregnancy rates compared to women with no endometriosis. Many treatment regimens have been suggested prior to performing IVF for these women. The present study confirms the fact that pretreatment of women with endometriosis with a GnRH Analogue or Dienogest yields similar IVF outcomes with the latter giving a better patient compliance. There was no significant difference in the amount of gonadotropin required between women in the two groups. Our study indicate that Dienogest Pre-IVF Treatment can safely replace GnRH-Analogue in endometriosis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – BTTB CENTRE.
Trial registration number: BTTBC/2014/04.
Keywords: endometriosis, IVF-ICSI, dienogest, pregnancy-rate, GnRH-analogue

P-377 Postoperative oral contraceptives (OCs) may help maintain fertility-enhancing effects after laparoscopic removal of endometrioma

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Study question: Young women with endometrioma who desire pregnancy in the future may opt to plan the laparoscopic removal at a later date, right before seeking conception, concerning the time-dependent diminishing of fertility-enhancing effects of laparoscopy. However, it is not known whether postoperative OCs can maintain the fertility-enhancing effects.
Summary answer: The cumulative pregnancy rate after removal of endometrioma was not lower in women who started to seek conception after having laparoscopic removal of endometrioma followed by postoperative OCs, in comparison with women who immediately sought conception after laparoscopy, indicated that postoperative OCs may help maintain fertility-enhancing effect after laparoscopy.
What is known already: Endometriosis may cause infertility. Laparoscopic removal of endometriosis improves spontaneous pregnancy rate, however, this fertility-enhancing effects may diminish in a time-dependent manner. Postoperative OCs reduces recurrence of endometrioma, and OCs prescription is recommended after removal of endometrioma for women not immediately seek conception.
Study design, size, duration: 112 patients who underwent laparoscopic removal of endometrioma between January 2009 and December 2011 were analyzed. Patients who sought conception immediately after laparoscopy (*n* = 87, Group A) were compared with patients who eventually sought conception after having taken postoperative OCs (*n* = 25, Group B). The mean observation period was 19.1 months.
Participants/materials, setting, methods: The cumulative pregnancy rates after starting to seek conception (after laparoscopy in Group A, after ceasing OCs in Group B) were analyzed using the Kaplan-Meier method and the Log rank test. In Group B, the correlation between the duration of OCs use and pregnancy rate was analyzed using Wilcocxon test.
Main results and the role of chance: Patients' backgrounds such as rASRM score at laparoscopy and age at seeking pregnancy were not different between groups. The cumulative pregnancy rate in Group B (52.0%, 13/25) was not lower than that in Group A (39.1%, 34/87). The average time to conception was

also equivalent between groups (11.2 ± 8.3 months in Group A and 12.0 ± 7.1 months in Group B). In Group B, the period of OCs use was not associated with the cumulative pregnancy rate (19.4 ± 9.5 months in the pregnancy group and 15.1 ± 6.7 months in the non pregnancy group).
Limitations, reason for caution: This is the retrospective study and therefore results may be biased. The sample size is also relatively too small to draw conclusive data.
Wider implications of the findings: Our results indicate that postoperative OCs help maintain fertility-enhancing effects of laparoscopy. Given the inherent risks of endometrioma such as rupture, infection or ovarian damage, laparoscopic removal of endometrioma followed by OCs may be recommended for the management of young patients with endometrioma who desire pregnancy in the future.
Study funding/competing interest(s): Funding by University(ies) – Funded by Ministry of Education, Culture, Sports, Science and Technology/none.
Trial registration number: NA.
Keywords: ovarian endometriosis, fertility, oral contraceptives

P-378 Retrospective observational cohort study of endometrial receptivity array (ERA)

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Study question: To evaluate the role of endometrial receptive array (ERA) in patients with recurrent implantation failure, previous one IVF failure and thin endometrium.
Summary answer: There was an improvement in reproductive outcome in all the three groups (Recurrent implantation failure (RIF), 1 failed IVF and thin endometrium).
What is known already: The endometrial window of implantation (WOI) is a short period in menstrual cycle, 5–9 days post-ovulation. Displacement of window prevents embryo implantation, resulting in implantation failure. Endometrial receptivity array [ERA] – a diagnostic test based on specific transcriptomic signature that identifies receptive endometrium in natural and artificial (hormonal replacement therapy) cycles. This identification of WOI allows personalization of embryo transfer. Personalized embryo transfer (pET) improves implantation and pregnancy rate in patients with RIF.
Study design, size, duration: This retrospective study examined 182 infertile women who underwent ERA between August 2013 to October 2014. The primary end point was ongoing pregnancy rate (OPR).
Participants/materials, setting, methods: Patients were divided into three groups. Group I (RIF)-76 patients, group II (one failed IVF) – 93 patients and group III (thin endometrium ≤6 mm)-13 patients. Patients with receptive ERA underwent frozen blastocyst transfer (BET) in hormonal replacement cycle. Patients with non receptive ERA, underwent repeat ERA, followed by pET.
Main results and the role of chance: ERA was non receptive in 28% in group I, 15% in group II and 23% in group III. In group I – 53 (70%) patients, group II – 68 (73%) and in group III – 9 (69%) patients underwent BET, giving overall OPR of 37, 58 and 66.7% respectively. After pET, OPR in group I was 45%, group II – 16% and group III – 100%. In patients where 1st ERA was receptive the OPR in group I was 35%, group II – 62% and group III – 57.1%. Further subgroup analysis done in 15 RIF patients who failed at least one frozen BET at our centre (to eliminate centre bias). Incidence of non receptive ERA was 40%. OPR was 50% after pET and 44.4%in patients with 1st ERA-receptive.
Limitations, reason for caution: The numbers are very small to draw any definite conclusion. Further larger studies are warranted.
Wider implications of the findings: In patients with 1st ERA receptive, improvement in reproductive outcome was found even when we removed centre bias (quality of embryo and transfer technique). This post ERA increase in Pregnancy rate needs further evaluation. Also, failure to conceive post pET needs to be further investigated. Both clinician and patient, in thin endometrium, proceeded with embryo transfer more confidently after finding it receptive.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – nova ivi fertility Delhi.
Trial registration number: NA.
Keywords: endometrial receptivity array, endometrium, personalized embryo transfer

P-379 Assessment of clinical features of neuropathic pain in women with chronic pelvic pain

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Study question: Do women with chronic pelvic pain (CPP) have a neuropathic pain (NeP) component to their painful symptoms?
Summary answer: Clinical features of NeP are present in >50% of women with CPP. Rates of NeP are not significantly affected by associated intra-pelvic pathology. Features of NeP can be assessed by questionnaires that are acceptable to women. NeP questionnaires as well as modified QST could be used to predict response to treatment of CPP with neuromodulators.
What is known already: CPP affects 5–24% women. 55% have no obvious pathology and 40% have associated endometriosis. NeP is defined as pain arising as a consequence of a lesion/disease affecting the somatosensory system. The prevalence of NeP in women with CPP is not known. Diagnosis of NeP is challenging because there is no gold-standard assessment. Questionnaires have been used the clinical setting to diagnose NeP and QST has been used in a research to identify abnormal sensory function.
Study design, size, duration: This was a prospective cohort study. The sample size was calculated assuming an expected 70% negative diagnosis for the presence of NeP (based on previous clinical audit data) for the presence of NeP and was powered for specificity of 85±10% (based on validation of NeP questionnaires outside the reproductive tract).
Participants/materials, setting, methods: Women (aged 18–55) attending a university hospital with a diagnosis of CPP underwent a clinician completed questionnaire (DN4) and completed the S-LANSS and PainDETECT® questionnaires. They underwent QST testing by a trained clinician. They also completed a patient acceptability questionnaire. Patient notes were interrogated for underlying aetiology, and drug use.
Main results and the role of chance: Clinical features of NeP were identified by both questionnaires and QST. Of the women who were NeP positive, 56, 35 and 26% were identified by the S-LANSS, DN4 and PainDETECT® respectively. When NeP was identified by questionnaire, the associated laparoscopy findings were similar irrespective of which questionnaire was used. No subject had entirely unchanged QST parameters. There were distinct loss and gain subgroups as well as mixed alteration in function but this is not necessarily clinically significant in all patients. 80% of patients were confident that questionnaires could diagnose NeP in the setting CPP and 90% found them easy to complete.
Limitations, reason for caution: NeP questionnaires have not been validated within the context of CPP. The lack of gold standard means formal validation is likely to be challenging. A modified QST-protocol was used to facilitate testing in our clinical setting but this reduces ease of comparison with previously published NeP literature out with the pelvis.
Wider implications of the findings: Early identification of NeP in women with CPP with a simple questionnaire could facilitate targeted therapy with neuromodulators, which are cheap, readily available and with good safety profiles. Furthermore, this could prevent unnecessary or fertility-compromising surgery and prolonged treatment with hormones.
Study funding/competing interest(s): Funding by University(ies) – University of Edinburgh.
Trial registration number: REC No: 12/SS/0149. Lothian R&D approved: 2012/R/RM/47.
Keywords: neuropathic pain, endometriosis, chronic pelvic pain

P-380 Developmental disturbances caused by endometriosis and normalizing effects of aromatase inhibitors on embryo morphokinetics: a pilot study

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Study question: This study asks whether any effect of endometriosis as a cause of infertility on embryo development kinetics and also evaluates if aromatase inhibitors can create developmental amelioration in cases with endometriosis by time-lapse analysis.
Summary answer: Compared to controls, endometriosis can significantly disturb embryo morphokinetics, cause retardations in early embryo dynamics. In cases where aromatase inhibitors were included in ovarian stimulation protocols, although such disturbances can partially be masked during early cleavage, apparent albeit not significant differences in morphokinetic parameters.
What is known already: It is well known that women with endometriosis have lower success with IVF, which is postulated to be related with impaired follicular microenvironment and reduced endometrial receptivity. Although such an impaired microenvironment has been known to have an impact on oocyte quality and embryo development, little is known about its effects on human embryo morphokinetics.
Study design, size, duration: In order to minimize confounding factors, cases which endometriosis was sole indication of infertility were included. Morphokinetic parameters of 119 embryos (18 cycles) with endometriosis were retrospectively analysed and compared with 48 embryos (4 patients) with unexplained infertility using time-lapse monitoring between January 2012-August 2014 in Bahceci Fulya-Umut IVF Centers.
Participants/materials, setting, methods: Embryos derived from endometriosis patients that used antagonist protocol (11 cycles, 78 embryos, Group I), endometriosis patients that used letrozole-antagonist protocol (7 cycles, 41 embryos, Group II) and control group that used antagonist protocol (4 cycles, 48 embryos, Group III) were analyzed. Analyzed parameters were: extrusion of the 2nd polar body, pronuclei appearance, pronuclear fading, early cleavage time points and duration in each cleavage from two cells – until hatching blastocyst stages, time interval between cleavages, PN duration and duration of blastulation.
Main results and the role of chance: Embryos of endometriosis patients showed significantly perturbed early and late cleavage timings compared to controls ($p > 0.05$). Compared with Group II and III embryos, embryo development in Group I embryos delayed at from tPB2 to t5 parameters. In Group II, inclusion of aromatase inhibitors minimized these perturbations and resulted in improved early embryo morhokinetics. On the other hand, although the pace of early embryo development was found to be similar with the controls, embryos in group II were found to reach tEB and tHB stages significantly earlier ($p > 0.05$).
Limitations, reason for caution: The number of embryos and cycles analysed are the main limitations of our study. Further research with larger sample sizes, according to the stage of endometriosis and technical setting are required to confirm our findings.
Wider implications of the findings: Our preliminary study indicates that there can be significant disturbances on embryo morphokinetics in patients with endometriosis. To the best of our knowledge, this is the first study that analyse human embryo morphokinetics in such cases. As the number of cases increase, a better understanding of the possible effects, degree of disturbances with respect to the stage of endometriosis as well as the possible corrective approaches can be obtained.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study received no funding and no conflicts of interests to be declared.
Trial registration number: This study was not an RCT and therefore there is no registration number.
Keywords: endometrosis, time-lapse monitoring, letrozole

P-381 A significant association between CCR5 and voltage gated sodium channel SCN3A in endometriotic lesions may allow communication between inflammation and sensory nerves

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Study question: Is there a relationship between inflammation and the regulators of nociceptive action potentials in sensory neurons in endometrial and endometriotic tissue?
Summary answer: A significant positive correlation between the expression of the RANTES receptor CCR5 and the voltage gated sodium channel SCN3A exists in endometriotic lesions.
What is known already: Nerve fibers have been identified in eutopic endometrium of women with endometriosis and are present around endometriotic lesions. Endometriosis creates a chronic inflammatory response and inflammatory cytokine receptors are present on sensory nerves. By binding to their receptors cytokines can influence the activity of sensory nerve through the expression and activity of voltage gated ion channels. An association between peritoneal inflammation and sensory nerve ion channel expression may be important in pain signals.
Study design, size, duration: A total of 30 women (17 with endometriosis, 13 without) undergoing laparoscopic surgery were included in the study. No hormonal treatment was reported at least 3 months prior to surgery and all samples were collected during the proliferative stage. Both eutopic endometrial biopsies and ectopic endometriotic tissue was collected.
Participants/materials, setting, methods: mRNA was isolated from both eutopic and ectopic endometrial tissue and gene expression determined with quantitative PCR. Cytokine receptors (TNFR1, CCR2, CCR5 LepR), purinergic ion channels (P2X2, P2X3, P2X4) and voltage gated sodium channel (SCN3A, SCN9A and SCN11A) mRNA expression was determined via quantitative Real-time PCR.
Main results and the role of chance: CCR5, the receptor for RANTES, was significantly increased in the peritoneal ectopic lesions compared to either the matching eutopic tissue and the ovarian lesions. The purinergic receptors were present in eutopic tissue with no significant difference between their eutopic and ectopic expression. The voltage gated sodium channels however were either not present, or present at very low levels in eutopic endometrial tissue and significantly increased in the ectopic lesions. Furthermore analysis between cytokine receptors and voltage gated ion channel identified a significant correlation between the cytokine receptors (CCR and TNFR1), the voltage gated sodium channels (SCN3A and SCN9A) and importantly between the cytokine receptor CCR5 and the SCN3A voltage gated sodium channel.
Limitations, reason for caution: The current results only show an association between cytokine receptors and voltage gated ion channels and not a cause-and-effect. Further *in vitro* experiments on sensory nerve cells will be needed to confirm a direct association between CCR5 and the SCN3A voltage gated sodium channel.
Wider implications of the findings: As voltage gated sodium channels were only present in ectopic tissue these results suggests that either sensory nerve are not present in the endometrium of endometriotic women, or that expression of these ion channels is induced in sensory nerves in an ectopic, inflammatory environment. A significant relationship between CCR5 and SCN3A raises the possibility that RANTES may induce SCN3A expression in endometriotic associated sensory nerves and thus could contribute to the painful response.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Frauenklinink, Inselspital Berne.
Trial registration number: 149/03.
Keywords: endometriosis, inflammation, RANTES, CCR5, SCN3A

P-382 Genome-wide pathway analysis highlights the role of Wnt signaling in endometriosis

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Study question: Do endometriosis genome-wide association study (GWAS) data provide insight in novel endometriosis-associated biological pathways, and to what extent do they provide evidence for the causal involvement of previously hypothesised pathways such as steroidogenesis or immune responses?
Summary answer: Pathway analysis of the largest GWAS conducted to date, in women of European ancestry, uncovers multiple interesting pathways that are statistically enriched for genetic endometriosis association signals. Hypothesis-free analysis involving all independent loci genome-wide uncovers the *Wnt signaling pathway* as the top pathway associated with endometriosis.

What is known already: Endometriosis is a complex disease with an estimated heritable component of ~51%. GWA studies have so far identified nine genome-wide significant ($P < 5 \times 10^{-8}$) common risk variants for endometriosis, which explain <4% of heritability, where as half of the heritability is estimated to such risk variants. Pathway analyses of GWAS data combine the evidence of single variants into gene-based measures, and adding to the evidence for certain pathways to be involved in disease pathogenesis.
Study design, size, duration: We conducted hypothesis-free and hypothesis-driven pathway analyses utilising the International Endogene Consortium GWAS data, comprising of 3,194 surgically confirmed endometriosis cases and 7,060 controls with genotype data imputed up to Phase3 1000-Genomes. Endometriosis cases were grouped into stage I-II, and stage III-IV (rAFS classification) for sub-phenotype analysis.
Participants/materials, setting, methods: We used two major pathway databases, MSigDB and PANTHER and tested for enrichment of genetic variants in (1) all curated pathways (Hypothesis-free), (2) pre-selected pathways defined by over-representation of endometriosis candidate genes and literature review of endometriosis biology (Hypothesis-driven). Genetic enrichment analysis within these pathways was performed in MAGENTA software.
Main results and the role of chance: Hypothesis-free analysis showed 72 genetically enriched pathways with a nominal $P < 0.05$, with the most significantly associated the *Wnt signaling pathway* (nominal $P = 0.018$, FDR = 0.018) comprising 80 known genes. In the hypothesis driven analysis *Insulin/IGF pathway-MAPK cascade* showed significant genetic enrichment for stage I-II endometriosis, and *Androgen oestrogen progesterone biosynthesis* –pathway for stage III-IV endometriosis (FDR < 0.05). There were no immunology-related pathways that showed significant enrichment of genetic variants associated with endometriosis, suggesting that immune response – although an important characteristic of disease progression and symptomatology – does not play a major part in initial causality. Results on sub-phenotype analyses (stage I–II and stage III–IV cases) will be presented at the meeting.
Limitations, reason for caution: The analysis is restricted to the gene-based pathway definition as registered in the databases, and by current knowledge of the constituting parts of these pathways. Only SNP associations within genes can be attributed to pathways, and are therefore included in the analysis.
Wider implications of the findings: The top ranked genetically enriched pathways for endometriosis are steroid hormone metabolism and steroid hormone related cell signalling pathways. Our highest ranked enriched pathway, *Wnt signalling* is involved in sex-hormone homeostasis and female tract development, offering us more knowledge on the underlying biology of endometriosis. With a recognised important role in endometrium biology, there should be an increased focus on the causal role of *Wnt signaling* in endometriosis pathogenesis.
Study funding/competing interest(s): Funding by national/international organization(s) – The Wellcome Trust.
Trial registration number: NAD.
Keywords: endometriosis, genetics, genome-wide association studies, biological pathway analysis, sub-phenotypes

P-383 Endometrial gene expression analysis related to the signaling network for uterine receptivity and implantation in patients with repeated implantation failure

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Study question: To analysis the endometrial gene expression related to the signaling network for uterine receptivity and implantation in patients with repeated implantation failure (RIF).
Summary answer: Gene expression pattern associated with signaling network for uterine receptivity and implantation can be used clinically in patients with RIF. This identification of the optimum window of implantation by using endometrial receptivity biomarkers can help to rescue repeated pregnancy failure resulting from misplaced dating of the endometrial WOI.
What is known already: Repeated implantation failure (RIF) is determined when embryos of good quality fail to implant following IVF-ET cycles in a minimum of 3 times. RIF is the most common cause of infertility couples. Many other studies that analyzed the human gene expression during different menstrual cycle have been published.
Study design, size, duration: Prospective controlled study. Analysis of the gene signature related to the signaling network for uterine receptivity and implantation in patients ($n = 3$) with repeated implantation failure.
Participants/materials, setting, methods: Human endometrial samples were collected throughout the menstrual cycle (LH + 5, LH + 7 and LH + 9) in patients with RIF. The profiling of transcriptomic signature and histologic evaluation were accomplished. Each total RNA sample was labeled and amplified. Labeled aRNA were placed on Agilent Sureprint G3 Human GE 8X60K array and analyzed.
Main results and the role of chance: There are different gene expression patterns between receptive and non-receptive histologically observed endometrium obtained on the same day of menstrual cycles. And its patterns were slightly different at each patient. Comparison of the data sets defined a common set were slightly different, also. In addition, in comparison with endometrial receptivity related 30 genes, various fold change were showed in endometrium of each patient with repeated implantation failure. From 6 to 15 genes were changed at LH + 5 phases versus LH + 7 phases and from 10 to 16 genes were changed at LH + 5 phases versus LH + 9 phases. Furthermore, there were strong different patterns in PCO patient with RIF.
Limitations, reason for caution: Since this was a prospective study with small sample size, randomized study in a larger scale will be necessary.
Wider implications of the findings: This result of the expression patterns of endometrial receptivity related genes are helpful for diagnosis and personalized embryo transfer in patient with RIF. Further more research is need to confirm and better understanding our data.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This study was funded by our own private infertility center.
Trial registration number: NA.
Keywords: implantation, Endometrium, gene expression, RIF

P-384 Increased expression levels of metalloprotease, tissue inhibitor of metalloprotease, metallothionein, and p63 in ectopic endometrium: an animal experimental study

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Study question: To characterize the patterns of cell differentiation and tissue invasion in topic and ectopic endometria of rabbits with induced endometriotic lesions via a well-known experimental model, 4 and 8 weeks after the endometrial implantation procedure.
Summary answer: Ectopic endometrial lesions seem to express greater power for cell differentiation and tissue invasion, compared with topic endometrial lesions, demonstrating a potentially invasive, progressive, and heterogeneous presentation of endometriosis.
What is known already: The etiopathogenesis of endometriosis is controversial and studies suggest that the endometrium of patients with endometriosis have an invasive and aggressive behavior, and higher expression levels of substances related to cellular invasion, cellular differentiation, and proliferation.
Study design, size, duration: Animal experimental study. Twenty-nine female New Zealand rabbits.
Participants/materials, setting, methods: Twenty-nine female New Zealand rabbits underwent laparotomy for endometriosis induction through resection of one uterine horn, isolation of the endometrium, and fixation of tissue segment to the pelvic peritoneum. Two groups of animals (14 and 15 animals in each group) were sacrificed 4 and 8 weeks after endometriosis induction. The lesion was excised together with the opposite uterine horn for endometrial gland and stroma determination.
Main results and the role of chance: Immunohistochemical reactions were performed in topic and ectopic endometrial tissues for analysis of the following markers: metalloprotease (MMP-9) and tissue inhibitor of metalloprotease (TIMP-2), which is involved in the invasive capacity of the endometrial tissue; and metallothionein and p63, which are involved in cell differentiation and proliferation. The intensity of the immunostaining for MMP 9, TIMP-2, metallothionein, and p63 was higher in the ectopic endometrium than in the topic endometrium. However, when the ectopic lesions were compared at

4 and 8 weeks, no significant difference was observed, with the exception of the marker p63, which was more evident after 8 weeks of evolution of the ectopic endometrial tissue.

Limitations, reason for caution: The results of an animal experimental study should be viewed with caution.

Wider implications of the findings: In this study, we demonstrated that the ectopic endometrial lesions showed higher expression levels of cellular invasion markers (MMP-9 and its inhibitor TIMP-2) and molecules involved in cellular proliferation and differentiation (MT and p63) than did topic the endometrial lesions in a rabbit experimental model of endometriosis, with good reproducibility and effectiveness. This indicates the invasive and progressive power of the illness, corroborating the other evidence described.

Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). FAEPA: Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo. CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Trial registration number: NA.

Keywords: experimental endometriosis, cell differentiation, tissue invasion

P-385 Mesenchymal stem cell-conditioned medium enhances trophoblast cell proliferation, migration and invasion: possible applications in improving implantation rate in assisted reproductive technology

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Study question: Embryo implantation is a complex process that requires adequate proliferation, migration and invasion of trophoblast cells. Since mesenchymal stem cell-conditioned medium (MSC-CM) can stimulate skin cell proliferation and migration, we investigated whether MSC-CM can enhance trophoblast cell proliferation, migration and invasion, and the underlying mechanisms.

Summary answer: Using mouse blastocysts and trophoblast stem (TS) cells, we found that MSC-CM could enhance trophoblast cell proliferation, migration and invasion. MSC-CM was found to be abundant in various growth factors and cytokines, among which thrombopoietin mediated MSC-CM-enhanced trophoblast outgrowth. ERK activation was shown to mediate MSC-CM-stimulated trophoblast cell proliferation.

What is known already: Our previous study has shown MSC-CM could significantly enhance skin cell migration. Sufficient migration capacity is crucial for trophoblast invasion to endometrium, and inadequate trophoblast invasion results in implantation failure or abortion. MSC-CM may provide a potential means to promote trophoblast functions, thereby increasing implantation rates and preventing abortion in assisted reproductive technology (ART).

Study design, size, duration: We first determined the effects of MSC-CM on mouse blastocyst outgrowth and on the proliferation, migration and invasion of TS cells. Then we analyzed growth factors and cytokines contained in MSC-CM. Finally, underling molecular mechanisms of MSC-CM-enhanced trophoblast cell proliferation and migration were studied.

Participants/materials, setting, methods: Mouse blastocyst outgrowth was assessed by measuring areas of trophoblast outgrowth at day4. Migration and invasion of TS cells were determined by Transwell assay. MTT was used to determine cell proliferation. Western blot and appropriate inhibitors were employed to study underlying mechanisms. Protein array detected protein contents in MSC-CM.

Main results and the role of chance: MSC-CM dose-dependently enhances blastocyst outgrowth, suggesting that MSC-CM could promote trophoblast cell migration. Transwell assays also revealed that MSC-CM promoted TS cell migration and invasion. Furthermore, MSC-CM significantly stimulated TS cell proliferation. MSC-CM was abundant in various growth factors and cytokines, including thrombopoietin. Anti-thrombopoietin antibody significantly inhibited the enhancing effect of MSC-CM on trophoblast outgrowth, and recombinant thrombopoietin also promoted trophoblast cell migration, indicating a role of thrombopoietin in MSC-CM-enhanced trophoblast cell migration. Extracellular signal-regulated kinases (ERK) were activated by MSC-CM, and ERK inhibitor PD98059 could block the MSC-CM-enhanced TS cell proliferation, indicating that ERK was involved in MSC-CM-enhanced trophoblast cell proliferation. This study suggested that MSC-CM could enhance trophoblast functions, findings that may be applied to increase implantation rate in ART.

Limitations, reason for caution: These findings were based on *in vitro* studies using mouse blastocysts and TS cells. Further studies using human TS cells may be performed after ethical issues are settled. Mouse model of recurrent miscarriage (CBA/J female X DBA/2 male) may be utilized to examine whether MSC-CM could prevent recurrent abortion.

Wider implications of the findings: Since MSC-CM significantly enhanced trophoblast cell proliferation, migration and invasion, it may be added in the embryo transfer medium to promote trophoblast functions, thereby enhancing implantation rate in ART.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by national/international organization(s). National Science Council (NSC101-2314-B-010 -031 -MY3). Taipei Veterans General Hospital (V102C-142).

Trial registration number: NA.

Keywords: embryo implantation, recurrent abortion, conditioned medium, mesenchymal stem cells, trophoblast stem cells

P-386 Combined treatments of intrauterine perfusion with G-CSF and injection of hCG enhance the endometrial growth, implantation and pregnancy rates in thin endometrium patients

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Study question: Intrauterine perfusion with G-CSF (granulocyte colony-stimulating factor) and intrauterine injection of hCG improved the endometrial growth and implantation rates, respectively. This study was examined the efficiency between endometrium perfusion of G-CSF and a combination of G-CSF and intrauterine injection of hCG in the patients.

Summary answer: For patients who had a thin endometrium (<8 mm) on the hCG day, the combined treatments of endometrium perfusion of G-CSF on the day of hCG and intrauterine injection of hCG before embryo transfer significantly improved the endometrial thickness, implantation and pregnancy rates.

What is known already: An adequate endometrial thickness is necessary for successful implantation. The treatment of Vitamin E, l-arginine, or sildenafil citrate was not sufficient to improve the endometrial growth in patients with a thin endometrium, but intrauterine perfusion with G-CSF was effective to patients showing a resistance to the treatment. More recently, intrauterine injection of hCG before the embryo transfer improved significantly both the implantation success and the pregnancy rates of the patients with recurrent implantation failure.

Study design, size, duration: Prospective study was examined using 58 patients who had a thin endometrium (<8 mm) on the hCG day and failed to conceive with high-quality embryos in previous IVF cycles in a private fertility clinic from 2011 to 2014. Groups were established according to the treatments.

Participants/materials, setting, methods: Group A (n = 34) was received G-CSF (filgrastim 300 μ/mL) by slow intrauterine infusion using embryo transfer catheter on the day of hCG. Group B (n = 24) was received G-CSF on the day of hCG and 500 IU of hCG intrauterine administration approximately 7 min before embryo transfer.

Main results and the role of chance: There were no significant differences in endometrial thickness on the day of hCG among both groups (Group A vs. Group B; 6.9 ± 0.8 vs.6.8 ± 7.2) whereas both groups showed significant increase of the endometrial thickness on the day of embryo transfer (8.6 ± 1.0 vs. 8.5 ± 0.8). The endometrial thickness was gradually increased from the hCG to the ET day, but it was not significantly differed on the day of ET in both groups. Interestingly, the rates of implantation (13.0 vs. 22.6%) and clinical pregnancy (29.4 vs. 41.7%) were significantly higher in Group B than those in Group A (P < 0.01). Thus, the combined treatments of G-CSF and hCG intrauterine infusion enhanced the endometrial thickness, implantation and pregnancy rates of the patients.

Limitations, reason for caution: The clinical results are necessary to confirm its efficacy and safety via further studies. Thus, further large cohort studies are required.

Wider implications of the findings: This study shows the most recent finding that the combined treatments of G-CSF and hCG intrauterine infusion enhance the increase of endometrial thickness as well as the implantation and pregnancy

rates in patients with thin endometrium. The combined treatments will improve the efficacy for patients with recurrent implantation failure.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ellemedi infertility clinics.
Trial registration number: NA.
Keywords: thin endometrium, G-CSF, injection of hCG

ETHICS AND LAW

P-387 Oocyte banking for future mother-to-daughter donation; how to assess these requests?

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Study question: How should requests for oocyte banking for future mother-to-daughter donation be assessed in the light of relevant ethical considerations?
Summary answer: Oocyte banking for future mother-to-daughter-donation is challenging and needs special ethical attention of the fertility specialists involved. Requests should be handled with thorough screening and psychological counselling. Further guidance is needed, especially with regard to concerns that those for whom oocytes were banked, may feel obligated to use them.
What is known already: The ethical aspects of gamete donation by family members ('Intrafamilial Medically Assisted Reproduction'; IMAR) have been discussed in ESHRE and ASRM documents. However nowadays, oocyte vitrification opens the door for a new form of IMAR: banking of mothers' oocytes for a possible future donation to her daughter in situations where premature ovarian insufficiency (POI) is diagnosed in girls and young women. This form of intergenerational IMAR raises additional concerns not yet discussed in those documents.
Study design, size, duration: We performed a systematic review of the literature regarding mother-to-daughter donation to extract the ethical dilemma's involved. We collected 3 requests of mothers who asked for oocyte banking for future donation to their daughters with POI in two academic fertility centres from 2011 up to 2014.
Participants/materials, setting, methods: Studies were identified by searching Medline, ISI Web of Science and Google scholar until January 2015. Studies were eligible when intergenerational donation or banking of oocytes was discussed. We identified 197 studies from the literature, of which 5 were included in the systematic review.
Main results and the role of chance: Three publications were ESHRE and ASRM documents on IMAR. One review discussed the possibility of oocyte banking for young galactosemic girls. One case-report described a mother who banked oocytes for her daughter with Turner syndrome. The reported ethical considerations were risk of role-confusion and coercion. The risk of role-confusion might be lower in mother-to-daughter-donation compared to sister-to-sister-donation, since the donor's life expectancy is limited. The risk of coercion might be reduced since the donor is in the more powerful position. No permission was given to the three mothers who requested oocyte banking in our hospitals. The staff was reluctant to bank oocytes since guidance was lacking with regard to their major concern: that young girls can feel obligated later in life to use the oocytes.
Limitations, reason for caution: The systematic review of literature revealed a limited number of studies. The search for requests for mother-to-daughter donation was limited to two academic fertility centres.
Wider implications of the findings: Overall; there is a shortage of literature regarding this specific form of IMAR. On the one hand, the mother's gift may help fulfill a later childwish of her daughter. On the other hand, young girls do not yet know if they want to have children and can feel obligated later in life to use the oocytes. The specific ethical dilemmas of oocyte banking for future mother-to-daughter donation should be further explored.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Academic Medical Center, Center for Reproductive Medicine.
Trial registration number: NA.
Keywords: mother-to-daughter-donation, fertility preservation, ethics, IMAR

P-388 The technological imperative in reproductive medicine

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Study question: As in many areas of medicine, also reproductive medicine is not immune to the dogma 'we can so we should'. Why is this ethically problematic, how can we explain it and what can be done about it?
Summary answer: Given the rising health care costs and given the increasing pressure on research funding, a more critical attitude towards new innovations should be adopted. Both on the level of individual treatment and on the level of research, responsibility is shared by several parties involved.
What is known already: It has previously been argued that technology is no longer merely a tool that is used to respond to challenges and demands in health care, but that technological possibilities also create demand. Moreover, new medical technology is the dominant driver of increasing health care costs.
Study design, size, duration: Existing literature on the technological imperative in health care was gathered and an assessment was made of whether or not this phenomenon is ethically problematic in the field of reproductive medicine, with a focus on recent achievements such as uterus transplantations and derivation of gamete precursors from stem cells.
Participants/materials, setting, methods: The model that is used to bring empirical data (as found in literature research) and normative ethics together is the Wide Reflective Equilibrium. This is essentially a coherence theory, where the justification of the components (moral judgments, moral principles and background theories) is achieved by their coherence.
Main results and the role of chance: The technological imperative is at play on two different levels in reproductive medicine. First, individual patients often wish to exhaust all treatment options to avoid future regrets. A rational assessment of success rates and risks is easily undermined. There is a shared responsibility of policy makers (reimbursement schemes), physicians and patients to weigh the pros and cons of each treatment option rather than 'trying everything possible'. Second, also research is not exclusively steered by patients' needs. Besides the technological imperative, factors such as prestige, publication pressure and commercial interests contribute to the trend to focus on groundbreaking new technologies, rather than – for example – making existing ARTs more affordable. In this case, responsibility stretches out to funding bodies, researchers, pharmaceutical companies, scientific journals and academic institutions.
Limitations, reason for caution: This is a philosophical assessment, not an empirical study.
Wider implications of the findings: Today more than ever before, the theoretical possibilities in health care are enormous. At the same time, research funding is limited and new treatment options are oftentimes very costly. Thus, we are evolving towards a society in which much will be technically feasible, but not necessarily affordable or desirable. Also in reproductive medicine, there is an increasing responsibility of several actors to maintain the right priorities for technological innovation and to resist the technological imperative.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Ghent University. Research Foundation – Flanders.
Trial registration number: NA.
Keywords: ethics, innovation, ART, technological imperative

P-389 The moral and legal status of human parthenogenetic entities and parthenogenetic derived stem cells after the ruling of the European court of justice

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Study question: The research project aims to clarify the legal and moral status of human parthenogenetic entities and parthenogenetic derived stem cells taking into account the patent ruling of the European Court of Justice.
Summary answer: At least in patent law human parthenogenetic entities have a different moral and legal status than human totipotent entities. This patent law related findings can be a blueprint for the overall determination of the moral and legal status of human parthenogenetic entities.
What is known already: Within the member states of the European Union (EU) there it is controversial whether early human totipotent entities have such a moral

status which protects them from purposeful production and destruction for stem cell research and/or medicine. Whilst techniques based on human embryonic stem cells extracted from human totipotent entities are unpatentable within the EU, techniques based on stem cells from human parthenogenetic entities are patentable, because human parthenotes are not considered to be totipotent.
Study design, size, duration: None.
Participants/materials, setting, methods: None.
Main results and the role of chance: The ruling of the European Court of Justice in its parthenote patent case indicates that human parthenogenetic entities have another moral status than human totipotent entities. This different moral status is connected to a smaller protection scope than the moral status of human totipotent entities provides. Whilst techniques based on embryonic stem cells extracted from human totipotent entities are unpatentable in the EU, techniques based on stem cells from human parthenogenetic entities are patentable. Therefore, therapies based on embryonic stem cells extracted from totipotent human entities are considered not to be in compliance with the ordre public of the EU as well as the related patent issues, therapies based on stem cells extracted from human parthenotes seem to be in compliance with current morals standards.
Limitations, reason for caution: None.
Wider implications of the findings: Since therapies based on embryonic stem cells extracted from human totipotent entities could cause moral and legal problems, therapies based on stem cells extracted from human parthenotes could be a viable alternative if such stem cells meet the necessary scientific and medical quality standards.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Translational Centre for Regenerative Medicine (TRM) Leipzig, funded by the German Ministry of Education and Research (BMBF 1315883).
Trial registration number: NA.
Keywords: parthenogenetic entities, parthenogenetic derived stem cells, moral and legal status

P-390

Abstract withdrawn by the author

P-391 The interest of gynaecologists in potential future fertility treatments involving artificial gametes for couples with ovarian failure

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Study question: What characteristics define whether gynaecologists would recommend potential fertility treatments with artificial gametes to couples with ovarian failure?
Summary answer: Gynaecologists are interested in treatments with artificial gametes allowing infertile patients to have genetically related children. Clinical introduction of such treatments would mainly depend on effectiveness and safety for the child. In addition, curing infertility, moral acceptability, naturalness, burden, safety for patients, costs, and diagnostic value are important characteristics.
What is known already: New fertility treatments with artificial gametes allowing infertile patients to have genetically related children are being developed in model organisms. Gynaecologists need to reflect on the conditions deemed necessary for introducing new treatments in clinical practice. Effectiveness, safety, costs and burden are classically considered relevant. Patients additionally value treatments' resemblance to natural conception and feeling cured. Which treatment characteristics define whether gynaecologists would recommend the use of artificial gametes to their patients is unknown.
Study design, size, duration: Exploratory qualitative study conducted in 2014. Ten gynaecologists selected by purposive sampling took part in a single face-to-face in-depth interview of 45–70 min. This sample size was sufficient to reach data saturation, defined as not identifying new treatment characteristics during three subsequent interviews.
Participants/materials, setting, methods: Gynaecologists were updated on the progress of developing artificial gametes and asked to reflect on treatment characteristics determining which treatment to prescribed to heterosexual couples with ovarian failure. The interviews were performed according to a semi-structured interview guide, recorded, transcribed and subjected to content analysis by two researchers.
Main results and the role of chance: The ten gynaecologists (6 women, 4 men) worked in University clinics in Belgium (n = 5) and the Netherlands (n = 5), had on average 17.2 (±9.9) years of experience. All participants had genetically related children, one had experienced fertility treatments herself and one had stepchildren. The gynaecologists were interested in artificial gametes and considered ten treatment characteristics as relevant: safety for the child, pregnancy rates, genetic parenthood, curing infertility, moral acceptability, naturalness, burden, safety for the patient, costs, and diagnostic value. The characteristics most commonly receiving their top-3-priority were: safety for the child (n = 10/10), pregnancy rates (n = 7/10) and genetic parenthood (n = 5/10). All gynaecologists indicated that the decision to offer artificial gametes in clinical practice would require reflection of and discussion among professionals from various fields.
Limitations, reason for caution: Data-saturation indicated that all treatment characteristics important to gynaecologists have been identified. Insight in how these treatment characteristics are traded-off against each other requires quantitative research on a large sample of gynaecologists.
Wider implications of the findings: Gynaecologists' interest encourages further research into fertility treatments with artificial gametes.
Study funding/competing interest(s): Funding by University(ies) – University of Amsterdam, Academic Medical Center.
Trial registration number: NA.
Keywords: artificial gametes, implementation, stem-cell based treatments, qualitative research

P-392 Time-lapse in reproductive medicine: outlining ethical prospects and challenges

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Study question: What are the ethical prospects and challenges of using time-lapse (TL) for different purposes in research, clinical service, care and commercial marketing in the assisted reproductive context? What possible professional or public policy implications follow from these?
Summary answer: The ethics of TL depends on its more exact use. Current knowledge indicates possible clinical benefits, but premature clinical application should be resisted. TL opens for, e.g., patient demands regarding embryo selection and implementation of TL involving display to patients mandates extra caution regarding care, counseling and marketing exploits.
What is known already: Using TL to capture the process of in vitro embryo development has quickly entered reproductive medicine, with prospective uses in research and clinical service. It has also been picked up as a possible marketing tool by commercial providers, and as an add-on of patient care and counseling. Assessment of the effects and efficiency of TL is ongoing and, as yet, displaying complex and partly unclear or contradictory results. care and counseling aspects remain uncharted.
Study design, size, duration: Explorative theoretical analytical applied ethics study.
Participants/materials, setting, methods: Systematic analysis of ethical aspects using literature on TL and relevant aspects of the ethics of assisted reproduction and reproductive medicine.
Main results and the role of chance: TL is valuable for research, but trials of TL-interventions aiming at improved IVF should not be launched prematurely or allowed to sneak into routine practice. Offering display of TL documentation of embryo development to patients during *ongoing* treatment has unclear benefits and risks from a care and counseling perspective and arises the issue of what to display. It may be a vehicle for increasing patient involvement, but may also create conflict between patients and clinicians regarding embryo selection, and will introduce care and counselling challenges as patients form their own reactions. *Retrospective* offers appear less risky, but may fuel into uncertainty

about the outcome. Marketing services with such offers risks biasing patients towards an "entertainment" view and away from more important considerations counseling wise.
Limitations, reason for caution: There are many unknowns regarding the more detailed effects on patient experience of incorporating TL display as part of assisted reproductive services. Such additions also have unknown cultural dimensions by making the living in vitro embryo more widely and intimately visible and acquaintable.
Wider implications of the findings: Besides the need to structure, monitor and limit attempts at using TL to boost IVF results, the idea of displaying TL output to patients is in need of much probing, not least regarding patient experience and structural effects on the perception of assisted reproductive services, but also clinical policy and counseling requirements. Professional and scientific organizations should act to prevent premature and reckless clinical use.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Funding by national/international organization(s). University of Gothenburg, the Sahlgrenska University Hospital, the Dutch Research Council (NWO): Practices of Responsibility in Change.
Trial registration number: NA.
Keywords: time-lapse, ethics, policy, counseling, IVF

P-393 Pre-implantation genetic diagnosis and the European convention on human rights: applying the 'consensus' and 'margin of appreciation' principles

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Study question: Is a national ban on preimplantation genetic diagnosis (PGD) compatible with the European Convention on Human Rights (ECHR)?
Summary answer: No, by applying the 'consensus' and 'margin of appreciation' principles commonly employed by the European Court of Human Rights (ECtHR), an absolute ban on PGD falls outside the allowable margin of variation in European countries' laws and is therefore incompatible with the European Convention on Human Rights.
What is known already: In the 2013 case *Costa and Pavan v. Italy*, the ECtHR struck down Italy's ban on PGD because it was internally incoherent; Italy permitted pregnancy termination if the resulting child would suffer from a serious genetic disease but would not permit the use of PGD to select for an embryo without the same disease. The Court did not address the legal question of whether a PGD ban would violate the ECHR *per se*.
Study design, size, duration: To determine whether a PGD ban violates the ECHR *per se*, I apply the principle that a high level of consensus among European national laws delineates a margin of appreciation for acceptable legal variation. I survey national laws in Europe on PGD in order to ascertain if consensus exists.
Participants/materials, setting, methods: The legal approach towards PGD was examined in the ECHR's 47 Contracting states. Existing national legislation or regulation on PGD was surveyed. A number of countries lacked explicit rules for the use of PGD, but fertility clinics freely offering PGD were identified, indicating that no ban was in place.
Main results and the role of chance: Of the 47 Contracting states to the ECHR, PGD is explicitly legal or otherwise available in 40. Only five prohibit PGD, and one is in the process of modifying its ART law to allow for the limited use of PGD. The final two countries do not appear to have IVF clinics. In the countries where PGD is available, the conditions for which it is performed varies, but at a minimum, it is utilized to select against serious heritable disorders. Based on these results and an analysis of the 'consensus' concept, I argue that consensus exists among the Contracting states that PGD should be allowed, at least for selection against serious genetic conditions. An outright ban on PGD would thus be in violation of the ECHR.
Limitations, reason for caution: The 'consensus' and 'margin of appreciation' concepts are not precisely defined by the ECtHR or ECHR experts. Determining how many Contracting states constitutes a sufficient consensus for arguing that a national measure falls outside the margin of appreciation, and hence violates the ECHR, is therefore a matter of judgment.
Wider implications of the findings: While an outright ban violates the ECHR, the Convention likely protects variation regarding the conditions for which PGD may be performed, given the lack of consensus in national laws. Legal diversity results in reproductive tourism, as couples seeking PGD travel abroad to obtain services forbidden at home. Reproductive tourism puts patients at risk for legal and health complications, and is already occurring within Europe and between Europe and the US, where PGD is not regulated.
Study funding/competing interest(s): Funding by University(ies) – This study was conducted under the auspices of Yale University's Department of Political Science, but did not require departmental funding.
Trial registration number: NA.
Keywords: PGD, European convention on human rights, reproductive rights, margin of appreciation, consensus

FEMALE (IN)FERTILITY

P-394 Comparative results of PGS cycles in patients needing two or three stimulations to provide for sufficient embryos for PGS

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Study question: Are different the results of Preimplantation Genetic Screening (PGS) cycles in patients needing to undergo two or three ovarian stimulations to yield at least ten mature oocytes?
Summary answer: Oocyte vitrification is a good strategy in patients with an inadequate response to PGS to improve their chances of a successful pregnancy. Patients who underwent 2 or 3 ovarian stimulations have different characteristics but the final outcomes were very similar.
What is known already: The main cause of infertility in recurrent miscarriage, implantation failure, severe mail factor and/or advanced maternal age is the high rate of embryo aneuploidy. PGS is a useful tool for improving treatment results in these couples. At least 10–12 mature oocytes (MII) are required to assured the availability of at least two normal embryos for transfer. Many women would need to undergo 2–3 ovarian stimulation cycles and oocyte accumulation to achieve this goal.
Study design, size, duration: An observational study of 112 patients undergoing two (2OS = 83) or three (3OS = 29) successive ovarian stimulations, oocyte accumulation, ICSI for PGS and fresh embryo transfer (ET) cycles performed between January 2012 and March 2014.
Participants/materials, setting, methods: Women undergoing several ovarian stimulations for PGS by CGH at Hospital Universitario Quiron-Dexeus. Ovarian stimulation was performed with rec-FSH or rec-FSH + HMG and GnRH-antagonist. Euploid embryos were transferred on day 5. Patient characteristics, ovarian response and clinical outcomes after two or three stimulations (20S vs 3OS) were compared by an apropriate statistical analysis.
Main results and the role of chance: Patients underwent a mean of 2.3 ± 0.4 stimulation cycles. Comparative characteristics of 2OS vs 3OS patients were: age 39.1 ± 3.4 vs 39.8 ± 4.0 years (*n*.s.), AMH 1.6 ± 1.2 vs 0.7 ± 0.7 ng/ml ($p < 0.5$); AFC 11.0 ± 5.0 vs 9.0 ± 4.0 ($p < 0.5$) respectively. Patients in 2OS group produced significantly more oocytes during second stimulation (7.5 ± 1.9 vs 10.3 ± 4.1; $p < 0.00$) regardless repeating the same stimulation protocol in 80.1% of the cases. Among patients in 3OS group, the mean number of oocytes recovered was identical in the three stimulation cycles (6.2 ± 2.5). Comparing 2OS vs 3OS, there were no significant differences in number of total oocytes (17.8 ± 4.7 vs 18.5 ± 6.3), number of embryos biopsied (9.0 ± 3.0 vs 7.0 ± 3.0), euploid embryos rate (17.6%vs18.6%) and number of embryos transferred (1.6 ± 0.5 vs 1.3 ± 0.5). There were no differences in clinical pregnancy rates per patient (34.9 vs 31.0%) and per embryo transfer (56.8 vs 60%) between 2OS vs 3OS patients.
Limitations, reason for caution: This is an observational study, performed at a single center, in a limited number of cases, with our population of patients, under our local stimulation protocols and our laboratory of oocyte cryopreservation and PGS.
Wider implications of the findings: Our results suggest that patients needing to undergo two or three ovarian stimulation cycles can expect similar results after the transfer of one or two euploid embryos. The accumulation of oocytes for PGS in patients with suboptimal ovarian response to stimulation

seems a feasible strategy to achieve the transfer of euploid embryos and clinical pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproduction Department of Quiron Dexeus Hospital, Barcelona, Spain
Trial registration number: NA.
Keywords: DGS, oocyte vitrification, aneuploidies, ovarian stimulation

P-395 The impact of thyroid stimulating hormone (TSH) on Anti Mullerian hormone (AMH) levels in infertile women. Does TSH affect follicular recruitment?

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Study question: Recent animal studies demonstrated impacts of thyroid function on follicular recruitment. Thyroxin co-culture within physiological ranges resulted in accelerated growth and reduced apoptosis of preantral follicles. To investigate whether thyroid function also influences follicular recruitment in humans, the impact of thyroid function on AMH in euthyroid infertility patients was investigated.
Summary answer: Euthyroid infertility patients with TSH ≥ 2.5µIU/mL demonstrated significantly lower AMH levels than euthyroid women with TSH < 2.5µIU/mL. These results suggest that thyroid function may influence follicular recruitment in infertility patients.
What is known already: Thyroid disorders are associated with menstrual irregularities and may cause anovulation. Even overt and/or subclinical hypothyroidism has been associated with female infertility. These observations have led to the commonly adopted practice of supplementing thyroxin in women with TSH ≥ 2.5 µIU/mL, desirous of pregnancy.
Study design, size, duration: All 225 infertility patients underwent AMH and thyroid function testing at a University-affiliated private fertility center between 2009 and 2014.
Participants/materials, setting, methods: In 225 infertile women the association of AMH and stratified TSH levels (TSH< vs. ≥2.5 µIU/mL) was investigated. Analyses were adjusted for patient age, sex hormone binding globuline levels and thyroid autoimmunity. Only euthyroid women with normal prolactin concentrations were eligible for enrollment.
Main results and the role of chance: Mean patients' age was 38.4 ± 5.0 years, mean AMH levels were 1.3 ± 2.0 ng/mL (median 0.4 ng/mL, [0.1–11.0 ng/mL]), mean TSH levels were 1.8 ± 0.9 µIU/mL. Adjusted for age and SHBG, women with TSH <2.5 µIU/mL presented with significantly higher AMH levels (1.4 ± 2.1 ng/mL) compared to those with TSH ≥2.5 µIU/mL (0.9 ± 1.5 ng/mL; $P = 0.02$), a finding remaining significant when the analysis was further adjusted for laboratory evidence of thyroid autoimmunity ($P = 0.02$).
Limitations, reason for caution: Women included in these study were predominantly of advanced reproductive age. Whether these findings are applicable to women of all ages remains to be elucidated.
Wider implications of the findings: The here presented significant association of AMH and TSH levels among euthyroid infertile women offers an explanation for previously observed effects of thyroid hormone supplementation on pregnancy potential in euthyroid women with TSH levels above 2.5 µIU/mL. Our findings also contribute to the ongoing discussion of whether thyroid function or thyroid autoimmunity represents the more important factor in affecting female reproduction. Further research is needed to evaluate whether L-thyroxin supplementation may have a beneficial effect on oocyte yield in the course of IVF.
Study funding/competing interest(s): Funding by national/international organization(s) – The Foundation for Reproductive Medicine.
Trial registration number: NA.
Keywords: thyroid function, anti Mullerian hormone (AMH), thyroid stimulating hormone (TSH), functional ovarian reserve, follicular recruitment

P-396 Prevalence of HPV infection in oocyte donors and women treated for infertility: a prospective study

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Study question: What is the prevalence of Human Papilloma Virus (HPV) infection in oocyte donors and women treated for infertility? Is there any correlation between HPV status, childlessness and female infertility?
Summary answer: In this study, HPV prevalence in oocyte donors was 26% compared to 15% in women treated for infertility.

The prevalence of HPV in oocyte donors was significantly higher than the prevalence in women treated for infertility. Younger childless oocyte donors were significantly more often HPV+ than donors who were parous. Length of infertility treatment was negatively related to HPV prevalence. Further studies are needed to clarify this issue.
What is known already: HPV infection could play a role in human fertility like other sexually transmitted diseases (STDs). Higher risk of spontaneous abortion and possible mother-fetus transmission has been also described in HPV positive women. Moreover, *in vitro* studies demonstrated increased apoptosis and delayed development in HPV positive embryos. Unlike other STDs, HPV is not tested obligatorily for gamete donors or infertile couples, although it could significantly affect fertility, pregnancy or the fetus itself.
Study design, size, duration: This was a prospective laboratory based study. Cervical smears of oocyte donors ($n = 158$) and women treated for infertility ($n = 610$) were collected between April 2013 and October 2014. All participants filled a questionnaire focused on their health status and sexual behavior.
Participants/materials, setting, methods: Cervical smears were analyzed for presence of 14 high-risk (hrHPV) genotypes by Cobas 4800 HPV system (Roche) and PapilloCheck HPV-Screening system (Greiner Bio-One) detecting 18 hrHPV. Data from questionnaires, clinical data and HPV screening results were analyzed by statistical software R.
Main results and the role of chance: Forty-one (26%) of oocyte donors were HPV+. Childlessness in HPV+ oocyte donors was more frequent than in the HPV- group (39 vs. 20%; $p = 0.016$). The average age was 25.6 in HPV+ vs. 27.4 in HPV-($p = 0.023$). HPV infection was detected in 90 (15%) women from infertile couples and increased with the number of sexual partners (median 4 vs. 5; $p = 0.002$). Interestingly, women treated for infertility ≤6 months were more frequently HPV+ than women treated ≥48 months (32.4 vs. 7.5%, $p = 0.001$). The prevalence of HPV was twice as high within oocyte donors as in infertile women (26 vs. 15%), which could be related to the lower age of oocyte donors (27.0 vs. 32.7; $p < 0.001$).
Limitations, reason for caution: Only cervical smears have been analyzed in this study. No data on embryos/fetus/child development of HPV positive women or recipients of oocyte from HPV positive donor were evaluated, which could contribute to clarification of the relation between HPV infection and female fertility.
Wider implications of the findings: The significantly higher prevalence of HPV infection in the group of oocyte donors is disconcerting. According to the literature, HPV positivity is a risk factor for pregnancy. HPV positive oocyte donors may be therefore a risk for the oocyte recipient and for the further development of the fetus.
Study funding/competing interest(s): Funding by national/international organization(s) – IGA_LF_2014_009, CZ.1.05/3.1.00/14.0307, CZ.1.05/2.1.00/01.0030.
Trial registration number: NA.
Keywords: Human papillomavirus, Female fertility, Oocyte donation

P-397 Direct ovarian stimulation (DOS) by intra-ovarian injection of corifollitropin-a for the patients with poor ovarian response in IVF-ET program

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Study question: The effects of DOS by intra-ovarian injection of Corifollitropin alfa on oocytes recovery, embryo development and pregnancy outcomes for patients with a history of poor response in previous IVF-ET cycles.
Summary answer: After DOS cycle, the number of good quality oocytes retrieved, good quality embryos and transferred embryos were significantly

increased. The clinical and ongoing pregnancy rates were statistically higher in DOS cycle (37.0 and 33.3%, respectively) compared with prior conventional GnRH antagonist cycle (0%). Our study showed that there was a significant improvement of IVF-ET outcomes in poor responders undergoing DOS.
What is known already: Poor ovarian response (POR) to stimulation usually indicates a reduction in follicular response resulting in a reduced number of retrieved oocytes. In general, these poor responders have reduced pregnancy rates compared with normal responders. There are numerous strategies that have been suggested to improve the probability of pregnancy in POR. However, pregnancy rates after in vitro fertilization (IVF) remain disappointingly low, with studies reporting around 10%
Study design, size, duration: This prospective study is conducted in the private infertility clinic between January and December 2013. Total thirty patients were enrolled and the criteria for the patient selection were as follows. a. Five or less retrieved oocytes in prior two consecutive IVF-ET cycles with conventional GnRH antagonist protocol. b. Serum AMH levels equal to and <1.0 ng/mL. c. And visible ovaries by TVS on the second day of menstruation.
Participants/materials, setting, methods: We conducted direct ovarian injection of FSH on aforementioned patients, and compared the reproductive outcomes with prior conventional IVF cycle. The outcome variables were the number of good quality oocytes retrieved (MI, MII) and embryos (G1, G1-1, G2), the number of embryos transferred, clinical pregnancy rate and ongoing pregnancy rate.
Main results and the role of chance: The effect of DOS was verified through comparison with the results of prior conventional GnRH antagonist protocol in the same study population. IVF-ET outcomes after DOS were significantly improved compared to their prior cycles. The number of retrieved oocytes (*3.37 ± 1.42* vs. *7.41 ± 3.34*), good quality oocytes (*2.19 ± 1.24* vs. *6.37 ± 3.20*), and good quality embryos (*1.37 ± 0.97* vs. *2.81 ± 1.24*) and successfully transferred embryos (*1.63 ± 0.74* vs. *3.00 ± 1.07*) were all increased to about two folds by DOS. Clinical and ongoing pregnancy rates improved up to 37.0 and 33.3%, respectively (Table 1).

Table 1 | IVF-ET and clinical outcomes

	Prior cycles (n = 27)	DOS cycles (n = 27)
No. of retrieved oocytes	3.37 ± 1.42[a]	7.41 ± 3.34[a]
No. of good quality oocytes (M1, M2)	2.19 ± 1.24[a]	6.37 ± 3.20[a]
No. of good quality embryos(G1, G1-1, G2)	1.37 ± 0.97[b]	2.81 ± 1.24[b]
No. of transferred embryos	1.63 ± 0.74[b]	3.00 ± 1.07[b]
Clinical pregnancy rate (%)	0	10 (37.0%)
Ongoing pregnancy rate (%)	0	9 (33.3%)

Mean ± SD; [a]$p < 0.001$ by Paired t-test; [b]$p < 0.05$ by Wilcoxon signed rank test.

Limitations, reason for caution: The limitation of the present study is that small number of patients would make it difficult to consider the available evidence for DOS. And the additional dose of rFSH or rLH might have influenced on the results. In spite of the limitations described above, the current attempt showed the remarkable outcomes for poor responders, which have not been suggested in most other studies. Furthermore, Cochrane reviews and meta-analysis revealed limited successes on quality of oocytes and embryos, pregnancy outcomes in patients using either numerous stimulation protocols or adjuvant therapy like GH and rLH in poor responders.
Wider implications of the findings: DOS by intra-ovarian injection of Corifollitropin alfa, as an alternative approach to ovarian stimulation, enhances the ovarian response, and thereby improves the number and quality of transferred embryos and clinical outcomes. This may be due to increased FSH concentration and sensitivity for early antral follicles with concomitant augmentation of angiogenesis by sufficient estrogen secreted in the treated ovaries. Consequently, it may enhance the ovarian response to the subcutaneous injected gonadotropins, several days later.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: direct ovarian stimulation (DOS), poor ovarian response (POR), corifollitropin alfa, ART

P-398 Evaluation of transvaginal hydrolaparoscopy (THL) as a first line investigation for tubal pathology: feasibility and prognostic capacity

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Study question: What is the prognostic capacity of THL as a first line investigation for tubal pathology to predict natural conception?
Summary answer: In an unselected group of subfertile women who undergo THL, the incidence of bilateral tubal occlusion and peritubal disease are 4.9 and 19.2%. These abnormalities strongly reduce natural fecundity.
What is known already: THL is a safe method to investigate tubal patency and explore the pelvis in subfertile women. It can be performed as an outpatient procedure and is well tolerated.
Study design, size, duration: Between 2000 and 2011, we performed THL as a first line diagnostic test in 952 subfertile women in four large hospitals in the Netherlands. Follow-up on fertility outcome during 36 months after the procedure was derived by examining medical records.
Participants/materials, setting, methods: We studied women with primary or secondary subfertility trying to conceive for at least 12 months. Cumulative treatment independent ongoing pregnancy rates were calculated for each category of findings, using Kaplan-Meier analysis. Furthermore we calculated fecundity rate ratios (FRR) to express the relative risk on natural conception.
Main results and the role of chance: Bilateral tubal patency was found in 82.7% One-sided tubal occlusion in 12.6% and bilateral tubal in 4.7% Cumulative spontaneous pregnancy rates after 36 months were 52% for women with bilateral patent tubes, 48% for women with one-sided tubal occlusion and 7% for women with bilateral tubal occlusion. Corresponding FRRs were 0.93 (95% CI 0.71–1.23) for one-sided tubal occlusion, and 0.10 (95% CI 0.03–0.31) for bilateral tubal occlusion. Endometriosis and adhesions were diagnosed in respectively 6.4 and 9.1 % of women, and both endometriosis and adhesions in 3.8%. Corresponding FRRs are 0.73 (95% CI 0.49–1.02), 0.62 (95% CI 0.43–0.90), and 0.35 (95% CI 0.19–0.71).
Limitations, reason for caution: In this prospective cohort, THL was used in a clinical setting and abnormalities at THL had medical consequences, thus generating informative censoring. We controlled this by doing time to event analysis.
Wider implications of the findings: The results of our study show that when bilateral tubal occlusion of peritubal disease are diagnosed at THL, chances of natural conception are significantly reduced. THL is a feasible procedure and because of its prognostic capacity it can be used as a first-line diagnostic test for tubal patency in subfertile women.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Not applicable.
Trial registration number: NA.
Keywords: Transvaginal hydrolaparoscopy, fallopian tube, culdoscopy, prognostic capacity

P-399 Alterations of myeloid cell populations in human follicular fluid correlate with ovarian response to gonadotropins

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Study question: We sought to determine whether ovarian stimulation leads to myeloid cell population alterations as seen in other proangiogenic inflammatory states.

Summary answer: In close similarity to changes observed in the placenta before labor, ovarian stimulation is associated with reciprocal alterations in myeloid cell populations within the follicular fluid (FF) that correlate with ovarian response to gonadotropins.

What is known already: Immature myeloid cells (IMCs) are bone marrow-derived cells that normally differentiate into granulocytes, macrophages, and dendritic cells (DCs) but expand in proangiogenic inflammatory states such as malignancy and pregnancy. For example, labor is preceded by a decrease in IMCs and an increase in DCs populating the placenta.

Ovarian stimulation and ovulation are associated with follicular inflammation and angiogenesis. The presence of inflammatory cell population shifts as seen in other proangiogenic conditions remain unexplored.

Study design, size, duration: An observation study; We analyzed 12 FF samples from 10 patients undergoing IVF. Patients received standard ovarian hyperstimulation protocols, and oocyte retrieval was performed 34 hours after hCG. In order to minimize blood contamination, only FF from the first aspirated follicle was analyzed. FFs with bloody contamination were discarded.

Participants/materials, setting, methods: FF single cell suspensions were immunostained with anti CD45; HLA-DR; lin-2; CD33 antibodies. The samples were run on FACS Calibur and analyzed using FloJo software. Bone marrow derived hematopoietic cells were detected by expression of CD45. DCs were defined as $CD45^+$ $lin2^-HLADR^+$ cells and IMCs were defined as $CD45^+$ $lin2^-$ $HLADR^-CD33^+$ cells.

Main results and the role of chance: FFs (n = 12) from 10 patients were analyzed. Patients were all normal responders 33.5 ± 0.9 years of age, BMI 26.7 ± 0.8 kg/m^2, day 3 FSH was 6.0 ± 0.3 IU/L, estradiol levels at day of hCG trigger was 1181 ± 111 pg/ml, number of eggs retrieved was 9.6 ± 1.0. We observed that both IMCs and DCs are highly abundant myeloid cell populations within the FF: 41.7 ± 4.5% and 20.2 ± 5.0% of total $CD45^+$ bone marrow derived hematopoietic cells respectively. We detected a significant negative correlation between IMCs and DCs in the FF (r = -0.60, p = 0.038), possibly indicating a process of differentiation of IMCs into DCs. Interestingly, the presence of DCs in FF correlated with the response to ovarian stimulation as reflected by estradiol levels at the day of hCG triggering of ovulation (r = 0.58, p = 0.044).

Limitations, reason for caution: This is an observational study and only associations can be determined. In vitro culture studies and mouse studies are underway to determine whether IMCs indeed differentiate into DCs upon follicular maturation.

Wider implications of the findings: Our study indicates that ovarian stimulation is accompanied by alterations of IMCs and DCs in the FF in a reciprocal fashion that correlates with ovarian response. Similar population shifts were observed by our group upon maturation of the placenta before labor, pointing at a universal phenomenon of sterile inflammation that accompanies physiologic angiogenesis. Specifically, in the ovary, differentiation of IMCs into DCs may lead to healthy development, angiogenesis and maturation of the ovarian follicle.

Study funding/competing interest(s): Funding by national/international organization(s) – Israel Science Foundation 142/09.

Trial registration number: NCT01083745.

Keywords: ovarian stimulation, myeloid cells, angiogenesis

P-400 Long-term effects of methamphetamine exposure in adolescent mice on the future ovarian reserve in their adulthood: a randomized controlled study

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Study question: Does a long-term exposure of methamphetamine (MA) in adolescent mice have negative impacts on their future ovarian follicle number and anti-Mullerian hormone (AMH) level?

Summary answer: Eight weeks of methamphetamine abuse from adolescent days significantly decrease the primordial and growing follicle number and increase the atretic follicle number in their later reproductive age. We also found a lower AMH secretion per cell after granulosa cell culture in the treatment group compared with the control group.

What is known already: Several studies have demonstrated that MA, alongside its adverse impact on nervous system, is teratogenic as well as embryotoxic. What's more, it could also negatively affect the male fertility by disrupting the function of hypothalamic-pituitary-testicular axis, decreasing levels of luteinizing hormone, and damaging sperm function as well as testicular structure. As for adult women, a long-term abuse of MA could result in the disruption of menstrual cycles and dysfunction of hypothalamic-pituitary-gonadal axis.

Study design, size, duration: This is a randomized controlled cross sectional study with a sample size of 11 mice of the treatment group and 11 of the control group. The duration of MA administration lasted for 8 weeks. Ovaries and blood serum of each mouse were obtained and further processed after MA/saline treatment.

Participants/materials, setting, methods: Methamphetamine (5 mg/kg) or saline was administered to ICR mice from the 21st PD for 8 weeks. Serum AMH level was measured by ELISA. Ovarian follicle numbers were counted after HE-staining. Expression of AMHR2 was valued by realtime-PCR and western-blotting. Granulosa' secretion of AMH was investigated by cell culture.

Main results and the role of chance: A significantly lower number of ovarian primordial follicles per section (22.5 ± 6.3 vs. 38.4 ± 6.0, p = 0.001), and growing follicles(5.4 ± 3.0 vs 10.0 ± 3.5 p = 0.001) as well as higher number of atretic follicles per section (13.6 ± 3.3 vs. 10.9 ± 3.7, p = 0.038) in the treatment group were found than those in the control group. After ovarian dissociation and cell culture, we found that granulosa cells from the treatment group showed a lower secretion of AMH per cell after rFSH stimulation (0.08 ± 0.02 vs. 0.11 ± 0.02 pg/L, p = 0.021). However, no significant difference was found in the serum AMH level, and the expression of mRNA and protein of ovarian AMH receptor-2 between the two groups.

Limitations, reason for caution: The long-term impact of methamphetamine to adolescents on their future ovarian reserve was only shown in murine with a relatively small sample size. A perspective RCT on human is difficult because of ethical restrictions.

Wider implications of the findings: It's generously accepted that methamphetamine abuse causes neurodegeneration. Some of the affected neurological pathways also contribute to the neuroendocrine system relating to fertility. But the potential effects of MA on the female reproductive functions have not been extensively studied. From a public health point of view, this is also an interesting topic as a considerable portion of the juvenile is consuming such a drug which could possibly compromise their future reproductive ability.

Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology.

Trial registration number: NA.

Keywords: methamphetamine, adolescent, ovarian reserve, ovarian follicle counting, anti-Mullerian hormone

P-401 Enrichment of functional pathways in cumulus cells surrounding oocytes whose embryos successfully implant

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Study question: The main objective of the study was to determine biomarkers expressed in human granulosa (GC) and cumulus (CC) cells that could be used for the prediction of pregnancy in IVF procedures.

Summary answer: There were no biomarkers expressed in GC or CC that could be used for the prediction of pregnancy after embryo transfer (ET) in IVF procedures. However, gene ontology (GO) analysis revealed pathways connected with embryo development are significantly overrepresented in CC samples whose embryos achieved pregnancy.

What is known already: The oocyte plays a dominant role in regulating GC and CC functions during folliculogenesis and it is believed that functions of GC and CC indirectly reflect oocyte's competence. For this reason, several published studies in recent years suggested that genes expressed in GC and CC have the potential to serve as biomarkers of pregnancy in IVF procedures. However, potential candidate genes differ between the studies and thus, a universal biomarker is still to be found.

Study design, size, duration: Forty-seven infertile women were included in the study. The study was approved by National Ethics Committee and all patients signed informed consent. Genome wide gene expression analysis of 64 individual GC and CC samples was performed using microarrays, followed by a qPCR validation of microarray data.
Participants/materials, setting, methods: GnRH antagonist protocol was used for ovarian stimulation in women with tubal and unexplained cause of infertility. Follicles were aspirated individually. After that, GC and CC were stored separately and oocytes cultured individually. Elective single embryo transfer was performed. Microarrays were used for genome wide gene expression analysis, followed by a functional analysis of gene expression profiles using Gene Set Enrichment Analysis (GSEA).
Main results and the role of chance: There were no significantly differentially expressed genes between non-implanted and implanted embryos in either of the cell types after the correction for multiple testing. Also, qPCR analysis was in accordance with microarray analysis and there were no differentially expressed genes. GSEA analysis revealed pathways as ectoderm and epidermis development, and keratinocyte differentiation are significantly overrepresented in CC samples, surrounding oocytes whose embryos achieved pregnancy. There were no overrepresented pathways in GC samples. This finding implies, that processes of embryogenesis are regulated already during folliculogenesis and perhaps the flaws in these processes lead to the development of less competent embryos with a lower ability to implant.
Limitations, reason for caution: Relatively small number of patients included in the study.
Wider implications of the findings: It has already been proposed that there are advantages of using the complete set of up and down-regulated genes gained from a microarray experiment, rather than focusing on the most affected ones, as physiological changes are often not translated into large gene expression variations. Therefore, genes from the same pathway moving in a cluster can serve as a better indication of the physiological condition studied than expression of a single gene.
Study funding/competing interest(s): Funding by national/international organization(s) – Slovene Research Agency, grant number P30326.
Trial registration number: NA.
Keywords: gene expression, cumulus cells, pregnancy

P-402 Anti-Mullerian hormone (AMH) as predictor of natural conception

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Study question: Does AMH predict the chances of natural conception and time to conception in women with unknown fertility status?
Summary answer: AMH does not predict the chances of conception in women <40 years and the time to conception independently from the age of women. AMH predicts the chances of conception in women aged 40 years or older.
What is known already: AMH is a marker of ovarian reserve and response to gonadotropins. Very few data are available in the literature on the use of AMH in predicting natural fecund ability.
Study design, size, duration: This was a prospective cohort study. Enrolment was offered to all women attending our clinic who were about to start trying to conceive between May 2012 and September 2014. Out of 191 couples enrolled in the study, 169 couples completed the follow-up and were included in the analysis.
Participants/materials, setting, methods: The study included women of reproductive age with no previous reproductive experience. Patients with risk factors for infertility were excluded from the study. A secretary contacted the patients by email or by telephone every 3 months up to 1 year from the enrolment in the study.
Main results and the role of chance: The median female age was 34 years; the median male partner age was 37 years. One hundred twenty-one couples conceived during the study period (63.4%; 95% CI, 56.1–70.2%). The mean (±SD) time to conception was 4.7 (±2.8) months. AMH levels were similar in women who conceived and did not conceive ($p = 0.129$). AMH levels were similar in patients who conceived and did not conceive when the analysis was restricted to women <30 years ($p = 0.255$), ≥30 and <35 years ($p = 0.569$) and ≥35 and <40 years ($p = 0.741$). In women ≥40 years, AMH levels were higher in patients who conceived (2.0 ± 1.5 ng/ml) than in those who did not conceive (1.0 ± 0.9 ng/ml; $p = 0.032$). There was no significant correlation between AMH levels and time to conception ($p = 0.610$).
Limitations, reason for caution: The major limitation of the study is the small number of women ≥40 years ($n = 29$).
Wider implications of the findings: If the results of this study will be confirmed by investigations including a larger number of women aged 40 years or older, measurement of AMH might be offered to these patients in order to predict the chances of natural conception.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Piazza della Vittoria 14 S.r.l.
Trial registration number: NA.
Keywords: anti-Mullerian hormone, time to pregnancy, fecundity, natural conception, age

P-403 Pregnancy outcomes of intrauterine injection of human chorionic gonadotropin before frozen embryo transfer in *in vitro* fertilization

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Study question: To investigate potential effects of intrauterine injection of human chorionic gonadotropin (hCG) before frozen embryo transfer (FET) in *in vitro* fertilization.
Summary answer: The clinical pregnancy rate and live birth rate were decreased in hCG treated group. No birth defect was found in this group.
What is known already: HCG is the first embryonic signal secreted by the embryo before implantation. It has been demonstrated that hCG played an important role in embryo invasion, implantation and uterine immune tolerance. Several previous studies have declared intrauterine administration of hCG before embryo transfer could increase the pregnancy rate *in vitro* fertilization/intracytoplasmic sperm injection in fresh cycles. However, its potential benefits in frozen embryo transfer cycles have not been investigated.
Study design, size, duration: This was a case control study of 143 cycles from 143 patients performed between May and September 2012. Primary endpoint was live birth rate. Secondary endpoints included clinical pregnancy rate, implantation rate, miscarriage rate and ectopic rate. Safety endpoint was birth defect.
Participants/materials, setting, methods: Infertility patients younger than 42 years endured at lest one failed cycle of either fresh or frozen embryo transfer. Thirty-eight patients in the study group received 500 IU of hCG via intrauterine administration before FET, and 115 patients in the control group underwent FET without hCG.
Main results and the role of chance: The clinical pregnancy rate was statistically significantly decreased in the hCG administration group compared with the control group (26.3 vs.46.1%, $P = 0.032$). The live birth rate (18.4 vs. 34.8%, $P = 0.058$) and implantation rate (13.0 vs.22.4%, $P = 0.054$) were lower in the hCG group than the control group, but with no statistical significance. There were no significant difference in miscarriage rate (20.0 vs. 24.5%, $P = 1.000$) and ectopic rate (10.0 vs.0%, $P = 0.159$) between the study group and the control group. None of birth defect infant was found in the hCG group.
Limitations, reason for caution: This was a retrospective case control study of limited samples from a single IVF center. Prospective analysis of large data sites from a multicentre study is necessary to confirm the effect on intrauterine injection of hCG.
Wider implications of the findings: The miscarriage rate and ectopic rate were not influenced by hCG intrauterine administrated before FET.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maternal and child health care hospital of Shaan Xi province.
Trial registration number: NA.
Keywords: frozen embryo transfer, human chorionic gonadotrophin, intrauterine administration, pregnancy outcome

P-404 Preeclampsia in autologous and oocyte donation pregnancy: is there a different pathophysiology?

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Study question: Is the extent of complement activation in pregnancies after oocyte donation (OD) complicated by preeclampsia different from complement activation in preeclamptic and uncomplicated autologous pregnancies?
Summary answer: In line with autologous preeclampsia pregnancies, there is excessive activation of complement in preeclamptic OD pregnancies. However, in contrast to autologous pregnancies this is not associated with a counterbalancing upregulation of complement regulatory proteins, suggesting another trigger or regulatory mechanism involved in placental C4d deposition in preeclamptic OD pregnancies.
What is known already: OD is accompanied with an increase in early and late obstetrical problems. The pathophysiological mechanism underlying these complications is thought to differ from autologous pregnancies, based on differences in histocompatibility, pathological findings and neonatal birth weight. However, both in autologous and OD pregnancies, the pathogenesis of preeclampsia is poorly understood. In autologous pregnancies, increasing evidence suggests that preeclampsia is associated with excessive complement activation.
Study design, size, duration: Case control study.
Participants/materials, setting, methods: Patients were selected who conceived by oocyte donation and delivered at our hospital. Thirty-three pregnancies were uncomplicated, 9 pregnancies were complicated by pre-eclampsia. We furthermore included 46 autologous pregnancies with preeclampsia and 20 autologous and uncomplicated pregnancies as controls. Paired samples of decidua basalis, maternal peripheral blood and umbilical cord blood from all pregnancies were obtained within 24 h after delivery.
Main results and the role of chance: A significantly ($p < 0.03$) higher incidence of C4d deposition was observed in placentas from women with preeclampsia compared to uncomplicated pregnancies, both in OD and autologous pregnancies. The level of complement factors in serum did not differ between the groups. We observed no fetal growth restriction in our preeclamptic OD group. The main difference between OD and autologous pregnancies was the significantly lower placental mRNA expression level of complement regulatory proteins in both uncomplicated and preeclamptic OD pregnancies.
Limitations, reason for caution: A limitation is the low number of included patients with OD. In the Netherlands commercial and anonymous donation is forbidden by law, which affects the number of women that apply for OD. Furthermore, many patients wish to conceal the fact that their pregnancy was conceived artificially and will not mention the OD to medical personnel.
Wider implications of the findings: To unravel the, possibly different, pathophysiological mechanism underlying preeclampsia in OD pregnancies. We hypothesize that the allogeneic nature of the fetus in OD pregnancies plays a role in the development of these pregnancy complications.
Study funding/competing interest(s): Funding by national/international organization(s) – now.
Trial registration number: No RCT.
Keywords: oocyte donation, pregnancy complication, complement activation

P-405 DEHP may alter ovarian function by induced mitochondrial fragmentation, increased reactive oxygen species and DRP1 expression in mouse granulosa cells

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Study question: Evaluate the effects of phthalates (PAEs) on mitochondrial function in ovarian granulosa cells (GCs).
Summary answer: Di-(2-ethylhexyl) phthalate (DEHP) affects female fertility by changing the steroidogenesis and altering mitochondrial function of GCs.
What is known already: PAEs are very widely used as plasticizers and solvents. Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant PAEs used in the world. Previous studies have demonstrated that DEHP interferes with reproductive system by inhibiting steroid hormone production.
Study design, size, duration: Six-week-old female ICR ovarian granulosa cells (GCs) were used to evaluate of DEHP on female reproductive system. Mice were treated with different concentration of DEHP for 2 or 4 weeks. Eighty mice were used in the study.
Participants/materials, setting, methods: Plasma estradiol and anti-Müllerian hormone (AMH) were measured by ELISA. Mitochondrial activity including oxygen consumption rate (OCR), glycolytic flux (extracellular acidification rate, ECAR) and ATP production rate were measured by a Seahorse XF24 extracellular flux analyzer. Mitochondria morphology of GCs was investigated by staining with MitoView green under confocal fluorescent microscope. Cell proliferation rate was determined by colorimetric MTT assay.
Main results and the role of chance: Oral administration with DEHP (0.05–500 mg/kg/day) for 2 weeks increased mouse estradiol levels. In contrast, a decreased estradiol levels was found after 4 weeks of DEHP treatment. Moreover, OCR and ATP production rate were also increased in GCs obtained from the mice treated with DEHP (5 and 500 mg/kg/day). The OCR was increased by 3-folds in the highest concentration of DEHP treatment group (347.0 ± 53.1 pMoles/min) compared with control (102.7 ± 32 pMoles/min). In contrast, ECAR of GCs was reduced after 2 weeks of DEHP treatment. DEHP treatment (5 and 500 mg/kg/day, respectively) showed a decrease of elongated mitochondria (43.9 and 46.2 vs. 54.2% as compared with vehicle group). Increased mitochondrial fragmentation were observed significantly after DEHP treatment. Moreover, expression of mitochondrial fission factor, dynamin-related protein 1 (Drp1) was elevated after 4 weeks of DEHP exposure. *In vitro* study of GCs, OCR also increased after DEHP (10 and 100 mM) treatment. The higher concentration of DEHP (100 mM) treatment inhibited the cell proliferation of GCs.
Limitations, reason for caution: It is hardly to distinguish the order or the correlation of ROS production and mitochondrial fragmentation.
Wider implications of the findings: PAEs could affect female fertility as time goes by.
Study funding/competing interest(s): Funding by national/international organization(s) – Ministry of Science and Technology.
Trial registration number: LAC-101-0022.
Keywords: di-(2-ethylhexyl) phthalate, mitochondria, reproductive toxicity, ovarian granulosa cell

P-406 A randomized double blind comparison of two hormone replacement regimens for thawed blastocyst transfer

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Study question: The two regimens of hormone replacement protocols used in thawed blastocyst transfer show different clinical outcomes?
Summary answer: There were significant differences for the clinical outcomes of thawed blastocyst transfer in the two regimens of hormone replacement protocols.
What is known already: The clinical outcomes of thawed blastocyst transfer in various regimens of hormone replacement protocols are controversial because large prospective randomized controlled trial is not yet conducted. A few observational studies suggested that the use of Estrogel (®) may be more effective for the clinical outcomes than Premarin (®).
Study design, size, duration: A large prospective, double-blind, randomized controlled trial in order to evaluate the outcomes in two different hormone replacement regimens for thawed blastocyst transfer was performed. A total of 1,000 women (median age 38.3 years), undergoing IVF at a clinic in Japan, were enrolled from July 2014 to October 2014.
Participants/materials, setting, methods: Participants were randomized according to a computer-generated randomization list as follows. Premarin (®) group ($n = 500$; 2.492 mg/day; from the second day of menstruation to the fifth day and 4.98 mg/day, after the sixth day). Estrogel (®) group ($n = 500$; 2.16 mg/day in Estrogen conversion, after the second day of menstruation).
Main results and the role of chance: Serum estradiol (E2) levels were 305.71 ± 7.70 pg/ml in Premarin (®) group, 783.31 ± 30.11 pg/ml in Estrogel (®) group. Therefore, serum E2 levels in Estrogel (®) group were higher than those in Premarin (®) group ($P < 0.001$). Furthermore, the pregnancy rate in Estrogel (®) group was higher than those in Premarin (®) group (39.0 versus 27.0%, $P = 0.0001$, rate ratio 1.73, 95% confidence interval: 1.32–2.26).

Moreover, the miscarriage rate in Estrogel (®) group was lower than those in Premarin (®) group (5.0 versus 10.1 %, $P = 0.002$, risk ratio 0.64, 95% confidence interval: 0.45–0.86). In addition, both regimens were well-tolerated, with no difference in serious adverse events.
Limitations, reason for caution: The conclusions are limited to the two hormone replacement regimens studied for the patient population examined in the present study.
Wider implications of the findings: Serum E2 levels contribute to the differences of pregnancy rate and miscarriage rate in the two different regimens. From the viewpoint, Estrogel (®) have an advantage as hormone replacement regimen for thawed blastocyst transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Oak Clinic Group.
Trial registration number: UMIN000015488.
Keywords: ART, thawed blastocyst transfer, hormone replacement regimens, a large prospective randomized controlled trial, pregnancy rate

P-407 Prevalence of unrecognized celiac disease in couples with unexplained infertility

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Study question: Is there any relation between celiac disease (CD) and unexplained infertile (UEI) couples? What is the prevalence of CD in unexplained infertile couples?
Summary answer: The prevalence of CD in UEI couples is not as high as that some reports. The study findings suggested that investigation of both of the couples with a diagnosis of UEI may be more beneficial in clarifying the etiology.
What is known already: CD is a chronic autoimmune disease characterized by small intestinal malabsorbtion and diarrhea, precipitated by ingestion of food products containing gluten. There are studies reporting that some nutritional deficiencies and some factors related to immunity may cause a decrease in fertility and some problems in sperm paramethers. In former studies, prevalence of CD was investigated in only women with UEI. There is not a knowledge about prevalence of CD in UEI couples.
Study design, size, duration: A total of 68 couples with UEI who had admitted at TDV 29 Mayis Hospital IVF Center between January and June 2014 were included in this prospective pilot study.
Participants/materials, setting, methods: The diagnosis of UEI was reached with basic infertility tests. A history of CD was questioned in the initial evaluation. Anti-gliadin, anti-endomysial, tissue transglutaminase antibodies and total IgA were tested. Gastroscopy was done to patients with positive serologic tests. Histopathological CD diagnosis was reached according to Marsh criteria.
Main results and the role of chance: The mean age of study population were 33.40 ± 4.59 years. Out of 65 couples that were taken into the study group, one of the 5 couples had a positivity in the autoantibidies (7.69%). Out of these 65 couples none of them had a autoantibody positivity at the same time in both partners. Antigliadin antibodies found positive for 2 females out of 5 couples and in 3 male partners for the same group. Out of these 5 couples only one male partner had all the antibodies positive (1.5%). In the histopathological examination of patients with positive autoantibodies, only the patient in whom all autoantibodies were positive had findings compatible with Marsh IIIa gluten enteropathy. Only one couple had a diagnosis of CD (1.5%).
Limitations, reason for caution: Our study is based on a limited sample size. Our data should be confirmed in a larger cohort of subjects.
Wider implications of the findings: CD was shown to affect the reproductive system in women in many studies. CD may also cause a decrease in fertility in men by affecting sperm motility and androgen levels. These results suggest that investigation of both of the couples with a diagnosis of UEI may be more beneficial in clarifying the etiology.
Study funding/competing interest(s): Funding by hospital/clinic(s) – TDV 29 Mayis Hospital.
Trial registration number: 2014294.
Keywords: unexplained infertile couples, celiac disease, prevalence

P-408 Association between vitamin D status in serum and follicular fluid and IVF-ET outcomes in Chinese infertile women

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Study question: This study aimed to assess the association between 25(OH)D levels in serum and follicular fluid and IVF-ET (*In vitro* fertilization-embryo transfer) outcomes in Chinese infertile women.
Summary answer: Serum vitamin D deficiency significantly reduced pregnancy rates and live birth rates of IVF treatment. Though there was a correlation between follicular fluid vitamin D and serum vitamin D, no significant difference were found between follicular fluid vitamin D and IVF outcomes.
What is known already: Significant controversy has emerged over the last decade concerning the effects of vitamin D on non-skeletal tissues, especially in reproductive field. Data regarding role of vitamin D in female reproduction are sparse and conclusions are conflicting. In particular, a gap on this topic in Asian women needs to be filled.
Study design, size, duration: This was a prospective cohort study. One hundred eighty infertile women undergoing IVF–ET cycles were recruited from March to December, 2012.
Participants/materials, setting, methods: All patients underwent IVF cycles using standardized regimens for pituitary down-regulation and controlled ovarian stimulation. Serum and follicular fluid samples were obtained on oocyte-retrieval day. 25(OH)D levels were quantitatively detected by enzyme-linked immunosorbent assay. Vitamin D deficiency was defined as serum 25(OH)D <50 nmol/L.
Main results and the role of chance: In total population, the number of women with vitamin D deficiency, insufficiency and replete were 51 (28.3%), 101 (56.1%) and 28 (15.6%), respectively. Both pregnancy rates (47.1 vs 67.4%, $p = 0.011$) and live birth rates (40 vs 63.3%, $p = 0.005$) of women with serum 25(OH)D <50 nmol/L were significantly lower than that in women with 25(OH)D ≥50 nmol/L. No significant differences were found in IVF outcomes between groups of 50 nmol/L ≤25(OH)D ≤75 nmol/L and 25(OH)D >75 nmol/L. IVF outcomes of women with different vitamin D status in follicular fluid had no significant differences. After controlling for multiple variables, serum 25(OH)D deficiency significantly associated with reduced pregnancy rates (OR: 2.997, 95% CI: 1.292–6.951) and live birth rates (OR: 3.373, 95% CI: 1.453–7.827).
Limitations, reason for caution: Our results refer only to patients with good ovarian function who have satisfactory responses to ovarian stimulation. For aging women with poor ovarian reserve, differences of pregnancy rates between vitamin D deficiency and those who are not might not be as high as what we have found in this study.
Wider implications of the findings: Serum vitamin D deficiency impairs pregnancy rates and live birth rates in Chinese IVF women. Clinical utility of vitamin D levels in follicular fluid seems to be limit. Future randomized controlled studies which focus on the effectiveness of vitamin D supplement on IVF women who are vitamin D deficient are interesting and meaningful. At the meantime, more studies are needed to confirm the mechanisms undergoing these findings from each aspect of follicular development to embryo implantation.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by the National Natural Science Foundation of China (Grant No. 81070466) and the Science Technology Research Project of Guangdong Province (Grant No. 2012A030400010). The authors have none declared.
Trial registration number: NA.
Keywords: vitamin D, 25(OH)D, IVF-ET, follicular fluid, live birth

P-409 Immunosuppressive treatment with tacrolimus improves reproductive outcome for repeated implantation failures patients who have elevated in Th1/Th2 cell ratios

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Study question: We evaluated the clinical efficacy of immunosuppressive treatment with tacrolimus for women with repeated implantation failure (RIF) and an elevated peripheral blood T helper (Th) 1/Th2 cytokine producing cell ratio.
Summary answer: This study indicates tacrolimus treatment significantly increases clinical pregnancy rate and live birth rate in RIF patients with shifted Th1 immune responses without increased maternal fetal complications.
What is known already: Pregnancy is established when an embryo, which is a semi-allograft, is successfully implanted to maternal decidua with an establishment of maternal immune tolerance. Th1 and Th2 cells play important roles in immune responses, such as immune rejection or tolerance. There is a general agreement that pregnancy is associated with Th2 dominance, and Th1 immune response is associated with embryonic rejection. An underling mechanism of embryo rejection is considered to be similar to an allograft rejection.
Study design, size, duration: This was a prospective cohort study of treatment for RIF patients ($n = 66$) with elevated peripheral blood Th1 ($CD4^+/IFN\text{-}\gamma^+$)/Th2 ($CD4^+/IL\text{-}4^+$) cell ratios in our clinic between November 2011 and April 2014.
Participants/materials, setting, methods: Thirty-four patients were treated with tacrolimus (treatment group) and 32 received no treatment (control group). Treatment group received tacrolimus between two days before embryo transfer and the day of the pregnancy test. The daily dose of tacrolimus (1–3 mg) was determined based on the degree of the Th1/Th2 cell ratio.
Main results and the role of chance: The clinical pregnancy rate of the treatment group was 58.8%, which was significantly higher than that of the control group (3.1%) ($P < 0.0001$). In the treatment group, the miscarriage rate was 5.0%, the live birth rate was 55.9% ($P < 0.0001$). There was no significant side effect from tacrolimus in treatment group. No one developed obstetrical complications during pregnancy.

	Treatment group ($n = 34$)	Control group ($n = 32$)
Age (years)[a]	36.2 ± 2.6	36.2 ± 3.9
Number of failed embryo transfer cycle (n)[a]	5.8 ± 2.7	5.9 ± 2.6
Th1/Th2 ratio[a]	16.0 ± 6.9	17.0 ± 4.9
Clinical pregnancy rate per ET (%)[#]	58.8	3.1
Live birth rate (%)[#]	58.8	3.1
Spontaneous miscarriage rate (%)	5.0	0

ET; embryo transfer, [a]mean ± SD, [#]$p < 0.0001$.

Limitations, reason for caution: This study however, has a limitation, since this is not a randomized controlled trial and a sample size is small. Moreover, endometrial changes or peripheral immune responses after tacrolimus treatment were not evaluated thoroughly. Further study is needed for tacrolimus effect on systematic immune responses and endometrial receptivity.
Wider implications of the findings: Tacrolimus has been utilized throughout pregnancy for women who have received an allogeneic organ transplant, and many female recipients have given birth while taking tacrolium. Tacrolimus inhibits T-lymphocyte signal transduction via creating a new complex. Tacrolimus is classified as class C drug by the FDA pregnancy category. The safety of tacrolimus for both mother and fetus/baby during pregnancy has been well established in many reports of female transplant recipients who achieved a post-transplant pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The authors have received no funding for this study, and they have no financial interest in any companies. There are no competing interests.
Trial registration number: The authors have received no funding for this study, and they have no financial interest in any companies. There are no competing interests.
Keywords: repeated implantation failure, immunosuppressive treatment, Th1/Th2 ratio, tacrolimus

P-410 Contraceptive pill and gonadotropin-releasing hormone (GnRH) flare-up agonist versus GnRH antagonist protocol in poor responders: Lessons to a prospective study

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Study question: Is a contraceptive pill and gonadotropin-releasing hormone (GnRH) flare-up agonist protocol better than a GnRH-antagonist protocol in poor responders.
Summary answer: The ongoing pregnancy rates per transfer were similar between the two protocols, despite a higher number of transferred embryos in the contraceptive pill and GnRH flare-up agonist protocol.
What is known already: In case of poor responders, several authors have reported advantage of the initial endogenous gonadotropin "flare" induced by GnRH-a, enhancing the effect of exogenous gonadotropins. The introduction of GnRH antagonists (GnRH-ant) have been a hope for the poor responder patients, because of some advantages such as the immediate suppression of LH, absence of flare-up effect, reduction of stimulation duration and the dose of gonadotropins.
Study design, size, duration: In our ART center, before the Bologna criteria, poor responders were defined as having <4 mature oocytes retrieved in the first stimulation IVF cycle with a GnRH agonist (GnRH-a) long protocol and 375 IU/day FSH or hMG stimulation (P1 protocol). The aim of this prospective study between 2004 and 2010 is to compare two other protocols in poor responder patients after failure using long GnRH-a protocol.
Participants/materials, setting, methods: After the P1 protocol and an interval less than 4 months, four hundred forty women were randomized between P2 protocol (with a contraceptive pill then flare-up GnRH-a and 375 IU/d FSH or hMG) and P3 protocol (with a multidose flexible GnRH-ant and 375 IU/day FSH or hMG). Cycle cancellation was recommended when less than three mature follicles were observed.
Main results and the role of chance: Twenty-two women who had an ongoing pregnancy with the P1 protocol (6.6% per transfer) were excluded to the prospective study. There were no significant difference between the two other protocols (demographic data, cancellation rate, total gonadotropin dose, fertilization and cleavage rates, grade A/B embryos), excepted to the estradiol levels on the hCG day ($p < 0.001$), embryos obtained ($p < 0.001$) and transferred ($p < 0.01$), higher in the P2 protocol. Despite these, the ongoing pregnancy rates per transfer were the same between the two groups (14.6 and 14.2%). Prognostic factors of pregnancy (multivariate analysis) were woman age <36 years old, no tobacco use, total FSH/hMG dose <5000 IU and endometrial thickness >10 mm.
Limitations, reason for caution: In 2011, an ESHRE consensus conference had defined the poor ovarian response by "Bologna criteria". In this study, the poor responders were defined before the Bologna criteria by a prior poor response (<4 mature oocytes retrieved after a long GnRH-a protocol with 375 IU/day FSH or hMG).
Wider implications of the findings: Despite the initial dose of gonadotropins was similar in the three protocols (375 IU/day), the number of oocytes retrieved and embryos transferred were significantly higher with the P2 and P3 protocols than with the first protocol. As the Polyzos results in 2013, the increase of the oocytes between P1 and P2/P3 protocols was the most important factor of pregnancy in the poor responders.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CHU Amiens Picardie.
Trial registration number: NA.
Keywords: IVF, poor responder, GnRH agonist, GnRH antagonist, pregnancy rate

P-411 The influence of female overweight and obesity on the outcomes of assisted reproductive technology treatment: the experience of a Portuguese center

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Study question: To investigate if female overweight and obesity have negative effects on the probability of achieving a clinical pregnancy after treatments with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
Summary answer: Compared with women with normal Body Mass Index (BMI) (18.5–24.9 kg/m^2), overweight and obese women required a higher dose of gonadotrophines, more days of stimulation, and had a lower number

of embryos to transfer. However, they had similar rates of clinical pregnancy $p = 0.5$, OR: 1.095 [0.84; 1.43] and miscarriage $p = 0.78$, OR: 1.09 [0.56; 2.06].
What is known already: Studies of outcomes from assisted reproductive technology (ART) treatments have detected negative effects of increased female BMI. The probability of live birth after ART was found to be 9–10% lower among overweight/obese women compared with normal-weight women, with obesity affecting the outcome more than overweight, and with suggestions of a dose response relationship. Otherwise, studies found no conclusive effect of increased female BMI on pregnancy rate after IVF or on deliveries after IVF/ICSI.
Study design, size, duration: Retrospective cohort study. 1110 IVF/ICSI cycles from one Portuguese Center collected throughout 2012–2014. BMI was recorded in the electronic medical chart. Study Group – 384 women with BMI ≥25 kg/m^2; Control Group – 384 women with normal BMI (18.5–24.9 kg/m^2).
Participants/materials, setting, methods: Women going through IVF/ICSI at the Reproductive Medicine Department of Maternity Dr. Alfredo da Costa – CHLC, Lisbon Portugal. BMI was calculated as body weight (kg) divided by height squared (m^2) and categorized according to the World Health Organization. Statistical analyses were performed with the use of SPSS version 22.0.
Main results and the role of chance: Both groups had similar mean age 33.9 versus 34 years ($p = 0.2$). Study Group had greater duration of infertility 5.6 versus 5 years ($p = 0.01$); more cases of polycystic ovary syndrome 16 versus 8% ($p < 0.001$); greater average antral follicle count ($p = 0.007$), needed higher doses of gonadotrophines ($p = 0.02$), more time for stimulation ($p < 0.001$), had low number of embryos 3.7 versus 4.2 ($p = 0.04$) and more cases with less than 4 embryos 57 versus 50% ($p = 0.04$ OR: 1.3 [1.01; 1.67]). However, with regard to clinical pregnancies, miscarriage and multiple pregnancies, we did not find statistically significant differences between both groups. Clinical pregnancies 36 versus 35% ($p = 0.5$; OR: 1.095 [0.84; 1.43]); miscarriage 12.3 versus 11.4% ($p = 0.78$ OR: 1.09 [0.56; 2.06]); multiple pregnancies 22.5 versus 21.3% ($p = 0.77$ OR: 1.07 [0.65; 1.77]). A sub-analysis to obese women alone (88 cycles) showed similar results.
Limitations, reason for caution: The sample size was too limited to draw firm conclusions regarding specific differences in live-birth rates between BMI groups.
Wider implications of the findings: While overweight and obese women have poorer results throughout the cycle, there is no visible difference in terms of final outcome. Therefore, BMI ≥25 kg/m^2 should not be used as a contraindication to IVF treatments. However, couples should be advised that controlling weight will improve chances of success and provide long-term health benefits.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Maternity Dr. Alfredo da Costa – CHLC.
Trial registration number: NA.
Keywords: female, overweight, obesity, ART, pregnancy

P-412 Assessment of uterine, subendometrial blood flows and endometrial gland vascular endothelial growth factor (EG-VEGF) in women with unexplained infertility

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Study question: Our study tried to investigate whether there was a defective expression of the EG-VEGF in women with unexplained infertility? And if we could correlate this EG-VEGF expression to non-invasive sonographic variables, namely endometrial thickness as well as subendometrial and uterine blood flows.
Summary answer: Women of the Unexplained Infertility Group had lower VEGF score, thinner endometrial thickness, higher subendometrial flow Resistance index (RI) and lower subendometrial flow Pulsatility Index (PI).
What is known already: It has been noted that a peak stromal VEGF expression occurs in the proliferative phase with a peak glandular VEGF expression occurs during the secretory phase. There is a significant increase in VEGF mRNA throughout the endometrial cycle in the non-pregnant patient with its expression increasing 3–5 times from the early proliferative phase to the late secretory phase.
Study design, size, duration: Cohort prospective study was conducted at Ain Shams University Maternity Hospital during the period between August 2010 and July 2012.The study included two groups of women: The unexplained infertility group, included fifty women and *the* fertile group, included fifty fertile parous women.
Participants/materials, setting, methods: On day 6 after detection of urinary LH surge, transvaginal ultrasound scan (TVS) was done for measuring endometrial thickness, uterine artery and subendometrial Doppler velocimetry indices. On the same day of TVS, endometrial samples were taken using office suction sampler for immunohistochemical detection of the vascular endothelial growth factor.
Main results and the role of chance: When compared to women of Fertile Group, women of the Unexplained Infertility Group had a significantly lower VEGF score [EG-VEGF score 1 (0–2) in infertile group compared to 2 (1–3) in fertile group; $p < 0.001$], significantly thinner endometrial thickness (8 mm compared to 12 mm; $p < 0.001$), significantly higher subendometrial flow RI and significantly lower subendometrial flow PI [1.2 (1–1.33) compared to 1.34 (1.22–1.44); $p = 0.001$]. The difference between both groups regarding the uterine artery RI and PI was statistically insignificant.
There was a significant positive correlation between EG-VEGF score and each of endometrial thickness and subendometrial PI. There was a significant negative correlation between EG-VEGF score and subendometrial RI.
Limitations, reason for caution: The main limitation for this study is the small sample size which affected the confidence interval (CI) of uterine artery Doppler variables, made it a wide interval.
Wider implications of the findings: VEGF may be an important paracrine modulator of the vascular growth, remodelling, and functional vascular permeability in the endometrium. Furthermore, functional studies suggest for the first time a direct role for EG-VEGF and human uterine fluid during initiation of embryo implantation in mid-secretory phase of the cycle.
Study funding/competing interest(s): Funding by University(ies) – Ain Shams university Hospital, Cairo, Egypt.
Trial registration number: NA.
Keywords: unexplained infertility, endometrial gland vascular endothelial growth factor (EG-VEGF)

P-413 Frozen embryo transfer protocols: a comparison of clinical pregnancy and delivery rates

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Study question: Is there a difference in outcome between natural and hormone replacement therapy (HRT) frozen embryo transfer (FET) cycles?
Summary answer: Natural cycle FET results in higher rates of both clinical pregnancies and deliveries than HRT cycles.
What is known already: There is conflicting evidence on the effectiveness of different FET protocols with some studies claiming that HRT protocols are superior and others claiming the opposite.
Study design, size, duration: This is a retrospective observational study of initial FET cycles over 14 years from a prospective IVF database. This included 9,860 single FET cycles and 4,235 double FET cycles.
Participants/materials, setting, methods: The initial FET cycle for each woman was extracted. This included both cleavage and blastocyst embryos. Cycles with incomplete data (3.1%) were excluded. Chi square testing was used to compare proportions (clinical pregnancy and delivery), and multivariate logistic regression was performed to account for potential confounding variables.
Main results and the role of chance: The clinical pregnancy rates were higher for initial cycle single embryo cycles comparing natural and HRT protocols (28.7 vs 23.6% $p < 0.001$) [odds ratio 1.31 (1.17–1.45)]. This was confirmed on multivariate logistic regression with adjusted odds ratio (aOR) 1.79 (1.58–2.03). Comparison of delivery rates provided further support with rates of 23.7 and 17.3% ($p < 0.001$) [OR 1.49 (1.32–1.68)] for natural and HRT protocols, respectively. Again, multivariate logistic regression analysis supported this [aOR 1.91 (1.65–2.21)]. Confounding variables allowed for included patient ages at

time of freeze and thaw, embryo age in days, years of freeze and thaw, patient's previous reproductive history, IVF cycle number, freezing technique, embryo grade, BMI and presence of polycystic ovaries.
Limitations, reason for caution: Although this study limited FET cycles to the initial one for each patient, used single FET cycles and employed logistic regression to allow for known potential confounders, there is still the possibility for bias, which would be better negated by a prospective randomised trial.
Wider implications of the findings: The lower clinical pregnancy and delivery rates with HRT FET cycles indicate that this protocol should be limited in use to women who are unable to cycle naturally.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Monash IVF.
Trial registration number: This study met the criteria for an audit and so was not assigned a registration number.
Keywords: FET protocol, natural FET, HRT FET, delivery rate

P-414 Blastocyst development and pregnancy outcome of the frozen-embryo transfers are similar after GnRH agonist or HCG triggering in patients at risk of OHSS

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Study question: Is there any difference between gonadotropin-releasing hormone agonist (GnRH-a) or human chorionic gonadotropin (HCG) triggering of final oocyte maturation (FOM) in patients at risk of developing ovarian hyperstimulation syndrome (OHSS) regarding blastocyst stage embryo development (BSED) and ongoing pregnancy rates (OPR) of frozen-thawed embryo transfer (FTET) cycles?
Summary answer: Blastocyst stage embryo development and ongoing pregnancy rates of the frozen thawed embryo transfer cycles are similar when final oocyte maturation is triggered with either GnRHa or HCG in patients at risk of OHSS.
What is known already: There are a few studies comparing pregnancy outcomes of FTET cycles after triggering of FOM with either GnRHa or HCG and vitrification of embryos at pronuclear or cleavage stage which reported acceptable chance of live-birth after GnRHa compared to HCG triggering. However, as far as we know comparison of the results of BSET after GnRH versus HCG triggering in combination with OPR has not been reported.
Study design, size, duration: A hundred and twenty-six patients undergoing controlled ovarian stimulation had their embryos frozen after triggering of FOM with either GnRHa or HCG due to OHSS risk. They returned for 150 FTET cycles between August 2011 and September 2014 and the results were analyzed retrospectively from Istanbul Memorial Hospital IVF database.
Participants/materials, setting, methods: Embryos at the blastocyst stage were cryopreserved in 62 and 64 patients after GnRHa and hCG administration, retrospectively. Twenty four patients returned for the second FTET cycle because of a negative result or abortion. BSED after triggering with GnRHa or HCG and OPR of the following FTET cycles were compared.
Main results and the role of chance: There were no differences between age and BMI between the groups. The serum AMH levels were higher in GnRHa compared to hCG group (9.8 ± 6.4 vs. 6.4 ± 3.9; $p \leq 0.001$) which resulted in significantly higher number of retrieved (31.5 ± 10.81 vs 25.5 ± 7.2) and matured oocytes (22.9 ± 8.3 vs 19.6 ± 6.4). However maturation and fertilization, and blastocyst and top quality blastocyst development rates were similar (53 vs 50% and 16 vs 16%) after hCG and GnRHa triggering, respectively. Ongoing pregnancy rate per embryo transfer was similar for hCG and GnRHa groups (53 vs 47%, $p = 0.597$).
Limitations, reason for caution: The major limitation of this study is the retrospective nature which resulted in group differences such as AMH levels. However we believe that this doesn't have any significant effect on our results, because as a known fact live-birth rates do not increase when more than 15 oocytes are retrieved.
Wider implications of the findings: As demonstrated by our study GnRH a triggering of FOM has comparable results regarding BSED and OPR in patients at risk of OHSS. These findings has important clinical implications for future use of GnRHa triggering as an alternative to hCG in patients at risk of OHSS who are patients with polycystic ovarian syndrome as well as patients undergoing COS for fertility preservation or for oocyte donation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Memorial Sisli Hospital.
Trial registration number: NA.
Keywords: ovarian hyperstimulation syndrome, GnRH analog trigger

P-415 Müllerian malformations in an Assisted Reproductive Technologies Center: clinical implications

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Study question: Are the Müllerian malformations associated with infertility and poor obstetric outcome?
Summary answer: Müllerian malformations can result in poor reproductive outcomes but the most important impact is observed in obstetric outcomes.
What is known already: Müllerian malformations are deviations from normal anatomy resulting from maldevelopment of the Müllerian ducts. Depending on the type and the degree of anatomical distortion they are associated with health and reproductive problems.
Study design, size, duration: We revised all the files of assisted reproductive technologies (ART) cycles in our center from June 2010 to August 2014 ($n = 2100$). The malformations found in this group were divided according to the ESHRE/ ESGE classification (2013) and the study only included the class U3 and the class U4.
Participants/materials, setting, methods: We performed a retrospective study and analysed variables such as women's age, type of infertility, type of malformation (bicorporeal uterus which included dydelphys uterus, and hemi-uterus), type of ART, pregnancy rate and outcomes of pregnancy.
Main results and the role of chance: Of the women submitted to ART cycles, 18 had uterine malformations; 14 had bicorporeal uterus (11 with bicornuate uterus and three with dydelphys uterus) and four had hemi-uterus. 61.1% of women had primary infertility. The main cause of infertility was male factor associated to the uterine malformation; only in two women the cause of infertility was the uterine malformation alone. These 18 women performed 33 cycles of ART: 17 Intracytoplasmic sperm injection (ICSI), 11 *In Vitro* Fertilization (two of them with donor sperm), four frozen embryo transfers and one intrauterine insemination. Pregnancy occurred in 11 cases after ART cycles (success rate of 33%). Seven of these pregnancies resulted in miscarriages (three in the first trimester and four in the second trimester). Two women had preterm labour and two delivered after 37 weeks of gestation.
Limitations, reason for caution: Müllerian malformations as bicorporeal uterus and hemi-uterus have a low incidence, even in women submitted to ART, limiting the power of the present study.
Wider implications of the findings: Bicorporeal uterus and hemi-uterus are major malformations which can result in poor reproductive outcomes but mainly in obstetric complications.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Centro Hospitalar do Porto.
Trial registration number: NA.
Keywords: Müllerian malformations, ART, pregnancy

P-416 Decreased healthcare resource utilization among women undergoing IVF/ICSI treated with GnRH antagonist protocols as compared to GnRH agonist protocols

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Study question: Is there is a difference in healthcare resource utilization between women undergoing IVF/ICSI treated with GnRH agonist protocols as compared to those treated with either corifollitropin alfa (CFA) in an antagonist protocol or treated by any antagonist protocol?
Summary answer: Fewer office visits, clinician visits, nurse visits, calls to the clinic, and transvaginal ultrasound scans were recorded in patients treated by CFA in an antagonist protocol or in patients treated with any antagonist protocol as compared to patients treated by agonist protocols.
What is known already: Agonist and antagonist protocols have been demonstrated to be equally efficacious in terms of live birth rate. Since their introduction, antagonist protocols have been generally recognized as patient friendly with fewer injections and days of treatment and hence less burdensome to the patient. The impact of these protocols on the clinic in terms of healthcare resource utilization has been less well investigated.
Study design, size, duration: Prospective observational study in 17 countries in Europe, Asia, Australia, and Israel; analysis includes women who met all inclusion and exclusion criteria, CFA ($N = 1647$), antagonist ($N = 2837$, including CFA patients), and agonist ($N = 465$). Healthcare resource utilization was captured during controlled ovarian stimulation (COS) at each visit.
Participants/materials, setting, methods: Healthcare resource utilization was assessed at each contact with the clinic over the course of COS. Number of office visits, physician and nurse consults, calls to the clinic, and transvaginal ultrasound scans were compared between protocols.
Main results and the role of chance: Both CFA and antagonist patients recorded fewer clinic visits (mean = 3.2 and 3.3, respectively) than agonist patients (mean = 3.7) ($p < 0.001$ for both CFA and antagonist vs agonist). 39.8 and 39.3% of CFA and antagonist patients, respectively, recorded 4+ office visits compared with 56.1% of agonist patients. Similar patterns were observed for physician and nurse consults. Fewer CFA and antagonist patients called the clinic at least once – 18.3 and 18.0%, respectively – compared with 27.7% of agonist patients ($p < 0.001$ for both comparisons). Fewer transvaginal ultrasounds were conducted in CFA and antagonist patients (mean = 2.7 for both) compared with agonist patients (mean = 3.2) ($p < 0.001$ for both comparisons). 28.3 and 28.7% of CFA and antagonist patients, respectively, had 4+ ultrasounds compared with 46.2% of agonist patients.
Limitations, reason for caution: While no clinically meaningful demographic or clinical differences were found at baseline, selection bias cannot be ruled out given that patients were not randomized. Healthcare resource utilization may be underreported as any omitted reports of resource utilization could not be queried or confirmed as reporting was event driven.
Wider implications of the findings: The finding of reduced healthcare resource utilization among women undergoing IVF/ICSI treated with GnRH antagonists compared with a GnRH agonist protocol demonstrates that antagonist protocols are beneficial in not only reducing the burden that ART treatment places on patients but also in optimizing utilization of clinic resources.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck & Co., Inc., Kenilworth, NJ, USA.
Trial registration number: NA.
Keywords: antagonist, corifollitropin, resource utilisation, agonist long protocol

P-417 Endometrial thickness after ovarian hyperstimulation in an intrauterine insemination program: a systematic review

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Study question: Does the stimulating agent used in ovarian hyperstimulation as co intervention in intra uterine insemination (IUI) affect endometrial thickness (EMT)?
Summary answer: Clomiphene citrate (CC) as a stimulating agent in ovarian hyperstimulation results in a thinner endometrium compared to other stimulating agents.
What is known already: EMT has been associated with success rates in medically assisted reproduction. The type of ovarian hyperstimulation might influence the EMT. It has been suggested that CC results in a lower endometrial thickness than gonadotrophins or aromatase inhibitors.
Study design, size, duration: We performed a systematic review and meta-analysis of studies comparing CC, gonadotrophins or aromatase inhibitors in an IUI program reporting on endometrial thickness in couples with unexplained subfertility.
Participants/materials, setting, methods: Medline, EMBASE and CINAHL were searched. Outcome measures were mean pre-ovulatory endometrial thickness and clinical pregnancy. Mean differences with 95% confidence intervals were calculated using a random effect model. Meta-regression was performed to determine if different stimulating agents interacted in the estimated effect of endometrial thickness.
Main results and the role of chance: We included 20 studies in our meta-analysis, of which 12 compared CC and letrozole, four compared gonadotrophins and letrozole and four used a single stimulation agent. There was evidence for a thinner endometrium in women treated with CC compared to letrozole [mean difference -0.36 mm (95% CI -0.72 to -0.01)]. There was no evidence for a difference in EMT in women treated with gonadotrophins compared to letrozole [mean difference 0.06 mm (95% CI -1.46 to 1.14), respectively]. Pre-ovulatory endometrium was thicker in pregnant than in non-pregnant women, although the difference was not statistically significant [6 studies, mean difference 0.55 mm (95% CI -0.11 to 1.21)]. Meta-regression could not rule out interactive effects of the type of stimulating agent used.
Limitations, reason for caution: There was considerable to substantial heterogeneity in the comparisons and the overall quality of the included studies was low, hampering firm conclusions. In addition, a cut-off value for optimal endometrial thickness is lacking.
Wider implications of the findings: Although clomiphene citrate appears to result in a thinner endometrium when compared to other stimulating agents, the influence of endometrial thickness on pregnancy remains unclear. Whether EMT could be used as a biomarker to guide the stimulation in future cycles should be evaluated in RCTs
Study funding/competing interest(s): Funding by hospital/clinic(s) – Academic Medical Center Amsterdam.
Trial registration number: NA.
Keywords: IUI, ovarian hyperstimulation, endometrial thickness

P-418 Is intra-uterine insemination (IUI) a cost-effective service? Evidence of success and patient satisfaction from a UK clinic specialising in IUI

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Study question: Does IUI treatment offer a sufficiently high success rate and patient satisfaction for the state to continue funding this treatment? This is particularly relevant question for the UK, where the National Institute for Clinical Excellence (NICE) has recommended that IUI should not be used as first treatment for unexplained infertility.
Summary answer: From 2009 to 2014, a live birth rate of 11.5% was achieved for all IUI patients. The estimated cost per live birth was 7800. 31.2% of patients achieved a pregnancy within three IUI attempts and 90.6% stated they were satisfied to attempt conception via IUI rather than go straight to IVF treatment.
What is known already: IUI is a low complexity therapy recommended by many specialists as first-line treatment for couples with mild or unexplained fertility problems. Pregnancy rates tend to be higher than for natural conception but lower than more complex treatments such as IVF. Advantages of IUI include its low cost, use of less drugs and no surgery. However, recent NICE guidelines recommend that IUI (with ovulation induction) should no longer be used as first treatment for unexplained infertility.
Study design, size, duration: Success rates (clinical pregnancy rate and live birth rate) were calculated from 859 couples having 1875 cycles of IUI combined with mild gonadotrophin stimulation. A questionnaire was then sent to a random sample of treated couples, whether successful or not, to seek their views on the IUI service received.
Participants/materials, setting, methods: Heterosexual couples (median female age 33.8 years, range 21–44 years) with unexplained infertility,

anovulation problems and/or borderline male factor received IUI treatment at a fertility clinic specialising in IUI with partner sperm. The questionnaire was posted to couples who had previously attended the clinic regardless of the outcome.

Main results and the role of chance: The overall clinical pregnancy and live birth rates per IUI were 14.9 and 11.5% respectively, resulting in an approximate cost per live birth of 7800. From the survey, 78.1% couples attended for unexplained infertility, whilst 18.8% had anovulation problems. 44.8% couples were offered IVF as an alternative to IUI at the initial consultation, whilst 51.7% were offered IVF after three IUI attempts. Expectations of success were low for 27.6% couples and moderate to high for 34.5% couples. 90.6% couples considered IUI to have a low level of discomfort and risk and were therefore satisfied with the decision to choose IUI over IVF treatment. 93.8% stated that they would prefer the offer of less invasive treatment via IUI rather than going straight to IVF.

Limitations, reason for caution: Only 32 patients from 50 responded to the survey. A wider survey might reveal different responses, although from the data provided, there was an overwhelming trend for patients supporting access to IUI treatment.

Wider implications of the findings: Despite the relatively low success rate of IUI compared to IVF treatment, the survey showed that couples prefer to be given the chance to conceive without invasive therapy. Moreover, with an 11.5% live birth rate, the cost per take home baby after IUI is significantly less than for IVF (we calculated this at € 7800 for IUI compared to €15400 for IVF, even with allowance for a generous IVF live birth rate of 40% cycle).

Study funding/competing interest(s): Funding by hospital/clinic(s) – Nottingham University Hospital, Nottingham, UK.

Trial registration number: NA.

Keywords: IUI, cost-effective, service provision

P-419 Myo-inositol during IVF: possible strategy for poor responders patients?

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Study question: The aim of the present study was to evaluate the effect of Myo-inositol on ovarian function in poor responders patients undergoing ovulation induction for ICSI cycle.

Summary answer: The results suggest that myo-inositol may improve ovarian response to stimulation in poor responder patients

What is known already: The management of poor responders remains a challenge in IVF techniques.

Inositol is a vitamin-factor of B group. The role of inositol in the streamlining of ovulatory process has been widely defined in patients with reduced insulin sensitivity. Recently it has been hypothesized that inositol could have a different way of action on different cell kinds. There is the evidence that inositol may improve ovarian function and optimize oocyte quality in IVF

Study design, size, duration: Prospective controlled observational trial.

Participants/materials, setting, methods: 72 women poor responder scheduled for an ICSI cycle, divided in two groups: group A: 38 patients treated with myo-inositol (4 g) + folic acid (400 µg) for the previous three months before the enrollment day; group B: 38 patients treated with folic acid (400 µg) for the same period.

Main results and the role of chance: The main goal was the definition of the number and the quality of the oocytes retrieved; secondary endpoints were the Ovarian Sensitivity Index (OSI: *n*° oocytes retrieved/total Gn units × 1000), oestradiol levels at the day of hGC administration, fertilization rate, implantation rate, ongoing pregnancy rate. There was no significant difference between the two groups regarding oestradiol level, but total rec-FSH units were significantly lower in group A (1975 ± 298 vs 2212 ± 312, $p = 0.004$). The results show a statistically significant increase in M2 oocytes rate (80.5 vs 66.6%, $p = 0.01$). The ovarian sensitivity index was higher, reaching a statistical significance, in the group A vs group B (1.88 ± 0.81 vs 1.54 ± 0.65, $p < 0.05$), showing an improvement in ovarian sensibility to gonadotropin.

Limitations, reason for caution: Small size of sample.

Wider implications of the findings: Results suggest that myo inositol therapy results in an increase in oocytes number that could be used for ICSI techniques, significantly increasing the number of oocytes recovered in MII in poor responder patients. Besides, the group pre-treated with myo inositol showed a significant increase of the gonadotropin ovarian sensitivity index (OSI), suggesting a myoinositol role in improving ovarian response to gonadotropins. Therefore myoinositol seems to be helpful in 'poor responders' patients undergoing IVF cycles.

Study funding/competing interest(s): Funding by University(ies) – Seconda Università degli studi di Napoli.

Trial registration number: Is not required.

Keywords: IVF, myoinositol, poor-responders

P-420 Effect of endometrial biopsy on intrauterine insemination outcome in controlled ovarian stimulation cycle

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Study question: The proposed hypothesis in our study is that endometrial biopsy preceding ovulation induction and intrauterine insemination (IUI) have the same beneficial effect on IUI outcome in patient undergoing controlled ovarian stimulation as demonstrated in IVF.

Summary answer: EB done in early follicular phase in same cycle of stimulation with IUI gives better CPR as compared to EB done in luteal phase of previous cycle.

What is known already: In controlled ovarian stimulation cycle uterine receptivity is diminished due to endometrial alteration and supraphysiological level of steroid hormones. Endometrial injury facilitates embryo implantation and pregnancy rates through local inflammatory and angiogenesis mechanism.

Study design, size, duration: prospective parallel randomized control study in a 1:1 allocation ratio conducted in a tertiary care centre from August 2012 to March 2014.

Assuming the clinical pregnancy rate in intervention and control Group as 32.7 and13.7% in previous study by Narvekar (2010) with alpha = 0.05 and power = 80% minimum 225 cases were to be included, but assuming that few subjects may be lost to follow up, a total of 251 subjects attending infertility clinic were included in the study.

Participants/materials, setting, methods: Subjects undergoing controlled ovarian stimulation with IUI were randomly allocated into 3 Groups. Block randomization with sealed envelope system was used. Group A: EB was taken between D 19-24 of the spontaneous menstrual cycles that precedes the fertility treatment and IUI which was done in next cycle ($n = 86$). Group B: EB was taken before D 6 of the menstrual cycle and fertility treatment and IUI was done in same cycle ($n = 90$). Group C: (Control Group) No EB in previous 3 cycle ($n = 75$).

Main results and the role of chance: Clinical pregnancy rate (CPR) was 19.77, 31.11, and 9.3% for Group A, Group B, and Group C, respectively. The results show a highly significant value for the paired t test of intervention Group B and control Group C of the cases ($p = 0.000957$). Clinical pregnancy rate was maximum after first cycle of OI and IUI following EB scratch in both Groups A and B ($p < 0.001$).

The relative risk (R/R) between Group A and Group C is 2.1179, 95% CI 0.9291–4.8278 ($P = 0.0742$) NNT-9.584. The relative risk (R/R) between Group B and Group C is 3.3333, 95 % CI 1.5442 to 7.1955 ($P = 0.0022$), NNT-4.592. The relative risk (R/R) between Group A and Group B is 1.5739, 95% CI 0.9308 to 2.661 ($P = 0.0905$), NNT 8.815.

Limitations, reason for caution: The study was not blinded and all due care was taken during randomization to prevent selection bias.

Wider implications of the findings: Most of the work related to endometrial scratching has been done in IVF cycles with promising results. In developing countries with lincreasing infertility rates there is requirement of affordable, low cost interventions like endometrial scratching which can be done as an OPD procedure prior to ovulation induction and IUI cycles, thereby improving pregnancy rates before proceeding for high cost alternative ART procedures.

Study funding/competing interest(s): Funding by hospital/clinic(s) – ESI-Postgraduate Institute of Medical Science & Research, Basaidarapur, Delhi.

Trial registration number: CTRI/2013/04/003521, WHO International Clinical Trials Portal
Keywords: endometrial biopsy, IUI, COS

P-421 Association between uterine perfusion and inherited thrombophilia in patients with unexplained infertility

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Study question: The aim of this study was to evaluate the possible association between impaired uterine arteries and genetic polymorphism of thrombophilic markers, like Factor V (FV) Leiden gene mutation, prothrombin (PT) mutation and homozygous mutation of methylenetetrahydrofolate reductase (MTHFR), in patients with unexplained infertility.
Summary answer: The polymorphisms taken individually are not sufficient to explain an alteration in blood flow, while combination of these could be the cause of a altered uterine flow, resulting in failure of the system both in a spontaneous pregnancy in a pregnancy induced by PMA.
What is known already: Uterine receptivity is one of the most important factors in determining successful implantation. Several studies showed that failure to conceive might be associated with an increased resistance in the uterine arteries. A reduced pregnancy rate (PR) has been reported in women with impaired uterine perfusion.
Study design, size, duration: This is a prospective study, in which the patients were computerized randomization. Between January 2013 and May 2014, 148 consecutive patients with unexplained infertility underwent basal uterine arteries Doppler velocimetry evaluation.
Participants/materials, setting, methods: Pulsatility index (PI) were automatically calculated and the patients were divided in two groups: Group A ($n = 49$) with PI >3 in at least one of uterine arteries; group B ($n = 99$) with normal uterine arteries. All patient underwent a screening for the three main thrombophilic markers.
Main results and the role of chance: Our results showed a statistically significant association between thrombophilic polymorphism mutations and impaired uterine arteries only with simultaneous presence of two or three mutations: FV Leiden and gene C677T of MTHFR (34.69 vs 1.01% respectively in group A and B, $P = 0.0001$); FV Leiden and mutation G20210A of PT gene (20.4% in Group A vs 2.02% in group B, $P = 0.0003$); gene C677T of MTHFR mutation and FV Leiden and mutation G20210A of PT gene (18.4% in study group vs 3.03% in control group $P = 0.0025$) and FV Leiden mutation, mutation G20210A of PT gene and gene C677T of MTHFR mutation (12.24 vs 1.01% in group A and B respectively).
Limitations, reason for caution: The limit of this study is the small number of the patients.
Wider implications of the findings: This study shows that the increase in resistance of the uterine arteries is positively correlated with the presence of at least two genetic polymorphisms for acquired thrombophilia. Knowing these polymorphisms allows to set the correct treatment immediately and thus avoid treatments that may not work. Also gives us important informations on the possible therapy to be set during the entire pregnancy.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Praxi DS, Praxi Provita.
Trial registration number: This study isn't RCT.
Keywords: uterine receptivity, utery artery perfusion, thrombophilia

P-422 Association between serum folate and vitamin B12 and success of assisted reproductive technologies

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Study question: Are pre-treatment levels of serum folate and vitamin B12 associated with assisted reproductive technology (ART) outcomes?
Summary answer: Higher serum levels of folate and vitamin B12 prior to ART treatment was associated with higher live birth rates among a population exposed to folate fortification. Moreover, women with higher levels of both serum folate and vitamin B12 had the greatest likelihood of reproductive success.
What is known already: Studies from European infertility cohorts suggest that folate may improve total and mature oocyte counts, embryo quality, and pregnancy rates; however, in studies that investigated live birth rates solely among women undergoing embryo transfer, no associations were observed. Several small studies and case reports have found associations between vitamin B12 deficiency and female subfertility yet a cohort study from the UK found no relation between plasma vitamin B12 and live birth following *in vitro* fertilization.
Study design, size, duration: A random sample of 100 women (contributing 154 ART cycles) participating in a prospective cohort study (EARTH) at the Massachusetts General Hospital Fertility Center (Boston, MA, USA).
Participants/materials, setting, methods: Serum folate and vitamin B12 were measured in blood samples taken between day 3 and 9 of treatment. Outcomes of ART were abstracted from medical records. Generalized estimating equations adjusting for age, BMI, and race were used to evaluate the association of folate and vitamin B12 with ART outcomes.
Main results and the role of chance: Women in the highest quartile of serum folate (>26.3 ng/mL) had 1.62 (95% CI 0.99, 2.65) times the probability of live birth compared to women in the lowest quartile (<16.6 ng/mL) (p-trend = 0.01). Women in the highest quartile of serum vitamin B12 (>701 ρg/mL) had 2.04 (95% CI 1.14, 3.62) times the probability of live birth compared to women in the lowest quartile (<439 ρg/mL) (p-trend = 0.008). Evidence of an additive interaction between serum folate and vitamin B12 was observed; women with serum folate and vitamin B12 levels >median had 1.92 (95% CI 1.12, 3.29) times the probability of live birth compared to women with folate and vitamin B12 levels ≤median. This translated into an adjusted difference in live birth rates of 26% (95% CI 10–48%) (p-value = 0.02).
Limitations, reason for caution: Residual confounding is still possible due to the observational nature of this study. The generalizability of our study to women presenting at infertility clinics worldwide is unclear as our women have much higher serum folate levels than comparable populations in Europe.
Wider implications of the findings: These findings support the importance of preconception folic acid supplementation and suggest the additional intake of vitamin B12. Given that live birth rates per initiated ART cycle have plateaued for approximately a decade, a randomized trial of high dose supplementation with folic acid and vitamin B12 before planned ART warrants serious consideration.
Study funding/competing interest(s): Funding by national/international organization(s) – NIH grants R01-ES009718, P30-DK046200, T32-DK007703-16.
Trial registration number: NA.
Keywords: folate, ART, vitamin B12, IVF

P-423 Gram staining, PCR or PNA FISH analysis for detection and classification of Bacterial Vaginosis – a prospective study in a Danish IVF setting

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Study question: Should BV be sub-classified with a molecular based tool instead of conventional Gram staining? What is the prevalence of Bacterial

Vaginosis (BV) in infertile Danish women? Finally, should a molecular based abnormal flora be taken into account prior to fertility treatment?
Summary answer: The BV prevalence was 18.9%. BV should be sub-classified using qPCR, enabling to cluster false-positives, constituting *Lactobacillus iners*, which is a lactobacillus species, morphologically indistinguishable from *Gardnerella vaginalis. Gardnerella vaginalis* abundance above a threshold level of 10^9 copies/mL may negatively affect spontaneous as well as assisted conception.
What is known already: BV is a serious reproductive health issue with an incidence of approximately 19% in the fertile population. BV often presents itself sub-clinically with a change of the vaginal microflora from protective and acidic *Lactobacilli* spp. to a more heterogeneous environment dominated by anaerobic bacteria, especially *Gardnerella vaginalis*. Few studies have been conducted in infertile women, and some have suggested a negative impact on fecundity in the presence of BV.
Study design, size, duration: A cohort of 200 asymptomatic infertile patients, primarily Caucasians, attending two Danish fertility clinics from April 2014 to December 2014 were prospectively enrolled in the trial.
Participants/materials, setting, methods: Vaginal swabs from IVF/IUI patients were obtained from the posterior fornix. Gram stained slides were assessed according to Nugents criteria. PCR primers were specific to four common *Lactobacilli* spp., *Gardnerella vaginalis* and *Atopobium vagina*. Threshold levels were established using AUROC analysis. PNA FISH slides were analyzed, using confocal microscopy.
Main results and the role of chance: The prevalence of BV by Nugent score was 18.9% 95% CI (13.7–25.1) versus 21.3% 95% CI (15.6–27.0) by qPCR. The molecular tools revealed that 13.5 % of Nugent positives were false positive due to the abundance of a certain *Lactobacillus* species called *L. iners*. We provide evidence that the non-pathological *L. iners* cluster is morphologically indistinguishable from *Gardnerella vaginalis*. Therefore, a molecular based diagnostic tool should be used when diagnosing an abnormal vaginal flora. Interestingly we observed that none of the patients with *Gardnerella vaginalis abundance* had a successful reproductive outcome. The full data analysis will be presented at the conference.
Limitations, reason for caution: Although a total of 200 patients were included in the study, a larger sample size is needed to draw firm conclusions regarding the reproductive outcome of *Gardnerella vaginalis* infected patients.
Wider implications of the findings: *Gardnerella vaginalis* seems to play a role in infertility, however, when using Gram staining, there is a significant number of false positives due to the presence of non-pathological *L. iners*. This justifies the future use of molecular based diagnostics. If a negative correlation between *Gardnerella vaginalis* and the reproductive outcome is corroborated, we suggest that all patients should be screened for *Gardnerella vaginalis* prior to commencing fertility treatment as this intervention might have significant socioeconomic impacts.
Study funding/competing interest(s): Funding by hospital/clinic(s) – The AP Møller Maersk Foundation for the advancement of Medical Science. The Foundation of medical research Hospital HE MIDT, Denmark. No competing interests.
Trial registration number: NCT02042352.
Keywords: vaginal microbiome, bacterial vaginosis, *Gardnerella vaginalis*, qPCR, nugent score

P-424 Does the endometrial secretory immunomodulatory profile differ between women with recurrent implantation failure (RIF) and fertile controls? A case control study

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Study question: Does the endometrial secretory immunomodulatory profile differ between women with recurrent implantation failure and fertile controls?
Summary answer: This study suggests that the uterine secretome is more tightly associated with RIF patients when compared with unstimulated healthy controls. Analysis demonstrated clear segregation of the two groups of women with eight immunomodulatory molecules, including Leukaemia Inhibitory Factor (LIF), being lower in RIF patients than controls.
What is known already: Human reproduction is tightly governed at the embryo-endometrial interface. Alterations in the constituents of endometrial secretions reflect changes in the underlying endometrial tissue of the peri-implantation environment. Endometrial fluid sampling can be performed accurately and noninvasively. Previous studies have examined general subfertile populations in cycles associated with ovarian stimulation, but the uterine secretion profile of women with RIF is unknown.
Study design, size, duration: A single blind prospective case control study in an *In vitro* fertilisation (IVF) centre, United Kingdom (August 2012 to December 2013). Sixteen women with RIF and twenty healthy fertile controls were recruited.
Participants/materials, setting, methods: Endometrial fluid samples were aspirated from RIF patients (the absence of pregnancy after transfer of ≥3 good quality embryos and over ≥2 IVF cycles) and healthy parous controls in follicular and luteal phases. Samples were investigated for 45 immunomodulatory factors and the data analysed with logistic regression modelling.
Main results and the role of chance: Univariate analysis comparing concentrations of 23 immunomodulatory molecules demonstrated lower βNGF in women with RIF (10.57 pg/µl ± 3.06) than controls (24.96 pg/µl ± 6.21). When five-parameter partial least squares logistic regression modelling was applied, there was a difference in eight immunomodulatory markers, including LIF.
Multivariate analysis demonstrated clear segregation of study patients from controls; principle Component (PC) analysis 1 explaining 55% of the variance and PC2 28%. Magnitude of clustering being tighter between closely clustered patients than controls.
Limitations, reason for caution: Our results may be confounded by a small sample size; a larger scale study in this specific subgroup of women is further needed to confirm our findings.
Wider implications of the findings: Our study demonstrated reduced levels of eight immunomodulatory molecules, including LIF, in RIF compared with controls. This suggests an attenuated inflammatory response in the endometrial peri-conceptual environment, consistent with established data. An understanding of the *in vivo* immunomodulatory milieu in women with RIF, reconfirmed in larger studies, may provide a method to discriminate women with a higher RIF propensity from those who will go on to have successful outcomes whilst serving to delineate plausible underlying mechanisms.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Complete Fertility Centre, Southampton, UK.
Trial registration number: Regional ethics committee number 12/SC/0568.
Keywords: RIF, implantation, receptivity, immunomodulatory, endometrium

P-425 Activator of fatty acid metabolism (AICAR) improves maturation rate, but affects spindle formation and chromosome integrity in *in vitro* maturing denuded mouse oocytes

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Study question: Does AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), an activator of the 5¢ adenosine monophosphate-activated protein kinase (AMPK), that is assumed to influence fatty acid metabolism, improve quality of *in vitro* matured (IVM) denuded (DO) or cumulus enclosed oocytes (CEO)?
Summary answer: AICAR enhanced dose dependently the rate of polar body (PB) formation of IVM DO and CEO. However, spindle immunofluorescence revealed severe disturbances in metaphase II spindle formation and caused precocious chromatin decondensation in DOs cultured in the presence of AICAR. In contrast, spindle formation was not visibly affected in CEOs.
What is known already: IVM of immature oocytes presents an alternative strategy to obtain fertilizable metaphase II oocytes during ART treatment (e.g., in cases of OHSS). AICAR is an activator of the AMPK that is involved in regulation of fatty acid metabolism. Studies in mammalian oocytes suggest that activation of the fatty acid pathway by AICAR supports meiotic resumption. So far, meiotic progression, spindle, chromosomes and chromosomal constitution of IVM oocytes exposed to AICAR have not been analyzed.

Study design, size, duration: DOs and CEOs from 3- to 6-month-old MF1 mice were matured for 16h i*n vitro* in absence (control), or presence of 0.01, 0.05, 0.1 and 0.2 mM AICAR.
Participants/materials, setting, methods: Spindle, chromosomes and aneuploidy were studied by immunostaining and confocal microscopy, or after spreading. Gap junctional communication was inhibited by 100 mM Carbenoxolone (CBX) in culture with our without AICAR. For pulse chase, IVM was for 7 h in AICAR and 9 h without AICAR (7 h+/9 h-), or with reverse pulse (7 h-/9 h+).
Main results and the role of chance: 0.1 mM AICAR increased the rate of PB oocytes from culture of DOs and CEOs compared to controls. However, there was a significant dose-dependent increase in PB-DOs without spindle or with aberrant spindle and unaligned chromosomes and such with decondensed chromatin, whereas spindles and chromosomes appeared normal in AICAR exposed CEOs. Inhibition of gap junctional communication lead to similar spindle abnormalities and decondensed chromosomes in CEOs as seen in DOs, but were significantly less frequent. First meiosis was only marginally susceptible to 0.1 mM AICAR and there was no induction of first meiotic error. Spindles were also normal in DOs exposed to AICAR for 7 h (up to metaphase I) followed by 9 h without AICAR, whereas the reverse pulse chase interfered with spindle reassembly after anaphase I.
Limitations, reason for caution: Stimulation of fatty acid metabolism via cumulus may increase maturation and possibly quality of oocytes when they are in bi-directional contact with cumulus. In contrast, IVM without cumulus with AICAR appears to critically affect MI/MII transition in mouse DOs, but further studies in other mammals are required.
Wider implications of the findings: IVM is still sub-optimal since oocyte developmental potential appears restricted. Whereas AICAR improves GVBD-rate in experimental studies, it appears to interfere with meiosis I/II progression in DOs, and thereby critically affects spindle formation. Hyperstimulation of AMPK and fatty acid metabolism can possibly contribute to increased ROS in DOs, whereas it may improve supply of metabolites by cumulus in CEOs and thereby increase oocyte maturation. Cumulus appears to protect oocytes from adverse influences of AICAR.
Study funding/competing interest(s): Funding by national/international organization(s) – DFG (German research foundation).
Trial registration number: No number.
Keywords: maturation, metabolism, spindle, chromosomes, oocyte

P-426 High response to Clomiphen citrate in intrauterine insemination cycles when converted into 'rescue' IVF results in higher implantation rates compared to minimal stimulation IVF cycles

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Study question: This study carried out retrospective comparative analysis of clinical pregnancy and implantation rates in young women who were originally slated for IUI but underwent rescue IVF as a result of high response to clomiphen citrate and human menopausal gonadotropin versus women undergoing standard minimal stimulation antagonist protocol with gonadotropin.
Summary answer: Converting IUI cycles of young women giving higher response to clomiphen citrate-human menopausal gonadotropin, to rescue IVF may be a safe, extremely cost effective strategy resulting in higher implantation and ongoing pregnancy rate when compared to age matched high responder women undergoing IVF with standard minimal stimulation protocol using r-FSH.
What is known already: Ovarian stimulation in IUI cycles may improve monthly pregnancy rate by increasing number of oocytes available for fertilization and implantation. However, this treatment modality may also give rise to supernumerary follicles in high responders exposing the women to risks of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). Although conversion of such IUI cycles to IVF eliminates frustration of cycle cancellation, cost effectiveness of such strategy remains questionable due to use of high dose gonadotropins.
Study design, size, duration: Out of 240 IUI cycles carried out from January to December 2013, 14 cycles in women (age: 23–32 years) with excessive follicular response to CC-stimulation were converted to "rescue" IVF. For the retrospective comparison, case matched women (n = 54) who had undergone mild stimulation protocol IVF were selected from our IVF unit.
Participants/materials, setting, methods: Women slated for IUI were stimulated with clomiphen citrate (100 mg daily, day 3–day 7) and injection hMG (75 IU daily, day8–day of inj.hCG 5000 IU) before they underwent 'rescue IVF' to avoid risk of multiple gestation/ovarian hyperstimulation syndrome. Case-matched control group women had undergone minimal stimulation IVF with 150 IU r-FSH/antagonist
Main results and the role of chance: Age, infertility period, BMI, basal FSH and AMH levels did not differ significantly between test and control groups. Day/hCG Sr. Estradiol levels and the number of eggs retrieved were significantly lower (p = 0.04; p = 0.01 respectively) in rescue IVF group compared with control IVF group. However, blastocyst formation rate and stage / grade of blastocyst transferred (p = 0.58; p = 0.21 respectively) were comparable between both study groups where all cycles involved transfer of d5 blastocyst. Although not statistically significant, clinical pregnancy rate was higher in rescue IVF group (57.1%) compared to control group (44%). Implantation rate was significantly higher in rescue IVF group than in control IVF group (58.3 vs. 25%; p = 0.03). Most significantly, gonadotropin dose used was significantly lower in test group compared to control IVF group.
Limitations, reason for caution: Higher implantation rate in test group is due to more number of twin pregnancies compared to control group despite comparable number of blastocysts transferred. This may be because in test group, supraphysiological levels of estradiol are not as raised as in control group where higher dose of gonadotropins is used.
Wider implications of the findings: We have presented a most cost effective strategy where ovulation induction in IUI with clomiphen citrate-hMG combination was converted to rescue IVF upon obtaining a hyper response. This protocol is cheaper than conventional IVF algorithms and also to IUI protocols where gonadotropins are used from day 3 onwards. If multicenter prospective studies affirm such strategy to give significantly higher clinical pregnancy rates, it may further facilitate transfer of single blastocyst, thus also averting multiple gestation risks.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Self Funded by our IVF clinic. Vaunshdhara Clinic and Assisted Conception Centre.
Trial registration number: NA.
Keywords: IUI, rescue IVF, clomiphen citrare, r-FSH, antagonist

P-427 Adverse obstetrical and neonatal outcomes in pregnancies resulting from oocyte donation

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Study question: To explore obstetrical and neonatal outcomes among relatively young women with optimal health status conceiving singletons with donated versus autologous oocytes (via IVF and spontaneously).
Summary answer: Oocyte donation is associated with hypertensive disorders of pregnancy, oligohydramnios, preterm delivery, labor induction, delivery by caesarean section, retained placenta, post-partum hemorrhage and longer hospital stay after delivery. However, neonates have similar probability for major congenital malformations and did not differ regarding birth weight and length among term infants.
What is known already: Oocyte donation as an infertility treatment among women with idiopathic, iatrogenic or natural menopause has been associated to gestational diabetes, hypertensive disorders, placental abnormalities, preterm delivery and increased rate of caesarean delivery while simultaneously being characterized by high rates of primiparity, advanced maternal age and multiple gestation constituting the individual risk of mode of conception difficult to assess.

Study design, size, duration: Retrospective cohort case study involving 289 pregnant women with singleton deliveries; 76 women conceiving with donated oocytes, 150 nulliparous women without infertility conceiving spontaneously and 63 women conceiving after non-donor IVF.
Participants/materials, setting, methods: Data on obstetrical and neonatal outcomes were retrieved from the National Birth Medical Register and the medical records of oocyte recipients from the treating University Hospitals of Sweden. Demographic and logistic regression analysis were performed to examine the association of mode of conception and perinatal outcomes.
Main results and the role of chance: OD pregnant women had a higher probability of hypertensive disorders [aOR 2.84, 95% CI (1.04–7.81)], oligohydramnios [aOR 12.74, 95% CI (1.24–130.49)], postpartum hemorrhage [aOR 7.11, 95% CI (2.02–24.97)], retained placenta [aOR 6.71, 95% CI (1.58–28.40)], caesarean delivery [aOR 2.95, 95% CI (1.52–5.71)] and induction of labor [aOR 3.00, 95% CI (1.39–6.44)], when compared to women who conceived spontaneously. Similar trends, though not statistically significant, were noted among OD and non-donor IVF pregnant women. Higher intervention during delivery was observed in women with diminished ovarian reserve but the risk for hypertensive disorders did not differ after adjustment. Despite higher likelihood of prematurity, similar mean birth weight and length among term infants were noted and the presence of congenital malformations did not differ between groups.
Limitations, reason for caution: One of the limitations of our study is the lack of power. Furthermore we did not take into account parameters such as donor age, paternal age, ART method (conventional IVF or ICSI) as well as if the pregnancy resulted from a cryopreserved or fresh embryo.
Wider implications of the findings: The selection process of recipients for medically indicated oocyte donation treatment in Sweden seems to be effective in excluding women with severe comorbidities and beneficial regarding the health status of the infant. Oocyte recipients-despite being relatively young and of optimal health status-need careful counseling preconceptionally and closer monitoring prenatally for the development of hypertensive disorders. Nevertheless neonatal outcomes seem to be favorable.
Study funding/competing interest(s): Funding by national/international organization(s) – Family Planning Fond in Uppsala and Swedish Research Council for Health, Working Life and Welfare.
Trial registration number: NA.
Keywords: oocyte donation, pregnancy complications, neonatal complications, hypertensive disorders, indication for oocyte donation

P-428 Three-dimensional assemble of endometrial tissue *in vitro*

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Study question: Can regenerative-medicine technique with cell sheets become a new treatment method for endometrial disorder causing female infertility? And can 'functional three-dimensional (3-D) endometrium-like tissues' be produced *in vitro* using cell sheet engineering?
Summary answer: The functional 3-D endometrium-like tissues consisting of epithelium and stroma were assembled *in vitro*. In the future, the tissues might contribute to an endometrial regeneration for severe endometrial adhesions.
What is known already: Endometrial disorder such as endometrial adhesion, Asherman's syndrome, is one of the factors that causes infertility. Severe endometrial adhesions are caused by massive defects of the endometrium including stem cells. Therefore the general therapy such as surgical synechiotomy is not effective.

Recently, a new approach called "cell-sheet engineering", which can harvest confluent-culture-cells as a contiguous cell sheet having intact cell-cell junctions and extracellular matrix without enzymatic treatment, has been developed for tissue regeneration.
Study design, size, duration: Endometrial-epithelial-cell-sheets and endometrial-stromal-cell-sheets were prepared from rat endometrial tissues. The 3-D tissue was assembled using total three cell sheets, which consisted of a single epithelial-cell-sheet on the upper side and two stromal-cell-sheets underneath; they were then layered. After that, the layered-cell-sheets were re-cultured *in vitro*.
Participants/materials, setting, methods: Endometrial tissues resected from SD rats were treated with trypsin/EDTA. The two kinds of cell sheets (endometrial epithelial and stromal cell sheets) were produced from the isolated cells separated by preplate method and harvested from temperature-responsive cell culture dish by reducing the temperature. After re-culture, the layered cell sheets were evaluated histologically.
Main results and the role of chance: Histological examination of the specimens showed two types of cell sheets. By immunostaining, one type of the cell sheets showed a superficial CK18 positive layer, and CK18 negative and vimentin positive layer. Another type of cell sheet showed only vimentin positive layer. And the two types cell sheets could be layered and re-cultured. The 3-D structure assembled by re-cultured cell sheets was similar to normal endometrial tissue constituted of endometrial epithelial and stromal layer.
Limitations, reason for caution: The function of 3-D assembled endometrium-like tissue *in vivo* is still unclear, because the results of this study show only a partial function *in vitro*.
Wider implications of the findings: Transplantation of endometrial-cell-sheets have a high possibility not only to prevent intrauterine re-adhesion after synechiotomy, but also to regenerate endometrium. And the regenerated endometrial tissues might have a normal function such as menstruation and implantation of a fertilized egg.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was partially supported by grant from Formation of Innovation Center for Fusion of Advanced Technologies in the Special Coordination Funds for Promoting Science and Technology "Cell Sheet Tissue Engineering Center (CSTEC)" from the Ministry of Education, Culture, Sports Science, and Technology (MEXST), Japan.
Trial registration number: NA.
Keywords: Asherman's syndrome, intrauterine adhesion, cell sheet, regenerative medicine, tissue engineering

P-429 Molecular biomarkers in cumulus and granulosa cells for oocyte quality estimation and pregnancy outcome prediction

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Study question: The aim of the present study is to monitor cumulus and granulosa cells gene expression profile focusing on genes selected as central hubs of networks generated by *in silico* proteomic analyses of follicular fluid.
Summary answer: The preliminary results obtained so far suggest a statistically significant correlation between ART outcome and selected gene expression levels.
What is known already: The granulosa and cumulus cells, contributing to follicular fluid formation, may affect follicle growth, oocyte maturation and competence acquiring. Nevertheless, follicular fluid protein composition remain to be clarified and no oocyte "quality" biomarkers have been still identified. Thus, up to now, oocyte and embryo quality estimation is unsatisfactorily limited to morphological parameters.
Study design, size, duration: In this study, 60 infertile women undergoing to IVF procedure were enrolled and granulosa and cumulus cells were carefully isolated in order to test gene expression level.
Participants/materials, setting, methods: DAVID for functional clusterization and Metacore for pathway analysis were applied to select follicular fluid proteins involved in key process in follicular milieu. In order to validate the specific role of these keys factors, we tested mRNA expression levels of selected genes both in granulosa and cumulus cells in a cohort of 60 women, in comparison with healthy subjects.
Main results and the role of chance: Networks generated by functional pathway analyses essentially converge on key central hubs such as: matrix metalloproteinases (MMPs), thrombin, vitamin D receptor and retinoid X receptor-alpha heterodimer (VDR/RXR-α), whose combined differential presence/functionality may be useful for oocyte quality estimation in IVF programs, in order to improve pregnancy and baby born rates.

Our preliminary results proved that the MMP2 expression in granulosa cells inversely correlates with reproductive outcome, since MMP2 mRNA levels significantly decrease in women with more than 4 oocytes retrieved at the pickup and in women whit ART positive outcome. Also VDR and RAR expression

levels significantly decreased in women with high oocytes retrieval at the pick-up day. These observations clearly highlight a significant impact of granulosa and cumulus gene expression on ART procedure outcome.
Limitations, reason for caution: A larger study needs to be carried out in order to validate these findings.
Wider implications of the findings: This combined *in-silico* approach and gene expression profile allowed us to depict a wide overview on the follicular dynamics and to identify some key factors whose expression levels could be related to positive ART outcome in different category of patients. Therefore our study, if replicated in a larger population, might contribute in improving oocyte selection and ART protocols in infertile women.
Study funding/competing interest(s): Funding by University(ies) – University of Siena.
Trial registration number: NA.
Keywords: follicular fluid, proteome, oocyte quality, gene expression, ART

P-430 Lifestyle factors associated with irregular menstrual cycle in Korean women

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Study question: Which lifestyle factors are associated with irregular menstrual cycle in Korean women?
Summary answer: Among various lifestyle factors, body mass index (BMI) and smoking habit were significantly associated with irregular menstrual cycle in Korean non-diabetic women.
What is known already: Physiological regulation of menstruation cycle depends on spatiotemporal interactions of signaling between endogenous and ovarian hormones in the hypothalamic-pituitary-ovarian axis. Alteration of these orchestrated interactions by individual lifestyle and unfavorable habits may cause of irregular menstrual cycle.
Study design, size, duration: This cross-sectional study with retrospectively collected data including 3,799 non-diabetic Korean women with 19–49 years old in data from the Fifth Korea National Health and Nutrition Examination Survey (KNHANES) 2010–2012.
Participants/materials, setting, methods: The participants of non-diabetic women did not take any oral contraceptives and female hormones. We examined association of age, body mass index (BMI), drinking and smoking habits with irregular menstrual cycle. Statistical analyses were performed by contingency tables and logistic regression using SPSS program. Estimates are given as adjusted odds ratios with 95% confidence intervals.
Main results and the role of chance: Age, BMI, marriage status, menarche age and smoking habit were significantly associated with the irregular menstrual cycle, respectively ($P < 0.01$). The prevalence of irregular menstrual cycle was 16.9% of 19–29 years, 9.2% of 30–39 years and 9.6% of 40–49 years, respectively. BMIs of Asian obesity criteria were strongly associated with menstrual irregularity. Obese class I group with 25.0–29.9 of BMIs (odd ratio, 1.565; 95% Cl, 1.134–2.161; $P < 0.05$) and obese class II group with ≥30.0 of BMIs (odd ratio, 2.042; 95% Cl, 1.160-3.594; $P < 0.01$) presented significantly higher risk of irregular menstrual cycle compared with normal BMIs of 18.5-22.9. We found significant higher prevalence of irregular menstrual cycle in young women (19–29 years old) with obesity group I and II as 25.4% ($P < 0.001$) and middle-aged women (30–39 years old) with smoking habit group as 16.1% ($P < 0.005$).
Limitations, reason for caution: Possible limitation in our study is the use of retrospective questionnaire information on lifestyles and irregular menstrual cycle. The higher odds of irregular menstrual cycle in severely obese women may reflect other health problems in these women rather than the diabetes on the menstrual cycle.
Wider implications of the findings: This study demonstrates that risk of irregular menstrual cycle was closely associated with BMI and smoking habit in Korean non-diabetic women. Weight control and smoking cessation should be recommended for promotion of women's reproductive health.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare (A120043).
Trial registration number: (KNHANES) 2010–2012.
Keywords: irregular menstrual cycle, cross-sectional study, BMI, smoking habit, life style

P-431 Influence of regional adiposity on assisted reproductive technologies outcome

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Study question: What is the influence of regional adiposity on assisted reproductive technologies (ART)?
Summary answer: Anthropometric measures associated with abdominal obesity were better predictors of ART outcomes than BMI (body mass index). However, neither of them was associated with lower pregnancy and live birth rates.
What is known already: Obesity has a negative effect on female reproductive function, including fertility and pregnancy. The influence on ART is controversial. Most studies have shown that BMI is directly associated with higher gonadotropin requirement, higher cancellation rates and lower number of retrieved oocytes (NRO). However, the effects of obesity on embryo quality, clinical pregnancy and live birth rates are not conclusive. These ART outcomes were based on female and/or male BMI and regional adiposity was not explored.
Study design, size, duration: Prospective cohort study. All the women ($n = 578$) selected in our institution for performing an assisted reproductive technique – *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) – were included in the study, which was conducted between October 2012 and August 2014.
Participants/materials, setting, methods: Women's BMI, waist circumference (WC) and waist-to-hip circumference were recorded at the beginning of the stimulated cycle. WC was measured by two different methods: midway between lowest rib and iliac crest (WC_1); just below the lowest rib (WC_2). Women underwent controlled ovarian hyperstimulation with long agonist or short antagonist/agonist protocols.
Main results and the role of chance: Mean BMI (23.9 ± 4.1), WC_1 (83.7 ± 10.8), WC_2 (79.4 ± 8.8) and waist-to-hip ratio (0.83 ± 0.08) were not associated with clinical pregnancy, live birth and cancellation rates. These variables were associated with lower levels of oestradiol at day 5 (E_2) and endometrial thickness. A hierarchical regression examined the additional contribution of regional adiposity. BMI didn´t predict total gonadotropin dose, number of follicles, NRO, number of mature oocytes, fertilization and implantation rates but predicted E_2 ($r = -0.118$, $p = 0.005$) and endometrial thickness ($r = 0.151$, $p < 0.001$). WC_2 increased the explained variance, including E_2 ($b = -0.188$, $p = 0.011$), number of follicles ($b = -0.163$, $p = 0.031$) and NRO ($b = -0.172$, $p = 0.023$). Final models explained <4% of the outcomes. Women with ovulatory dysfunction ($n = 62$) were analyzed separately and outcomes were similar between obese and non-obese. However, fertilization rate was lower in WC_2 >80 cm group when compared with WC_2 ≤80 cm (50.9 vs 71.6%, $p = 0.034$).
Limitations, reason for caution: Visceral adiposity was indirectly evaluated by anthropometric measures, although we measured waist circumference just below the lowest rib, which correlates better with visceral fat. This study has also potential confounding factors, such as woman's age, origin of infertility, duration of infertility, male BMI, smoking habit and number of IVF cycles.
Wider implications of the findings: This study added the importance of fat distribution, especially in women with ovulatory dysfunction, which could translate the clinical effect of oxidative stress on follicular fluid induced by

abdominal obesity, described by some authors. Despite these results, BMI was not a predictor of adverse ART outcomes and adiposity measures had a small relative contribution. Therefore, BMI should not be used to select women for ART treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – N/A.
Trial registration number: NA.
Keywords: obesity, IVF

P-432 The effectiveness of hysteroscopy prior to IVF: a randomized controlled trial (inSIGHT study)

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Study question: Does routine hysteroscopy prior to the first IVF treatment cycle improve live birth rates?
Summary answer: Routine hysteroscopy prior to IVF does not increase ongoing pregnancy rates leading to live birth.
What is known already: Hysteroscopy is thought to improve ongoing pregnancy rates in subfertile women entering an IVF program. However, a recent randomized controlled trial did not show any beneficial effect from hysteroscopy performed in women with at least two failed IVF treatment cycles. The effectiveness of routine hysteroscopy prior to the first IVF cycle has never been assessed.
Study design, size, duration: The inSIGHT study was a multicenter, randomized controlled trial in 22 hospitals in the Netherlands, positioned in the Dutch consortium for studies in women's health. Subfertile women with a normal transvaginal ultrasound scheduled for a first IVF treatment were eligible. Women with recurrent miscarriage or intermenstrual blood loss were not included.
Participants/materials, setting, methods: Participants were randomly allocated to hysteroscopy with treatment of detected abnormalities before IVF (intervention group) versus no hysteroscopy before IVF (control group). The follow-up (FU) period was 18 months. Since completed live birth data are not available yet, we report in this abstract ongoing pregnancy rates. Analysis was by intention-to-treat.
Main results and the role of chance: Between 2011 and 2013, we randomized 750 women (intervention group: $N = 373$, control group: $N = 377$). Here, we report on 741 women (99%). Hysteroscopy revealed abnormalities in 17% of the women, which were all instantly treated. Cumulative ongoing pregnancy rates at 18 months of FU were 0.55 in both groups [risk difference (RD): 0.008 (95% CI -0.077, 0.093)]. Cumulative clinical pregnancy rates at 18 months of FU were 0.65 versus 0.62 [RD: 0.028 (95% CI: -0.054, 0.110)] for the intervention and control group, respectively. Miscarriage rates were 0.20 in the hysteroscopy group and 0.16 in the control group (RD: 0.04 [95% CI -0.40, 0.55)]. One complication was reported after the hysteroscopy procedure (endometritis).
Limitations, reason for caution: At the moment of writing the abstract, data on live birth have not been collected completely. Complete follow-up on live births will be available in June 2015.
Wider implications of the findings: In subfertile women with a normal transvaginal ultrasound scheduled for IVF, routine hysteroscopy does not improve outcome.
Study funding/competing interest(s): Funding by national/international organization(s) – ZonMW, the Dutch Organization for Health Research and Development.
Trial registration number: NCT01242852.
Keywords: hysteroscopy, IVF, infertility, pregnancy

P-433 Immunologic and inflammatory markers in prediction models of clinical pregnancy and live birth in association with *in vitro* fertilization (IVF) in women receiving immune-suppressive therapy

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Study question: Are baseline titers of immune and/or inflammatory markers in infertile women, receiving immune-suppressive therapy during IVF cycles, predictive of clinical pregnancy and live birth?
Summary answer: Immune-suppressive therapy during IVF cycles appears to negate IVF outcome associations with immune but not with inflammatory markers.
What is known already: When maternal immune systems malfunction, as for example in association with maternal autoimmunity, female immune systems appear to lose the ability to be reprogramed from rejection to tolerance, mounting an allogeneic immune response against the implanting embryo. Such an allogeneic-immune response potentially negatively affects implantation, increases miscarriage risks in early pregnancy and disrupts the inflammatory tissue environment surrounding a normal implantation site.
Study design, size, duration: Immune and inflammatory markers were drawn at first office visits. An initial IVF cycle was completed weeks later at our academically affiliated infertility center, during which patients with positive immune and/or inflammatory markers were treated with immune suppressive treatments (prednisone and, on occasion also IV-Ig).
Participants/materials, setting, methods: We performed a retrospective evaluation of 184 patients undergoing fresh, non-donor IVF cycles between November 2012 and October 2013. All laboratory tests were performed by commercial laboratory assays. Logistic regression models, adjusted for age, were used to estimate the effect of these markers on pregnancy and live birth.
Main results and the role of chance: Testing panels included as inflammatory markers adiponectin, leptin, C-reactive protein (CRP) and interleukin 6 (IL-6). As markers of immune system activation we assessed a panel of thyroid autoantibodies [ant-thyroid peroxidase (TPO), anti-thyroglobulin, anti-thyrotropin receptor antibody], total immunoglobulins (IgA, IgE, IgG and IgM) and a panel of anti-phospholipid antibodies [anti-phosphatidylserine (anti-PS), anti-b2 glycoprotein and anti-cardiolipin (in IgA, IgG and IgM isotypes)]. Increasing CRP ($P = 0.02$) and IL-6 ($P = 0.034$) were associated with decreasing odds of live birth. Increasing total immunoglobulin IgG was associated with increasing chances of live birth ($P = 0.03$). Increasing IgG anti-PS was associated with decreasing ($P = 0.03$), while increasing IgM anti-PS was associated with increasing ($P = 0.04$) chances of pregnancy.
Limitations, reason for caution: Since patients with positive immune and/or inflammatory markers were actively immune-suppressed, here documented associations between primarily inflammatory markers and IVF outcomes apply only to so-treated patients. Untreated patients may demonstrate additional associations.
Wider implications of the findings: In women with activated immune systems, immune-suppression appears to eliminate potential effects of immune markers on IVF cycle outcomes but does not eliminate the effects of inflammatory markers, suggesting a need for better and more aggressive anti-inflammatory treatments in such patients.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for Human Reproduction, Foundation for Reproductive Medicine.
Trial registration number: NA.
Keywords: *in vitro* fertilization (IVF), immune markers, inflammatory markers, implantation

P-434 Effect of follicular fluid from infertile women with endometriosis on oocyte maturation, meiotic spindle morphology and markers of DNA damage in mouse oocytes

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Study question: Does incubation with follicular fluid of women with endometriosis alter oocyte maturation, meiotic spindle morphology or cause DNA damage?
Summary answer: We find that incubation of mouse oocytes in follicular fluid from women with severe, but not mild or no endometriosis, reduced maturation

rates. However, this reduction in maturation is not attributed to defects in spindle morphology or increased DNA damage.
What is known already: Recent meta-analysis showed that women with endometriosis have a lower oocyte yield; it is not known if this is related to impaired oocyte quality attributed to the altered composition of follicular fluid in endometriosis. Quality of an oocyte is closely related to nuclear maturation and this depends on the presence of a normal cell spindle. To date, no study has examined the spindle morphology and level of DNA damage in human oocyte in those with endometriosis.
Study design, size, duration: Samples of follicular fluid were obtained from January 2014 to December 2014 from 20 infertile women during oocyte retrieval at their IVF/ICSI treatment. From August to December 2014 we performed IVM experiments using immature mouse oocytes as described below
Participants/materials, setting, methods: FF containing mature oocytes was obtained from 20 infertile women during oocyte retrieval procedure. Immature mouse oocytes were incubated in the absence of follicular fluid (No-FF) and in the presence of follicular fluid with control (CN-FF), mild (ME-FF) and severe (SE-FF) endometriosis. Five replicates experiments were performed each one using follicular fluid from a control patient and a patient with endometriosis. After 14–16 h of incubation, oocytes were washed, fixed and immunostained for visualization of chromosomes, DNA damage and polar body.
Main results and the role of chance: A total of 555 (SE-FF, n = 167; ME-FF, n = 97; CN-FF, n = 130; No-FF, n = 161) mouse GVs were collected. Following maturation, we found a trend in polar body extrusion percentage: No-FF, 70.8%; MF-FF, 67.7%; ME-FF, 66.0% and SE-FF, 55.1%. We found a statistically lower maturation rate in women with severe endometriosis when compared to either control or no follicular fluid groups ($P < 0.03$ and $P < 0.01$ respectively; CI 95%, Fisher's Exact Test). We found there is no difference in progression from GV to MI stage and there is no difference in MII spindle morphology between all groups. Following image analysis (SE-FF, n = 21; ME-FF, n = 28; CN-FF, n = 36 and No-FF, n = 44) we found no difference in nuclear gH2AX fluorescence between all groups ($P > 0.05$).
Limitations, reason for caution: This study is limited due to its small sample size, and investigations using large cohort of patients is required to verify these results. This study was using animal models and may not necessarily be extrapolated to humans.
Wider implications of the findings: Our result showed that follicular fluid of women with severe endometriosis has detrimental effect on oocyte progression to metaphase II but not from GV to MI stage and may contribute to the pathophysiology of infertility in women with endometriosis.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Ministry of Education, Malaysia. Complete Fertility Centre.
Trial registration number: NA.
Keywords: meioisis, oogenesis, follicular fluid, DNA damage, endometriosis

P-435 Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist (GnRHa) and human chorionic gonadotropin (hCG) in normal responder patients undergoing IVF/ICSI cycles: a retrospective analysis

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Study question: Does dual trigger significantly improves the laboratory and clinical outcome for normal responders in GnRH-antagonist cycles?
Summary answer: Similar clinical pregnancy rate is achieved when compared dual trigger for oocyte maturation to standard (hCGr) triggering. The number COCs and metaphase II retrieved were reduced with this approach of triggering.
What is known already: Recent data from retrospective studies suggest that the use of gonadotropin-releasing hormone agonist (GnRHa) and human chorionic gonadotropin (hCG) for oocyte maturation is promising and significantly improves the clinical outcome for normal responders in GnRH-antagonist cycles
Study design, size, duration: This single center retrospective case study, including 220 normal responder patients undergoing IVF/ICSI in antagonist cycles, was undertaken during the period January 2013–December 2014.
Participants/materials, setting, methods: Exclusion criteria were: Poor (<4 COCs) or high (>20 COCs) response to ovarian stimulation; >39 years; AMH <1.1 ng/ml; BMI >30 kg/m^2. Dual trigger (6500 IU hCGr + triptorelin 0.2 mg) was employed in 103 patients (January 2014–December 2014) and the results compared to 113 control patients using 6500 IU hCGr-only for triggering (January 2013–December 2013).
Main results and the role of chance: When compared dual trigger vs hCG; the number of COCs retrieved (8.83 ± 4.74 vs 10.39 ± 5.06 $p = 0.019$) and number of metaphase II (ICSI cycles) (6.68 ± 3.23 vs 8.56 ± 4.35 $p = 0.002$) was statistically different. The clinical pregnancy rate per transfer was similar between both groups 29.3% (dual trigger) vs 42% (hCG) $p = 0.07$.
Limitations, reason for caution: This is a retrospective study, moreover case and control cycles were performed in consecutive but different years. Future prospective randomized controlled trials are needed to clarify whether the addition of GnRH agonist to standard hCG is effective in improving the outcomes for normal responders in GnRH antagonists cycles
Wider implications of the findings: Dual trigger for final oocyte maturation in normal responders doesn't appear to improve outcomes. This approach should be validated in properly designed RCT before its application in clinical practice in order to avoid unnecessary extra medication in this population.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu.
Trial registration number: NA.
Keywords: dual trigger, GnRH antagonists, GnRH agonist triggering

P-436 Low and high relative number of top quality embryos is associated with decreased cumulative live birth rate – a cohort study of over 20 000 cycles

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Study question: How does the proportion of top quality embryos (TOPs) affect cumulative live birth rate (cLBR) after IVF/ICSI with frozen-thawed embryo transfer (FET)?
Summary answer: The proportion of TOPs does not change in subsequent stimulations, and low (<25%) but also high (>85%) percentage of TOPs is associated with lower cLBR.
What is known already: The transfer of TOPs is associated with higher chance of live birth after IVF/ICSI and most studies have compared outcomes of different numbers of TOP or TOP vs. non-TOP transfers. However, the impact of the quality of the entire embryo cohort on cLBR has not been studied.
Study design, size, duration: This cohort study analysed data from four infertility clinics. IVF/ICSI cycles with one TOP (n = 6177) or 1–2 non-TOP embryos (n = 5935) transferred on day 2 or 3 were performed during January 2000 – June 2011 (n = 12112). These were followed by 10188 FET cycles during January 2000 – June 2013.
Participants/materials, setting, methods: Regression models for the proportion of TOPs of all cleaved embryos were performed, followed by logistic regression for cLBR/stimulation. Patient and stimulation characteristics and consecutive treatment cycles were used as independent factors. Modeled probabilities were used to determine cutoff points for the probability of cumulative live birth ≥40%.
Main results and the role of chance: The percentage of TOP/all cleaved embryos was 21.1 ± 25.1 % (1.41 ± 1.88 TOP/stimulation). It remained stable with consecutive stimulations of the same patient (ANOVA, $P = 0.7$). Logistic regression showed that the percentage of TOP had a reverse-U-shaped (quadratic) effect on cLBR meaning that chances for live birth were lower with both low and high proportions of TOPs ($P < 0.0001$). The regression model included the number of collected oocytes, which had independent effect ($P < 0.0001$ for all oocyte groups). Modeled cLBR was 25.4% in cases with no TOPs, increased to 40% (at 25% TOPs) and to 46% (55% TOPs), but thereafter a decreasing trend was observed. The probability of live birth at the end of treatment was ≥ 40% if the proportion of TOP was between 25 and 85%.
Limitations, reason for caution: About 8% of women would have needed more time to complete all possible FET cycles. The total duration of the study

was long, but there was no trend in yearly fluctuations of cLBR/stimulation during the study period.
Wider implications of the findings: Present results suggest that a low number of TOPs in the first fresh cycle is associated with low relative number of TOPs in subsequent stimulations regardless of increased gonadotropin dose. cLBR is best at 55% TOPs and good (≥40%) at 25–85% TOPs. The decreasing trend observed thereafter may reflect suboptimal endometrial receptivity. This may be one explanation why some women with high numbers of TOPs do not become pregnant.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). Funding by national/international organization(s). University of Helsinki, Helsinki University Hospital, the Sigrid Juselius Foundation, Academy of Finland, University of Oulu, Oulu University Hospital.
Trial registration number: NA.
Keywords: live birth, embryo quality, IVF/ICSI

P-437 Personalized IVF outcome predictions provide more optimistic and precise outlooks than age-based national averages

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Study question: The likelihood of achieving live birth after IVF treatment is often reported on a per-cycle basis using age-based national averages. Our study aimed to assess whether couples' personalized, cumulative likelihoods of success accurately reflect observed events, and could therefore be used to report more precise, journey-wide outlooks.
Summary answer: Provided they did not discontinue treatment, 98.4% of patients predicted to respond well to IVF treatment achieved live birth by the time their cumulative chances of success plateaued. Moreover, on average these patients achieved live birth more than 2 cycles earlier than this plateau point.
What is known already: Estimated success rates for couples beginning IVF treatment are based on average success rates per treatment cycle adjusting for maternal age. Such estimates do not reflect couple-specific chances of having a baby over an entire treatment journey, and do not account for possibilities of treatment discontinuation or the multiple rounds of treatment often required. They could therefore discourage many patients from continuing or even starting treatment altogether.
Study design, size, duration: We performed a retrospective cohort study on 9,750 patients undergoing infertility treatment at three clinics between April 2002 and June 2014. We excluded patients with natural parity ≥1, age ≥45, as well as donor-IVF and RE cycles.
Participants/materials, setting, methods: Diagnostic data of each couple, taken before treatment, was used to calculate cumulative likelihoods of live birth after IVF using a Cox proportional hazards model constructed using retrospective data from more than 14,000 IVF treatment cycles. We compared these likelihoods to observed rates of live birth and patient dropout.
Main results and the role of chance: We ranked patients according to their calculated probabilities of live birth after one IVF cycle. For the 1,950 patients in the top 20% of this ranking, the average probability of success was 53.8% (±3.2). We calculated that, for these patients, cumulative likelihoods for live birth did not increase by more than 5% beyond cycle 4, representing a plateau point of cumulative probabilities of success. In keeping with this, of the 1444 patients (74%) achieving live birth, 98.4% did so by cycle 4. However, the average number of cycles to achieve live birth was 1.37 (±0.75), much earlier than the plateau point. The 506 (26%) of patients discontinuing treatment did so after an average of 1.91 (±1.02) cycles, also preceding their expected plateau point of cumulative probabilities of success.
Limitations, reason for caution: One component of our data model, diagnosis, is subjective across the clinics from which patients were recruited. Out-of-pocket expenses, not biological factors, largely explain dropouts in our IVF cohort.
Wider implications of the findings: Our data suggest that almost all patients achieving success while undergoing IVF treatment do so by the time they reach the point where their predicted cumulative chances of success are at their maximum. Patients who are considering discontinuation of treatment may be encouraged to continue if presented with this information, at least until they reach their own point of maximum cumulative likelihood of success.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Celmatix Inc.
Trial registration number: NA.
Keywords: IVF, infertility, personalized medicine, data modeling

P-438 The therapeutic effect of hysterosalpingography (HSG) as a tubal patency test: a multi centre cohort study

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Study question: Does, in women with unexplained subfertility, tubal patency testing with HSG during a basic fertility work-up increase ongoing pregnancy rates compared to no tubal patency testing?
Summary answer: Tubal patency testing with HSG as part of a basic fertility work-up leads to higher ongoing pregnancy rates, compared to no tubal patency testing during the basic fertility work-up.
What is known already: A possible therapeutic effect of diagnostic tubal patency testing has been debated in literature for more than 50 years. Results from both randomized and non-randomized studies are conflicting.
Study design, size, duration: We performed a secondary analysis of a prospective cohort study among 7,860 couples with subfertility. Couples were included in the cohort after the basic fertility work-up had been completed. Couples with ovulation disorders, previous tubal surgery or severe male factor (TMC $<1 \times 10^6$) were not included in our study.
Participants/materials, setting, methods: Couples had been recruited in 38 Dutch clinics between January 2002 and December 2004. Depending on local protocols and patient preferences, some women underwent an HSG, while others did not. Couples were followed until conception, or until start of treatment. We analysed whether HSG had impact on ongoing pregnancy rates.
Main results and the role of chance: From the 7,860 couples included in the cohort we analysed data of 3,164 couples. Mean age of the women was 32 years. Mean duration of subfertility was 22 months. A total of 1,706 women underwent tubal patency testing by HSG during the basic fertility work-up and 1,458 women had no tubal patency test at all. The probability of natural conception after HSG was increased as compared to no tubal patency testing (adjusted Hazard Ratio 1.68, 95% CI 1.38–2.04)
Limitations, reason for caution: This was an observational cohort study in which women had not been randomized, thus bearing the risk of selection bias. We tried to reduce this bias by performing an inverse probability weighting (IPW) analysis.
Wider implications of the findings: Flushing of the tubes during patency testing is likely to have a therapeutic effect in subfertile women.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Not applicable.
Trial registration number: NA.
Keywords: hysterosalpingography, ongoing pregnancy, therapeutic effect, subfertility, diagnostic tubal test

P-439 A granulosa cell line that acquires cumulus oophorus properties – Early characterization

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Study question: Roles of granulosa cells (GCs) and *cumulus oophorus* cells (CCs) in the oogenesis process have been proposed but the underlying mechanisms require elucidation. As the number of primary cells is usually scarce, can a cell line as GC1a be used to enlighten CCs interactions with the oocyte?
Summary answer: Our findings suggest that GC1a cell line is a useful model for the study of follicular development. They show that GC1a cells differentiate in culture with features that mimic CCs, including OFS receptors expression and response to OSFs (oocyte secreted factors) and FSH (follicle stimulating hormone).
What is known already: Oocyte quality, achieved during the development of follicular microenvironment, is crucial for assisted reproductive technologies (ART) success. The microenvironment includes GCs that multiply along the cycle and differentiate into two structurally and functionally distinct types: mural GCs and oocyte surrounding CCs. The oocyte becomes regulator of itself by producing OSFs that modulate follicular cells function and survival that, in turn, modulate oocyte growth. However, the cellular details of such interchange are essentially unknown.
Study design, size, duration: To tackle the study question, granulosa derived GC1a cells were cultured in DMEM supplemented with FBS (fetal bovine serum). Early and late cell passage numbers were elected for experiments performed by adding GDF9 (50 ng/ml), BMP15 (100 ng/ml) and FSH (200 ng/ml) to the culture media and comparing with the controls.
Participants/materials, setting, methods: The presence of OSF and their receptors was evidenced by q-PCR, and the ability of OSFs and FSH to affect proliferation and cell cycle was studied by sulforhodamine-B (SRB) assay and flow cytometry, respectively; their ability to promote the activation of ERK1/2 and SMAD2 signaling was assessed by Western blot.
Main results and the role of chance: We report, for the first time in this cell line, the transcript of the OSF, such as GDF9, and the expression of OSFs receptors, such as BMPRI, BMPRII and TGFbRI. We also demonstrate that OSFs and FSH do not affect GC1a cell proliferation and cell cycle progression, compared with controls. The data indicate that passage number results in cell morphological change and different modulation features. In fact, in early passages, FSH and GDF9 activate ERK1/2 signaling, whereas in late passages GDF9, but not FSH, activates SMAD2 cascade.
Limitations, reason for caution: Despite the relevance to dissect the transduction pathway and the structural features, secretion products assessment is required, at early and late passages, to better support the model.
Wider implications of the findings: The data suggest that in this GC1a model, GCs can differentiated in CCs, which will allow further studies in a reproducible manner. Unveiling details of signaling pathways of oogenesis will evidence specific regulatory points. These are likely to be amenable to useful modulation, aiming at fostering successful oocyte development or, eventually, blocking its progression.
Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies). Universidade do Porto. GFI – Merck Serono Foundation.
Trial registration number: NA.
Keywords: granulosa cells, cumulus oophorus, GC1a cells, oocyte secreted factores, FSH

P-440 Human embryos from overweight or obese women develop slower from the time to compaction until the start of blastulation

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Study question: Is there a difference in morphokinetics of embryos from overweight or obese women compared to those from women with a healthy body mass index (BMI)?
Summary answer: Embryos from overweight or obese women show a trend of developing at a faster rate until the 6-cell stage than those from women of a healthy BMI. From the time to compaction until the start of blastulation, embryos from overweight or obese women develop at a significantly slower rate.
What is known already: Women with a higher BMI have been associated with an increased prevalence of infertility, as well as reduced clinical pregnancy rates following assisted reproductive treatment. Female BMI has also been negatively associated with several aspects of fertility, such as ovarian stimulation, uterine receptivity and miscarriage rates. However, there is limited information regarding its effect on embryo morphokinetics. The purpose of this study was to identify possible correlations between female BMI and embryo development.
Study design, size, duration: We performed a retrospective study analysing the developmental timings of 371 embryos from 219 consecutive couples attending IVF Hammersmith for ICSI treatment between April 2012 and November 2014. The Embryoscope™ was used to measure developmental kinetics. The timings to each developmental stage were related to female BMI.
Participants/materials, setting, methods: Women who underwent ICSI treatment between April 2012 and November 2014 whose embryos were cultured in the Embryoscope™ were included. We compared morphokinetics of transferred embryos between healthy (BMI = 19–25, $n = 242$, average age = 36.0), overweight (BMI = 26–29, $n = 76$, average age = 35.2) and obese (BMI ≥ 30, $n = 53$, average age = 34.9) groups, established according to NHS guidelines.
Main results and the role of chance: A trend of faster embryo development was observed in embryos from obese women compared to healthy women in the early stages of development, with a significant difference identified in the time to second polar body extrusion ($P < 0.05$) and to 2-cells ($P < 0.05$). However, embryos from obese women were slower to compact compared to healthy and overweight women ($P < 0.005$ and $P < 0.01$, respectively). Embryo development to the morula stage was also slower in overweight compared to healthy women ($P < 0.05$), and slower in obese compared to overweight women ($P < 0.005$). There was no significant difference in clinical pregnancy rate between the three groups, however a significantly higher implantation rate (47.1 vs 26.9%, $P < 0.01$) was observed in obese women compared to healthy women.
Limitations, reason for caution: This was a retrospective analysis of transferred embryos with different sample sizes between the groups. In order to ascertain the effect of maternal BMI on developmental timings, a prospective cohort study with an evenly distributed population is needed, analysing all embryos created.
Wider implications of the findings: We propose that increased metabolic activity in the embryos could explain the faster early development observed in the obese group, however later epigenetic events which coincide with blastocyst formation may be compromised causing slower development. In spite of slower embryo development in the obese group, the higher implantation rate observed may be explained by the lower average age in this group.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Hammersmith.
Trial registration number: NA.
Keywords: BMI, morphokinetics, embryo development, obesity

P-441 Thyroid-stimulating hormone is increased in infertile women with low anti-Müllerian hormone

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Study question: What is the prevalence of (i) hypothyroidism assessed by plasma thyroid stimulating hormone (TSH) and (ii) autoimmune thyroid disease assessed by the presence of thyroid peroxidise antibodies (TPOAb); and are these conditions associated with the ovarian reserve estimated by anti-Müllerian hormone (AMH) in infertile women of reproductive age?

Summary answer: In a cohort of 543 infertile women aged 20–40, 10% had autoimmune thyroid disease (with or without hypothyroidism), and another 5% had hypothyroidism without TPOAb. Three-quarters were diagnosed as a result of our screening prior to fertility treatment. TSH was significantly higher in women with an AMH below 5 pmol/l.

What is known already: Female infertility is associated with hypothyroidism and the presence of TPOAb, but the pathophysiology remains uncertain. Whether infertility is caused by a disturbance in the endocrine- and/or the immune system has yet to be established. Infertile women have been shown to have higher levels of TPOAb and TSH compared to fertile women. Studies investigating the correlation between AMH and thyroid function in infertile women are few and have limited sample size.

Study design, size, duration: Baseline data in a prospective cohort study of 543 infertile women. The women were newly referred to fertility treatment with their male partner. All women were aged 20–40 years and recruited between September 2011 and October 2013.

Participants/materials, setting, methods: On Cycle Days (CD) 2–5, prior to start of stimulation, women were examined by transvaginal ultrasonography (antral follicle count (AFC), ovarian volume, pathology) and blood sampling to assess serum-AMH, FSH, LH, TSH, thyroxine (T4), free-T4 and TPOAb. Medical and reproductive history, and lifestyle factors were recorded.

Main results and the role of chance: The mean (SD) age was 32.7 (4.0) years. TPOAb was observed in 56 (10%) women. Another 26 (5%) TPOAb-negative women had a serum-TSH above normal (>4.0×10^{-3} IU/l). Of these 82 women, 19 (23%) had previously been diagnosed with thyroid disease. Twenty-nine (5%) women had a very low AMH-level (<5 pmol/l). TSH was significantly higher in women with an AMH <5 pmol/l ($p = 0.01$), which was also associated with increased levels of FSH ($p < 0.001$), but not associated with LH, T4, free-T4 or TPOAb. Women with an AMH <5 pmol/l were older ($p < 0.001$), had a lower AFC ($p < 0.001$) and a shorter menstrual cycle ($p < 0.001$). In a multiple logistic regression analysis, the following covariates remained significantly associated with very low AMH-levels: TSH (OR: 2.31, 95% CI: 1.17; 4.56), FSH (OR: 1.3, 95% CI: 1.04; 1.49), and AFC (OR: 0.55, 95% CI: 0.44; 0.69).

Limitations, reason for caution: Since the number of TPOAb-positive women with a very low AMH was limited, we could not investigate the correlation between impaired ovarian reserve and autoimmune thyroid disease. Women already diagnosed with thyroid disease may have declined the offer of screening and the prevalence would, thus, be underestimated.

Wider implications of the findings: The prevalence of autoimmune thyroid disease in our cohort is similar to previous findings. A recent study found AMH to be inversely correlated to TSH in infertile women, which corresponds to our results. Untreated hypothyroidism is associated with a decreased fertility and an increased risk of early pregnancy loss. Our results emphasize the importance of screening infertile women prior to fertility treatment, as undiagnosed hypothyroidism and autoimmune thyroid disease may impair their success rate.

Study funding/competing interest(s): Funding by national/international organization(s) – Funding by commercial/corporate company(ies). The study received funding from MSD and through the European Union (EU) Interregional projects "ReproSund" and "ReproHigh". The authors have no conflict of interest.

Trial registration number: NA.

Keywords: autoimmune thyroid disease, hypothyroidism, ovarian reserve, female infertility, anti-Müllerian hormone

P-442 Inhibition of sirtuin activity and impaired synthesis of the sirtuin NAD + co-factor disrupts oocyte meiotic maturation

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Study question: Does sirtuin de-regulation brought about by either the sirtuin inhibitor, nicotinamide (NAM), or reduced NAD^+ production induced by inhibiting NAM phosho-ribosyltransferase (NAMPT) in the NAD^+ synthesis pathway impair oocyte maturation?

Summary answer: Sirtuin inhibition severely impaired two key developmental phases during oocyte maturation, entry into meiosis I and establishment of a proper meiosis II-arrested state conducive to fertilisation. Remarkably, the interval between these two extremes of maturation was completely unperturbed pointing to requirements for sirtuins at defined stages of oocyte development.

What is known already: Sirtuins are NAD^+-dependent deacetylases crucial for multiple functions that collectively combat aging in somatic cells. Surprisingly little is known about sirtuins in mammalian oocytes. Highly significantly, the effects of neither NAM nor NAMPT-inhibition have previously been studied during oocyte maturation. Formation of a fertilisable egg requires entry into and progression through meiosis I, transition from meiosis I-to-meiosis II and finally, establishment of a meiosis II-arrest-state characterised by a properly assembled spindle with aligned chromosomes.

Study design, size, duration: Fully-grown germinal vesicle (GV)-stage mouse oocytes ($n > 30$ per group in triplicate) were cultured in media treated with the sirtuin inhibitor, NAM. As an alternative for impairing sirtuin activity we inhibited production of the sirtuin co-factor, NAD^+, using FK866 to specifically inhibit NAMPT, an enzyme required for NAD^+ synthesis.

Participants/materials, setting, methods: Oocytes from hormonally-primed 7- to 9-week-old Swiss mice were cultured in media treated with NAM or FK866. Rates of GV breakdown (GVBD) following release from the GVBD inhibitor, 3-isobutyl-1-methylxanthine (IBMX), and first polar body extrusion (PBE) were assessed. Spindles and chromosomes were analysed using four-colour, high-resolution confocal imaging.

Main results and the role of chance: NAM-treatment significantly reduced GVBD, from 55 to <10% at 1 h ($P < 0.0001$) and from ~80 to 67% at 2 h ($P = 0.0011$) following release from IBMX. In contrast, PBE rates were indistinguishable between treated and untreated groups ($P = 0.22$) at 14 h post-GVBD. Strikingly, although polar bodies appeared morphologically normal, confocal analyses revealed complete failure of spindle assembly in ~35% of NAM-treated oocytes, a defect never observed in untreated controls. Furthermore, chromosomes decondensed and a nucleus formed. Although NAM induced defects at extremes of maturation, there were no defects in spindle assembly and chromosome alignment during the intervening period. Entirely consistent with NAM-induced defects, FK866 treatment markedly reduced GVBD at 1 h ($P < 0.0001$) and 2 h ($P = 0.0002$), providing independent support for the importance of sirtuins during GVBD.

Limitations, reason for caution: Although we corroborated our findings using two independent approaches for disrupting sirtuin function, it is possible that NAM or FK866 could lead to effects on targets other than sirtuins in oocytes.

Wider implications of the findings: This is the first evidence that sirtuins regulate GVBD and the establishment of the meiosis II-arrest state required for fertilisation. Our data are consistent with recent evidence that depletion of Sirtuin-2 induces spindle defects at meiosis II, raising the possibility that at least some NAM-induced defects reflect Sirtuin-2 deregulation. Sirtuin deregulation could underpin cases in which normal-appearing eggs fail to fertilise. Importantly, these data suggest that sirtuin-modulators may constitute novel adjuvants for improving oocyte quality.

Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). University of New South Wales, Australia. Ramaciotti Centre for Genomics.

Trial registration number: NA.

Keywords: oocyte, meiosis, oocyte maturation, sirtuins, spindle

P-443 Impact of uterus contraction frequency and elastography assessment on pregnancy rate after intrauterine insemination

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Study question: Do uterine contraction (UC) frequency and intensity at the time of intrauterine insemination (IUI) modify the fertility outcome of this technique?

Summary answer: Lower frequency and higher intensity of uterine contractions at the day of IUI were associated with a higher pregnancy rate. These two factors, with woman's younger age, thickness and ultrasound aspect of the endometrium were independently predictive of pregnancy rate arisen after IUI.

What is known already: Even without pregnancy, the uterus contracts in a cyclic and painless way. In spontaneous cycle, uterine contractions are common during periovulatory period and rare through the implantation window. In IVF, high contractility at embryo transfer reduces pregnancy rate. However, the impact of uterus contractility in IUI outcomes has never been directly studied. We aimed to assess the impact of uterine contractility, in frequency (contraction count) and intensity (elastography measurement) on the success rate after IUI.
Study design, size, duration: During a 2 years period, we conducted a pilot prospective cohort study including 100 infertile women eligible for IUI. At the time of IUI, we prospectively measured the frequency of UC, and assessed their intensity by elastography. These factors with other parameters entered a predictive model for pregnancy after IUI.
Participants/materials, setting, methods: Patients underwent transvaginal sonography with 5 min sagittal uterus recording, elastography, and 3D endometrial reconstruction, 1 h before IUI. Advanced ultrasound parameters were measured: UC frequency, sub-endometrial elastography, endometrial thickness, volume and aspect. Classical IUI parameters were recorded. With logistic regression stepwise down, we developed a predictive model for pregnancy.
Main results and the role of chance: The median frequency of UC was 2.8/min. Quantitative elastography estimates of sub endometrial area (area of interest) and endometrial area (control) were performed. The ratio of these two respective values was significantly higher (i.e., with a uterine contraction of higher intensity) in women with subsequent pregnancy after IUI. In multivariate analysis, UC number (OR = 0.039; $p = 0.021$; 95% CI 0.002–0.6), patient age (OR = 0.001; $p = 0.004$; 95% CI $1.48.10^{-5}$–0.12), the elastography ratio (OR = 63.26; $p = 0.01$; 95% CI 2.6–1532.0), endometrial thickness on the ovulation triggering day (OR = 28.21; $p = 0.03$; 95% CI 1.3–590.6) were predictive of pregnancy after IUI.
Limitations, reason for caution: The reproducibility of UC profile between several cycles of IUI is uncertain. To draw any conclusion using our predictive model of success after IUI, it should be validated in a new population.
Wider implications of the findings: In women with high uterine contractility and no pregnancy in IUI, fertility outcome after IVF should also be studied in order to state if a quicker appeal to IVF is indicated in this case. There is no consensus to date for the use of utero relaxation treatment during IUI. A randomized study should assess the benefit of this treatment by reducing UC frequency and improving success rates in IUI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Poissy ART Center.
Trial registration number: NA.
Keywords: uterine contractility, elastography, intrauterine insemination

MALE AND FEMALE CONTRACEPTION

P-444 Nano-puerarin: a prospective candidate for emergency contraceptive formulation

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Study question: What is the mechanism underlying the post-coital contragestative potential of poly lactic-co-glycolic acid-puerarin nanoparticles (PNP)?
Summary answer: PNP disrupts endometrial receptivity and render it hostile to embryo implantation. Central to this array of events is the attenuated Indian hedgehog (Ihh)–chicken ovalbumin upstream promoter transcription factor (COUP-TFII)–bone morphogenetic protein (BMP2) signaling axis that adversely impacts endometrial expression of estrogen receptor subtypes with consequent repression of progesterone receptor.
What is known already: The currently available post-coital contraceptive formulations are mostly steroid-based. The untoward effects attributed to the proliferative actions of estrogenic component of the pill, or higher failure rate and menstrual abnormalities associated with progestogen-only pills demands the development of non-steroidal EC formulations. Puerarin, a selective estrogen receptor modulator, exerts anti-implantation effects in rats, albeit at unacceptably higher doses. Furthermore, PNP exerts post-coital contragestative effect in rats at 1/4th dose of puerarin.
Study design, size, duration: Pregnant rats were orally administered with PNP ($n = 5$) or void-polymer ($n = 5$) for day 1–2 (D_1–D_2). Human endometrial stromal cell lines (hESCs) was cultured initially for 24 h in presence or absence of PNP and then for a further period of 72 h in the presence of medroxyprogesteroneacetate and db-cAMP.
Participants/materials, setting, methods: The effect of PNP on endometrial receptivity and decidualization was studied in day 5 pregnant rat endometrium (75 mg/kg/day) or cultured ESCs (10–50 nM) by *immunofluorescence*, RT-PCR/western blot analysis, gelatin zymography, and ESC migration assay.
Main results and the role of chance: Oral administration of PNP for day 1–2 of pregnancy led to total failure of implantation ($p < 0.05$). Disruption of endometrial receptivity was evidenced by attenuated endometrial expression of desmin, decidual prolactin, and insulin-like growth factor binding protein-1 ($p < 0.05$). There was down-regulated ($p < 0.05$) uterine expression of Ihh, BMP2, and COUP-TFII, and adversely altered expression of estrogen receptors (ERα, ERβ) and progesterone receptor AB ($p < 0.05$). PNP significantly suppressed the directed migration of hESCs by attenuating ($p < 0.05$) the expression of extracellular signal regulated kinases (ERKs), pERK, and myosin light chain, while the expression of Ras homolog gene family, member A (RhoA), and Rho-associated protein kinase was up-regulated ($p < 0.05$). Gelatin zymography analysis demonstrated attenuated endometrial expression of proMMP-9 ($p < 0.05$).
Limitations, reason for caution: The effective dose of PNP seems considerably high. Also, the *in vivo* effects are based on a rat model.
Wider implications of the findings: PNP exerts its anti-implantation activity by way of prohibitory effect on endometrial bed preparation and decidualization process. PNP is perhaps a prospective candidate molecule that may be further explored for future development of non-steroidal emergency or post-coital contraceptive formulation.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by the Council of Scientific and Industrial research–University Grants Commission, New Delhi, India.
Trial registration number: NA.
Keywords: contraception, endometrium, implantation, decidualization

P-445 The effect of hormonal contraceptives and bleeding pattern on migraine headache

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Study question: Preliminary data on headache diaries in migraineurs suggest that the progestin-only pill has a positive effect on the course of both MA and M0, especially reducing the number of days with migraine, the number of analgesics and the intensity of migraine-associated symptoms (including nausea or vomiting).
Summary answer: The Pearson correlation test revealed a relationship between duration of menstrual cycle and the intensity of headache pain ($p = 0.012$). The duration of oral contraceptive use was correlated with the intensity and duration of headpain ($p = 0.001$ and $p = 0.021$).
What is known already: Preliminary data on headache diaries in migraineurs suggest that the progestin-only pill has a positive effect on the course of both MA and M0, especially reducing the number of days with migraine, the number of analgesics and the intensity of migraine-associated symptoms (including nausea or vomiting).
Study design, size, duration: The questionnaire based study have been performed in the Outpatient Unit of Department of Neurology. We invited all women with M0 and MA in the Outpatient Headache Unit of the Department of Neurology to participate in the questionnaire-based study. We collected a data in two time periods: between 2006–2009 and 2013–2014 and we received the answers via 3 ways: personal face-to-face interviews, online and postal way. All participants completed a questionnaire containing 25 items compiled by our research team based on our clinical practice and literature search. The questions referred to socio-demographic data, the menstruation cycle pattern (mean age

at the first menses, duration of menses, characteristic of menstrual cramps and bleeding, the contraception habits, use of reliable and less reliable contraceptive methods) and migraine characteristic (including number of headache days, intensity of headpain, use of acute and prophylactic anti-migraine drugs). The validity process of the questionnaire was performed and internal reliability was also calculated.

Participants/materials, setting, methods: Our study group consisted of 108 women with migraine without aura (58%) and 78 women with migraine with aura (42%).All participants completed a questionnaire containing 25 items compiled by our research team based on our clinical practice and literature search. The validity process of the questionnaire was performed and internal reliability was also calculated. The associations between oral-contraceptive use, bleeding patterns and clinical characteristics migraine were compared by the Pearson correlation tests.

Main results and the role of chance: The average age of the patients at the diagnosis of migraine was 18.79 ± 6.97 years. The mean age at menarche was 12.3 ± 2.1 years. The majority of patients (59.4 %) had regular menstrual cycle (mean duration of the cycle: 28 days, length of bleeding: 3–5 days. Only 27 patients (11.54%) had a large amount of bleeding, 39.31 % of women suffering from menstrual cramps and 44.45% of patients had changes in body mass index in the last year. Regarding the sexual activity, 155 women (83.3 %) had regular sexual life in our study population. Thirty-one women (16.7%) does not have sexual activity during the study period. Regarding contraceptive practice of the migraineurs 54.3% of women used COC, 13.4% of women reported that used levonorgestrel containing intrauterine system and 3 women (1.61%) used vaginal ring. 26.61% of the 186 women have been used not reliable contraceptive methods as condom (22%), withdrawal (2.15%), spermicides (0.53%) and calendar method (1.61%). 3.27 % of women not used any contraceptive methods during the study period. The Pearson correlation test revealed a relationship between duration of menstrual cycle and the intensity of headache pain ($p = 0.012$). The duration of oral contraceptive use was correlated with the intensity and duration of headpain ($p = 0.001$ and $p = 0.021$)

Limitations, reason for caution: Limitations of the present study was the retrospective sample collection. We are going to introduce the prospective follow-up trial to understand better the impact of different type of oral contraceptives and their impact on the headache course.

Wider implications of the findings: NA.

Study funding/competing interest(s): Funding by University(ies) – Albert Szent-Gyorgyi Clinical Centre, University of Szeged.

Trial registration number: NA.

Keywords: migraine with and without aura, bleeding pattern, hormonal contraception

MALE AND FEMALE FERTILITY PRESERVATION

P-446 Controlled ovarian hyperstimulation in cancer patients candidates for fertility preservation is not associated with changes in the Follicular Output RaTe (FORT)

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Study question: What is the follicle responsiveness to exogenous FSH, assessed by the Follicular Output RaTe (FORT) in cancer patients, candidates for fertility preservation (FP) by oocyte vitrification following controlled ovarian hyperstimulation (COH)?

Summary answer: The present findings indicate that the follicle responsiveness to exogenous FSH, as far as it is measurable by FORT, is not modified by the cancer status, in young women seeking FP using COH.

What is known already: Oocyte and/or embryo cryopreservation after COH represents an established method of FP before cancer treatment. Whether these patients have a normal ovarian capacity to respond to exogenous FSH is controversial. It is conceivable that the oncologic status may, by itself, be associated with changes in antral follicle sensitivity to FSH. The FORT may be a remarkable tool to evaluate the follicle responsiveness to FSH, independently of the size of pretreatment cohort of small antral follicles.

Study design, size, duration: From July 2013 to December 2014, we prospectively studied 71 cancer patients, aged 20–40 years, candidates for oocyte vitrification following COH (FP group). Ovarian stimulation outcome was compared with that of 86 infertile women (Control group) matched for age, antral follicle count (AFC) at baseline and starting dose of FSH.

Participants/materials, setting, methods: All patients had 2 ovaries, no previous chemotherapy, no endometriosis and underwent COH using GnRH antagonists protocols. Antral follicles were counted before FSH administration and on the day of ovulation triggering (dOT). FORT was determined by the ratio pre-ovulatory follicle count (16–20 mm) on dOT × 100/AFC at baseline.

Main results and the role of chance: By design, mean age and FSH starting dose were similar in FP and Control groups (31.5 ± 0.6 vs. 32.3 ± 0.4 years; 269 ± 9.6 vs. 246 ± 6.0 IU, *NS*, respectively). Whereas antral follicle counts on baseline were comparable in both groups (17.4 ± 1.2 vs. 16.5 ± 0.9 follicles, *NS*, respectively), after a similar total amount of exogenous gonadotropins (2931 ± 141 vs. 2583 ± 120 IU, *NS*), FORT did not differ significantly in cancer patients when compared to controls (35.8 ± 2 vs. 36.9 ± 2%, *NS*). Finally, the total number of oocytes recovered (11.6 ± 1.1 vs. 10.7 ± 0.6 oocytes, *NS*, respectively) and vitrified (8.9 ± 0.8 vs. 8.1 ± 0.4 oocytes, *NS*, respectively) was comparable in FP and Control groups.

Limitations, reason for caution: FORT presents inherent limitations. Indeed, FORT calculation assumes that small antral follicles respond in a coordinated manner to FSH and that only follicles having reached diameters ranging between 16 and 20 mm on dOT effectively responded to FSH. In addition, the sample size may limit the generalization of these findings.

Wider implications of the findings: Further investigation is needed to assess the FORT according to the type of malignant diseases, and their impact on the general condition of patients.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Jean Verdier Hospital.

Trial registration number: NA.

Keywords: fertility preservation, oocyte vitrification, cancer, FORT

P-447 Oocyte cryopreservation in consecutive Natural Cycles in older women increase their chances of achieving a pregnancy

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Study question: Does collecting and cryopreserving oocytes with natural cycles (NC) and performing a subsequent thaw cycle help >38 years old women achieve a pregnancy.

Summary answer: Implantation and clinical pregnancy rates recorded in this study demonstrate that storing oocytes and performing a subsequent thaw cycle seems to be beneficial for this group of women.

What is known already: It is estimated that the NC costs 75–80% less than a stimulated cycle and can be safely used to collect and store oocytes for later use with IVF to prevent fertility decline due to age. Using vitrification as a cryopreservation procedure, oocytes can be stored and thawed with high survival rates and good pregnancy results.

Study design, size, duration: This is a retrospective study, from January 2011 until December 2014, including women performing NC and subsequent freezing oocytes, aged more or equal than 38 years old.

Participants/materials, setting, methods: All 174 women participated in this study performed NC and collected oocytes in order to freeze them and plan a subsequent thaw cycle. Vitrification was performed to store one oocyte in separate canes. When thawed, survived oocytes were fertilized using ICSI and embryo transfer (ET) was performed 3–5 days post thaw.

Main results and the role of chance: A total of 516 oocytes were frozen and stored for later use out of 174 patients using NC. Out of these, 34 women (mean age being 41.0) decided to perform an ET, using their vitrified

oocytes, 106 oocytes were thawed and 79 survived giving a survival rate of 74.5%. Following fertilization with ICSI, 69 oocytes were fertilized (87.3%) and 67 of them were transferred, with a mean number of embryos per ET equal to 1.97. Pregnancy was achieved in 11 of them (32.4%) with an implantation rate of 17.9%.

Limitations, reason for caution: Since there are a lot more cases of women with frozen oocytes performing thaw and embryo transfer in the following months, in our Unit, we intent to continue this study in order to increase the number of patients and compare survival rates and success rates to matched stimulated cycles.

Wider implications of the findings: NC can be a reasonable alternative. The benefits are, more friendly approach, significantly less cost, avoidance of anesthesia and oocyte freezing with NC can be suggested in older women with good success rates.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Embryogenesis IVF Unit, Greece.

Trial registration number: NA.

Keywords: natural cycle, oocyte cryopreservation

P-448 The Irinotecan metabolite SN38 is highly cytotoxic to germ cells in the mouse testis but has little ovotoxicity

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Study question: Does SN38, the most potent Irinotecan metabolite, affect germ cells in male and female mouse gonads when exposed to concentrations equivalent to patient plasma range?

Summary answer: Germ cells within the testis are much more sensitive to SN38 than those in the ovary. Male germ cells were present in significantly reduced numbers when exposed to concentrations equivalent to patient plasma range, whilst the ovary was largely unaffected even when exposed to much higher concentrations.

What is known already: Irinotecan is used therapeutically for several tumour types, including paediatric tumours. Irinotecan is metabolised into several compounds, of which 7-ethyl-10-hydroxycamptothecan (SN38) is the most important, displaying stronger cytotoxic action than Irinotecan itself. Plasma concentration of SN38 in patients ranges between 10 and 100 ng/ml within the first 10 h after administration. Long-term side effects on fertility of this drug are not well described.

Study design, size, duration: The study involved culture of neonatal ovarian or testicular tissue for four days, with tissue exposed to varying concentrations of SN38 on Day 2 of culture.

Participants/materials, setting, methods: Whole ovaries from pnd (postnatal day) 4 mice and fragments of testis from pnd 5 mice were placed in serum-free culture media. On Day 2, a range of concentrations of SN38 were added to the media for 24 h (0.002–1 μg ml^{-1} for testes and 0.1–5 μg ml^{-1} for ovaries), with subsequent culture in drug-free medium for a further 2 days. Male germ cells were identified by expression of MVH (mouse vasa homologue), whilst ovarian follicle counts were assessed histologically. SN38-exposed tissues were compared with vehicle- (DMSO-) exposed controls; results are shown as mean ± SEM.

Main results and the role of chance: The pre-spermatogonial population decreased dramatically in SN38-exposed testes, with significantly lower MVH-expression levels than controls. Compared to the control group ($n = 7$), MVH-expression was reduced to: 44 ± 10% of control levels in 0.002μg ml^{-1} ($n = 5$; $p < 0.01$), 25 ± 7% of control levels in 0.01 μg ml^{-1} ($n = 4$; $p < 0.005$), 19 ± 7% of control levels in 0.1 μg ml^{-1} ($n = 7$; $p < 0.001$), and 5 ± 1% of control levels in 1 μg ml^{-1} ($n = 3$; $p < 0.001$). In marked contrast, SN38 exposure did not significantly affect ovarian follicle numbers, even at high concentrations. Follicle numbers were: 1257 ± 215 in 100 ng ml^{-1} ($n = 10$), 627.4 ± 107 in 1 μg ml^{-1} ($n = 8$), 771.9 ± 76 in 2.5 μg ml^{-1} ($n = 12$) and 1051 ± 143 in 5 μg ml^{-1} ($n = 12$), with treated groups not significantly different to the 1134 ± 160 follicles in the control group ($n = 10$).

Limitations, reason for caution: As for any animal *in vitro* model, caution is necessary for extrapolation of the findings to humans.

Wider implications of the findings: Our data demonstrate that male germ cells are particularly sensitive to SN38, even at concentrations lower than those typically found in the plasma of treated patients. This is of particular concern for pre-pubertal male cancer patients, for whom there is currently no option to protect or restore fertility in the case of gonadal damage caused by cancer therapy.

Study funding/competing interest(s): Funding by national/international organization(s) – Medical Research Council UK grant G1002118.

Trial registration number: NA.

Keywords: chemotherapy, ovary, testis, tissue culture

P-449 What is the best protocol to cryopreserve testicular cell suspensions?

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Study question: Establishing a successful method for testicular cell cryopreservation would be of immense benefit to patients undergoing sterilizing treatments. In view of a clinical application (CA), what would be the best protocol to cryopreserve and store testicular cell suspensions?

Summary answer: Our best protocol to cryopreserve immature mouse testicular cell suspensions (TCS) was found to be a non-controlled freezing procedure performed in an insulated container using vials with a cooling rate of 1°C/min till -80°C. It resulted in an average of 50% recovery of viable cells re-stablishing spermatogenesis in 75% of the transplanted testis.

What is known already: Uncontrolled-slow-freezing is the endorsed method to cryopreserve spermatogonial stem cells (SSCs), reaching 68.3 ± 2.8% post-thaw viability after purification, proliferation and freezing of calf type A SSCs permitting a repopulation efficiency of 43% after transplantation (Izadyar et al., 2002*)*. However, in perspective of a CA, an effective protocol for adult human testicular tissue is already established (Baert et al., 2013)*,* for TCSs, only 55.3 ± 23.8% post-thaw viability has been reported after performing vitrification (Sà et al., 2012), therefore, further improvement is needed.

Study design, size, duration: Three groups of samples ($n = 84$) enclosing five conditions were compared. The first group compared two controlled-slow freezing (CSF) protocols and vitrification. A second evaluated the combination of permeating and non-permeating cryoprotective agents (CPAs) and a third compared two commercial vessels used in cryopreservation. After thawing, TCS were transplanted to recipient mice ($n = 46$) for further functional assay.

Participants/materials, setting, methods: Donor prepubertal SV129xC57BL (GFP+) mice testes were digested to obtain TCSs. These TCSs were frozen under different conditions and thawed in a 37°C water bath. The number and viability of cells was counted immediately after digestion (fresh), after adding CPAs and after thawing. Thawed cells were transplanted to recipient infertile mice for functional assay.

Main results and the role of chance: Our results were compared in terms of the percentage of recovered viable cells and number of positive tubules after transplantation. Both CSF procedures did not differ significantly (41 ± 37% and 38 ± 25%), thus we opted for the best time- and cost-efficient procedure. Adding a non-permeating CPA (sucrose) did not result in a higher viable cell recovery (41 ± 26%), although, post-thaw cell viability presented no differences. When assessing different vessels, cells frozen in vials recovered better than those frozen in straws ($P = 0.046$) permitting to reach 50 ± 25% recovered viable cells. Testes transplanted with cells frozen-thawed in vials presented an average of 67 ± 6 positive tubules per 10^5 cells injected with 75% (6/8) of the transplanted testes having re-stablished spermatogenesis.

Limitations, reason for caution: Since human testicular tissue is scarce, the present protocol was developed in the mouse model. As the paramount perspective of this study is the translation of this protocol to human testicular cell suspensions, supplementary research is required in order to develop xeno-free cryopreservation media.

Wider implications of the findings: An optimal cryopreservation protocol enhances the chances for successful fertility restoration. TCS cryopreservation

is an option to preserve fertility in patients for whom sperm freezing is not an option (e.g., prepublertal boys undergoing gonadotoxic treatments). So far, cryopreservation protocols for TCSs in the human model are still suboptimal, thus, developing an easy, reproducible and cost-effective cryopreservation method is of dire need.

Study funding/competing interest(s): Funding by national/international organization(s) – European FP7 – Marie Curie Actions-Initial Training Network (ITN) Grants – 2014. Flammish league against cancer VLK.

Trial registration number: NA.

Keywords: fertility preservation, germ cells, male infertility, spermatogonial stem cell transplantation, immunohistochemistry

P-450 A 7 year follow-up of multinational counselling of patients prior to chemo- or radiotherapy – an analysis of >5,000 consultations

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Study question: What are the main reasons for the decision making of individual patients to opt for different fertility preservation techniques? Which patients decide to perform multiple fertility preservation techniques to increase their chance for later conception? Did the tendency to perform individual fertility preservation techniques change over the years?

Summary answer: Fertility preservation has become increasingly important as cancer treatments have improved significantly and the 5-year survival rate for the most common premenopausal cancers are above 85%. Therefore, being able to conceive after survival of cancer treatment has become an important part of quality of live for women.

What is known already: Fertility preservation has become increasingly important as cancer treatments have improved significantly and the 5-year survival rate for the most common premenopausal cancers are above 85%. Therefore, being able to conceive after survival of cancer treatment has become an important part of quality of live for women.

Study design, size, duration: An anonymous retrospective analysis from 2007 to 2013 was performed with 5,159 patients counselled for fertility preservation procedures before chemo- or radiotherapy in 39 (2007) to 85 (2013) university and non-university centers, being part of the network FertiPROTEKT (www.fertiprotekt.com). Of these, 4,060 women received fertility preservation therapies (78.7%).

Participants/materials, setting, methods: The fertility preservation offered included GnRH-antagonists, ovarian stimulation and ovarian cryopreservation. The individually chosen therapy was analysed according to: indication for consultation (cancer/benign disease), setting of consultations, patients' age, marital state, prior children, the number of fertility preservation techniques chosen. Data were analysed with ANOVA followed by SNK post-hoc test.

Main results and the role of chance: Fertility preservation counselling in young women receiving gonadotoxic therapies increased significantly over the years. Most of the counselled patients were childless women under 35 years. The frequency of techniques used changed from GnRH agonist to more invasive treatments like ovarian stimulation and ovarian cryopreservation from 2007 to 2013. The most common reasons for consultation were breast cancer (preferring cell and tissue cryopreservation) and lymphomas (using predominantly GnRH agonist therapy). While women between 20 and 40 years chose all kind of treatment options, younger women (<20 years) predominantly received GnRH agonists and tissue cryopreservation, older women (>40 years) GnRH agonists only. As expected the average number of aspirated oocytes per stimulation cycle decreased with age.

Limitations, reason for caution: Data about pregnancy rates following the individually performed fertility preservation techniques are missing, therefore the efficacy of the chosen therapy cannot be assessed. Only the decision making process could be analysed and correlated to the individual patients characteristics.

Wider implications of the findings: Due to increasing scientific progress more invasive techniques for fertility preservation are chosen by German speaking women, with the hope to further improve their chance of later conception. The fertility preservation options, including usage of GnRH-antagonist, ovarian stimulation and cryopreservation of ovarian tissue however still depend on time available until cancer treatment needs to be initiated, in order not to postpone crucial therapy.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by the participating centers. No competing interests.

Trial registration number: NA.

Keywords: fertility preservation, decision making process, changes over time

P-451 Safety assessment of cryopreserved ovarian tissue transplantation in breast cancer patients by molecular techniques and in vivo study

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Study question: The aim of this study was to develop an in vivo breast cancer model to validate the sensitivity and specificity of a developed molecular novel panel, for the detection of disseminated cancer cells (DCC), in ovarian cortex (OC) and medulla (OM) from breast cancer (BC) patients, which improve the security of ovarian tissue transplantation (OTT).

Summary answer: These findings confirm the sensitivity and specificity of molecular tools for the detection of DCC in OC and OM from BC patients regarding to histological methods and corroborate that the OTT in BC patients is a safe technique to preserve the fertility.

What is known already: We developed a novel molecular markers panel to increase the sensitivity and specificity of DCC detection in ovarian tissue (OT) from BC patients. After analysed of 60 OC and OM from we were able to confirm the absence of malignant cells in OT from these patients. However, although these methods provided good sensitive and sensible results, it is essential to be validated by an in vivo model.

Study design, size, duration: A total of 500.000 cells were injected in left renal capsule of nude mice divided in 3 experimental groups: A) BC cell-line MDA-MB-468 (n = 1,positive control), B) OC and OM cells from 5 BC patients (stages:I–IIIa; n = 10) and C) healthy ovary from caesarean (n = 1,negative control). Animals were sacrificed at 6 months.

Participants/materials, setting, methods: OT and MDA-MB-468 cells were cultured and transfected with fluorescent protein mCherry. Metastatic development was monitored by In-Vivo Image System (IVIS). Mammaglobin-1(MGB1), Gross Cystic Disease Fluid Protein-15 (GCDFP15) and Small Breast Epithelial Molecule (SBEM), were analysed by immunohistochemistry and RT-PCR in all samples before mice transplantation and in each organ´s mice after sacrificed.

Main results and the role of chance: Mice with OC and OM cells from BC patients and healthy ovary cells did not present any suspicious metastatic signal by IVIS study or tumour development after 6 months. Morphological, histological and molecular analysis of the different organs confirmed the absence of DCC. The GCDFP15, MGB1 and SBEM expression were absent in right/left adrenal gland, liver, kidney, pancreas and spleen. However, the positive control mouse, which received a cancer cell line injection, developed palpable tumour masses

of 2.5 cm. SBEM expression in positive control mouse was detected in tumour masses (ΔCt = 11,92), right adrenal gland (ΔCt = 11.98), spleen (ΔCt = 4.21) and pancreas (ΔCt = 7.5) corroborating the presence of infiltrating malignant cells in these mouse´s organs which had been previously detected by IVIS at day 104 post-injection.

Limitations, reason for caution: In this study we estimated, based on reported cancer models, that the injection of 500.00 cancer cells was enough to developed early metastasis. We do not know if a lower cell dilution could develop an early tumour mass.

Wider implications of the findings: This study validates, by in vivo model, a final panel with the most accurate metastatic markers for using it as diagnostic tool of micrometastases in OC from BC patients and corroborates the safety of OTT from BC patients. This study also shows for the first time that the OM from BC patients is free from malignant cells. The IVIS provides a non-invasive method for malignant cells in situ detection without sacrifice the mouse.

Study funding/competing interest(s): Funding by national/international organization(s) – This work was funded by the Spanish Government, grants FIS-PI13/02353, GV/2014/115, AP-2010-0675 and CD11/00292.

Trial registration number: NA.

Keywords: ovarian cortex cryopreservation, breast cancer, ovarian metastases, xenograft

P-452 Ex vivo tumour purging of ovarian cortex fragments to enhance the safety of ovarian tissue autotransplantation

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Study question: Ovarian cortex tissue of cancer patients that is cryopreserved for reasons of fertility preservation, may contain metastasized tumor cells that may lead to reintroduction of the malignancy after autotransplantation of the ovarian tissue. Is it possible to purge these metastasized cancer cells from the ovarian cortex tissue fragments?

Summary answer: Using various types of ex vivo chemotherapy, we were able to efficiently eliminate experimentally induced small tumours derived from leukaemia and breast cancer cells from ovarian tissue fragments.

What is known already: Autotransplantation of ovarian tissue fragments that contain metastasized tumour cells may reintroduce the malignancy to the recipient. Tumour cells have been detected in human ovarian tissue fragments, and transmission of the disease via transplantation of these contaminated fragments has actually been demonstrated for ALL (human) and lymphoma (mouse).

Study design, size, duration: Tumours of breast cancer and leukaemia were experimentally induced in ovarian cortex tissue fragments and cultured for 4 days. The inoculated tissue fragments were subsequently treated ex vivo with various regimens of chemotherapy, and the effect on the cancer cells and ovarian tissue was monitored.

Participants/materials, setting, methods: Experiments were performed with ovarian tissue obtained after prophylactic salpingo-oophorectomy in BRCA carriers. Bovine tissue was derived from an abattoir. Human cancer cell lines were purchased from various sources. The efficiency of chemotherapy (doxorubicin, cis-platin or Imatinib) on tumour elimination in ovarian tissue ex vivo was assessed using (immuno) histochemistry.

Main results and the role of chance: Our results show that purging of metastasized leukemia or breast cancer cells in ovarian cortex fragments by *ex vivo* chemotherapy is feasible. Treatment of contaminated fragments with either widely used chemotherapeutics or tumour-specific kinase inhibitors, showed mitotic arrest and large scale necrosis of the tumour cells. Histological examination revealed no deleterious effect of the purging procedure on the ovarian tissue. Our results using the kinase inhibitor Imatinib are especially relevant. As this compound is highly tumour specific, it is likely that the follicles, oocytes and the ovarian stromal will not be negatively affected by the *ex vivo* treatment. In addition, the severe side effects that are observed when Imatinib is administered systemically, will obviously not occur when applying *ex vivo* treatment to tissue fragments.

Limitations, reason for caution: The tumour model we used is based on the growth of human cancer cell lines. It is unknown whether these cells behave differently from malignant cells derived from primary tumours. Furthermore, the condition of the ovarian tissue after *ex vivo* chemotherapy requires further structural and functional investigation.

Wider implications of the findings: Our results indicate that it is possible to efficiently purge contaminating cancer cells from ovarian cortex fragments intended for fertility preservation purposes. Optimizing purging procedures will greatly increase the safety of ovarian cortex cryopreservation for cancer patients. Furthermore, by using these purging procedures ovarian cortex cryopreservation might also become a viable fertility preservation option for cancer patients suffering from types of malignancies for which ovarian cortex autotransplantation is currently considered unsafe.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Unconditional funding was received from Ferring pharmaceuticals.

Trial registration number: NA.

Keywords: female fertility preservation, safety, ovarian metastases, tumour purging, autotransplantation

P-453 Gonadotoxic chemotherapy agents impact ovarian stroma and vasculature

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Study question: Are gonadotoxic chemotherapy agents detrimental to ovarian vascular endothelium and stroma?

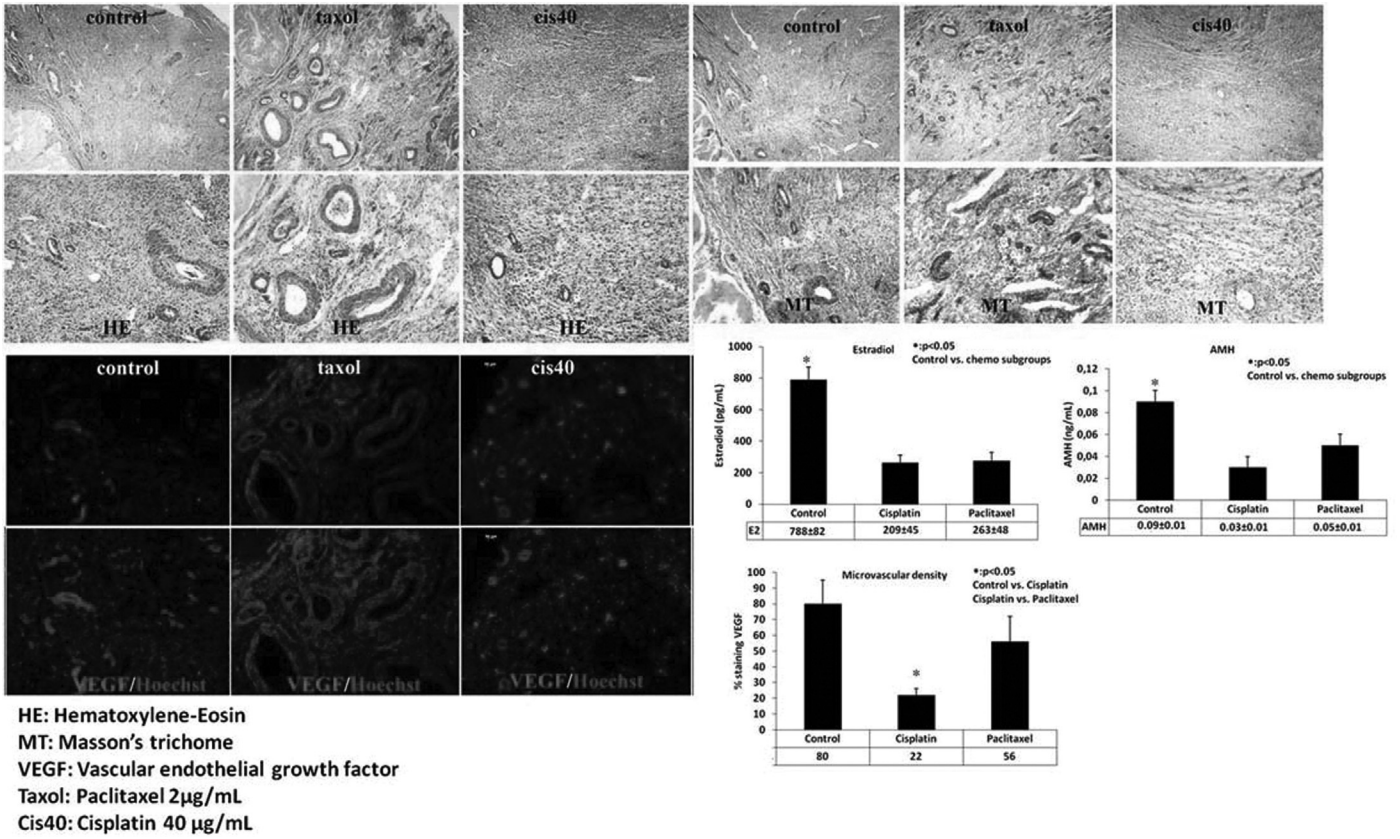
Summary answer: Yes, cisplatin, a chemotherapy agent with a high gonadotoxic potential causes a more extensive destruction in the ovarian stroma and vascular structures compared to control and less gonadotoxic chemotherapy drug paclitaxel.

What is known already: It is well known that massive and accelerated apoptotic death of the follicles is the main pathogenetic mechanism in chemotherapy related premature ovarian failure and ovarian aging in human. There is some evidence albeit not well substaintiated that chemotherapy induced damage in the microvascular endothelium and stroma may contribute to ovarian failure and aging in human. We therefore in this study analyzed the impact of two different chemotherapy agents on the microvascular structures and stroma in human ovary.

Study design, size, duration: An vitro cytotoxicity model of human ovary.

Participants/materials, setting, methods: Ovarian cortical pieces (n = 7, age 24-34) were obtained from patients undergoing laparoscopic ovarian cyst excisions for benign reasons. The samples were cultured for 24 hrs in 24 well plate using DMEM-F12 culture media supplemented with 10%FBS. Cisplatin (40mg/mL) and paclitaxel (2000ng/mL) were used at concentrations corresponding to their therapeutic blood levels. After culture, the ovarian samples were fixed, cryosectioned and stained with H&E, Masson's trichome, VEGF and Hoechst 33342 for examination of ovarian stroma and vasculature. The supernatants of cultured samples were assayed for estradiol and AMH productions by ELISA.

Main results and the role of chance: Ovarian cortical samples treated with cisplatin and paclitaxel produced significantly less E2 and AMH compared to control. The reduction in E2 and AMH productions were more pronounced in the cisplatin treated group compared to paclitaxel. Control ovaries preserved well their structure after 24 hr culture period. Ovarian stroma was stained uniformly HE and MT with many interstitial cells and easily identified microvascular structures. By contrast, the samples treated with cisplatin were characterized by a less cellular, more fibrotic stroma with a marked disarray of the cells and extracellular matrix. Interstitial cells were sparser. The histomorphological changes in ovarian stroma and vasculature in paclitaxel treated samples were



HE: Hematoxylene-Eosin
MT: Masson's trichome
VEGF: Vascular endothelial growth factor
Taxol: Paclitaxel 2µg/mL
Cis40: Cisplatin 40 µg/mL

less remarkable compared to cisplatin. Stroma and vascular structures were preserved to some point. The microvascular density was significantly reduced in the cisplatin group compared to control and paclitaxel (Figure).
Limitations, reason for caution: None
Wider implications of the findings: These results support the view that chemotherapy induced damage in ovarian stroma and vasculature may have additional roles in premature ovarian failure and aging.
Study funding/competing interest(s): Funding by University(ies) – Koc University School of Medicine and the Graduate School of Health Sciences, American Hospital Comprehensive Cancer Care and Fertility Preservation Programs, Istanbul Turkey.
Trial registration number: NA.
Keywords: ovary, chemotherapy, stroma, vascular damage, VEGF
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P-454 Nano-porous microtube array membranes loaded with sphingosine-1-phosphate promote ovarian graft survival: a cross-field in vivo transgenic mouse study

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Study question: Do ovarian grafts survive better with nano-porous microtube array membranes (MTAMs) loaded with sphingosine-1-phosphate (S1P)?
Summary answer: Nano-porous MTAMs loaded with S1P promote ovarian graft survival proved by in vivo bioluminescence imaging (BLI).
What is known already: Ovarian tissue cryopreservation and autotransplantation is a promising option for fertility preservation of female cancer patients. However, the post-transplantation window of ischemia limits the life span of the ovarian grafts. S1P can protect ovarian grafts from ischemic reperfusion injury. The biocompatible scaffolds can be served as connections between the host and grafts and as vehicles for drug delivery to improve the graft survival.
Study design, size, duration: Eight-week-old wild-type mice were transplanted with ovaries from age-matched FVB/N-Tg(*PolII-Luc*)Ltc transgenic mice with or without MTAMs loaded with S1P under the back skin of either side. All animal were sacrificed 5 weeks later.
Participants/materials, setting, methods: Poly-L-lactic acid (PLLA) MTAMs with a nano-porous wall structure were prepared by electrospinning. S1P (2 mM, 5 µL) was loaded into the MTAMs. Ovaries with or with MTAMs were transplanted under the back skin, and the graft survival was tracked in vivo by BLI for 5 weeks.
Main results and the role of chance: Histological examination showed larger size of the ovaries encapsulated by MTAM loaded with S1P with more surrounding vessels compared with those without scaffolds. In vivo BLI also demonstrated stronger intensity of bioluminescence from the ovaries with MTAM loaded with S1P, indicating better survival of the grafts. Nano-porous scaffolds made of biocompatible and biodegradable PLLA biomaterial provided good connection between the graft and host.
Limitations, reason for caution: The effective dosage and the role of S1P should be identified. We used nanotechnology in this study to fabricate delicate scaffolds to promote tissue survival. Although the results are promising, the effect of nanostructure on the tissue still needed to be investigated.
Wider implications of the findings: Scaffolds mimicking the structure and biological function of native extracellular matrix are beneficial for tissue growth. Multi-interdisciplinary approaches bring together chemistry, pharmaceutical science, biology, and medicine to design biodegradable biomaterials for their biological, medical, and pharmaceutical applications. Applying nanotechnology may overcome many limitations in regenerative medicine.
Study funding/competing interest(s): Funding by national/international organization(s) – The Ministry of Science and Technology, Taiwan, R.O.C.
Trial registration number: MOST 103-2321-B-038-008.
Keywords: ovarian transplantation, fertility preservation, scaffolds, tissue engineering, sphingosine-1-phosphate

P-455 Expression of angiogenic factors in cryopreserved mouse ovaries after heterotopic autotransplantation

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Study question: This study was aimed at investigating the effects of cryopreservation techniques (vitrification and slow-freezing) on ovarian expression of angiogenic factors by determining the levels of VEGF and angpt-2 in successful heterotopic ovarian autotransplants of mice.
Summary answer: In ovarian tissue, expression of angiogenic factors is thought to vary by method of cryopreservation. Despite declines in follicular densities due to necrosis, angiogenic factors rose to similar levels after ovarian autotransplantation, regardless of cryopreservation technique.
What is known already: Ovarian tissue banking is a promising option for preserving fecundity in young female cancer patients facing sterilization by chemotherapy and/or radiotherapy. However, the limited functional duration of some nonvascularized ovarian grafts may be due in part to ischemic injury sustained until revascularization is adequate. Revascularization may thus be a critical step in successful ovarian tissue transplantation. Vascular endothelial growth factor (VEGF) and angiopoietin-2 (angpt-2) are the principal mediators of neovascularization.
Study design, size, duration: Design: Prospective study Size: Twenty ICR mice aged 5 or 6 weeks old were sacrificed, and their ovaries were placed in sterile dish. Duration: Autotransplant be done one week after cyropreservation. After two weeks later, autotransplanted ovaries were evaluate for angiogenic factors.
Participants/materials, setting, methods: Ovarian tissues harvested from ICR mice at 5-6 weeks of age were stratified as follows: 1) no cryopreservation (control, group I), 2) vitrification in VFS-40 (vitrification, group II), and 3) gradual freezing in DMSO (slow-freezing, group III). Frozen specimens were thawed at room temperature, assaying VEGF and angpt-2 levels 1 week after cryopreservation and 2 weeks after autotransplantation.
Main results and the role of chance: VEGF and angpt-2 protein levels were significantly lower in cryopreserved ovaries of groups II and III than in controls (group I; $p < 0.05$), whereas groups II and III did not differ significantly in this regard. After autotransplantation, protein levels were similar in all groups, although primary and antral follicular densities of autotransplanted ovaries were again lower in groups II and III than in group I ($p < 0.05$) and did not differ significantly by cryopreservation technique.
Limitations, reason for caution: Since the experimental animal clinical application is limited. The activation of vascular factors do not correlate with the success of the ovary transplant. Although we have optimized freeze/thaw and autotransplantation procedures, not all ovaries so-treated regained their function, and a certain portion became atretic. This suggests that additional improvements in methodology are in order to enhance ovarian tissue survival.
Wider implications of the findings: We hope to improve the success rate of ovarian tissue transplantation after cryopreservation using pretreatment angiogenic factors.
Study funding/competing interest(s): Funding by University(ies) – Gyeongsang National University Hospital.
Trial registration number: NA.
Keywords: ovary, cryopreservation, vascular endothelial growth factor

P-456 Metabolites of cyclophosphamide seem to activate dormant human primordial follicles in culture

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Study question: How do cyclophosphamide active metabolites [4-hydroxy-cyclophosphamide (4hc) and phosphoramide mustard (PM)], affect dormant human primordial follicles in culture?
Summary answer: Cyclophosphamide metabolites seem to cause enhanced, uninhibited activation of primordial follicles in culture, thus "burning out" the ovarian reserve.
What is known already: The mechanism chemotherapy affects quiescent primordial follicles is unclear. Apoptosis and damage to follicular structure and vascular support are suggested mechanisms, but they have not been consistently proven. The "follicular burnout" theory suggests that chemotherapy targets developing follicles with high mitotic activity. Their removal results in lack of inhibition inducing continuous recruitment of ovarian primordial follicles, thus "burning out" the ovarian reserve. So far, there is no proof of the "burn out" theory in humans.
Study design, size, duration: Frozen-thawed ovarian tissue samples obtained from 10 women, aged 12 ± 5 years, undergoing ovarian cryopreservation were sliced. One slice was fixed immediately [uncultured controls] and other slices were cultured with basic culture medium [cultured controls] or with 4hc/PM (3mM, 10mM) [treated samples] for 24-48 hrs.
Participants/materials, setting, methods: Sliced samples were removed from culture every 24hrs, and spent media were collected at 48hrs. Evaluation of histological slices included follicular counts and classification, Ki67 immunohistochemistry and an apoptosis assay. In addition 17β-estradiol (E2; radioimmunoassay) and antimullerian hormone (AMH; enzyme-linked immunosorbent assay) were measured in spent media samples.
Main results and the role of chance: Samples treated for 48hrs had a significantly lower primordial follicle ratio (20-32%) in parallel with a significantly higher primary/secondary follicle ratio (50-55%) compared with cultured untreated controls (48% and 32%, respectively). At 24hrs these contrasts were less pronounced, and no differences were found between the two concentrations. Most atretic follicles in treated samples were primary/secondary. E2 and AMH levels were more than doubled in treated samples, compared with untreated. There were no traces of apoptosis in cultured follicles including those treated. Most activated follicles (80-100%), including treated samples, were stained positively for ki67 in granulosa cells. Role of chance: as we used organ culture, follow up of the same follicles was impossible, and different follicles were evaluated histologically at various levels of sample.
Limitations, reason for caution: The study included a limited specimen number that was cultured for 48 hrs. We, therefore, could not identify changes occurring beyond this period. Moreover, the organ culture technique does not enable a follow up of the same follicle at all culture time points (uncultured, 24 hrs, 48 hrs).
Wider implications of the findings: It seems that cyclophosphamide metabolites cause enhanced, continuous, uninhibited primordial follicle activation *in vitro*. Our report supports the "burnout theory" as the cause of cyclophosphamide follicular toxicity in humans. Yet, the mechanism by which cyclophosphamide metabolites cause uncontrolled recruitment of primordial follicles was not investigated, and further experiments should be conducted.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Infertility and IVF Unit, Beilinson Women Hospital, Rabin Medical Center.
Trial registration number: 5875, 0100-10-RMC.
Keywords: in vitro culture, cyclophosphamide metabolites, follicular burnout, anti-mullerian hormone, 17ß-estradiol

P-457 A single centre's experience of fertility preservation services: access to care and patient outcome

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Study question: To assess the fertility preservation service including reasons for referral, access and the corresponding outcomes of these patients following controlled ovarian stimulation (COS) as well as identify the challenges associated with developing a streamlined oncofertility service.
Summary answer: Over a 4 year period, 129 patients were referred for the purpose of fertility preservation and of these 42% were seen within a week of referral. Nearly 80% ($n = 103$) of referrals were for women diagnosed with cancer awaiting treatment and of these 30% did not proceed with treatment.
What is known already: Although national guidance recommends all patients should have access to this service, practice across the UK is variable and recent surveys suggest rate of referral of patients is poor. There is evidence from US models that a structured oncofertility program is successful in not only improving access for patients but also in providing adequate support. Some specific

challenges in COS in these patients include concerns in relation to oestrogen sensitive cancers and minimising OHSS.
Study design, size, duration: Retrospective cohort study reviewing a four year period of data in a large fertility clinic in Mersey UK
Participants/materials, setting, methods: participants were all patients referred for fertility preservation over a four year period. Data collection was retrospective interrogation of patient records.
Main results and the role of chance: There was poor adherence to national guidelines for referral for fertility preservation as evidenced by the small number of referrals in relation to both the population size served and the prevalence of cancer in females of reproductive age in the area. Of the referrals received, 80% (n = 103) were for cancer. The remaining cases related to patients at risk of losing their ovaries or requiring chemotherapy for reasons other than malignancy. Of the referrals received, 42% were seen within a week of referral and treatment was completed within four weeks. The average oocyte yield in the cancer group was 10 which was significantly higher than those requiring fertility preservation for reasons other than malignancy and OHSS rates were low.
Limitations, reason for caution: This is the experience of a single centre so extrapolating to the national or European level is hampered by loco-regional characteristics and population attributes. A multi-centre study is needed to identify problems with accessing care and assessing ovarian response to stimulation in these women. It will also inform on use of currently experimental methods for fertility preservation such as ovarian tissue cryobanking but also help to determine long term risk in hormone sensitive cancers.
Wider implications of the findings: A structured oncofertility program is necessary to provide optimum care for patients as well as regular updates for clinicians looking after patients with regards to available fertility preservation options. The high rate of cancellation may be related to fear of treatment and lack of appropriate counselling. This is a rapidly expanding area which requires robust practice guidelines and further research to optimise current fertility preservation techniques as well as establish experimental techniques into routine practice.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Liverpool Women's Hospital NHS trust.
Trial registration number: NA.
Keywords: fertility preservation, cancer, ovarian stimulation, premature menopause

P-458 Sperm banking in male oncological patients over 17 years' experience

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Study question: To evaluate the role of sperm cryopreservation in the fertility preservation of oncological male patients, regarding characterization of patients and semen parameters, as well as outcomes of thawed samples and intentions of storing after the legal period (3 years).
Summary answer: Sperm cryopreservation is a valid methodology of fertility preservation, besides the low number of patients requering it. Testicular tumor was the main indication for sperm banking and, also, presented the worse semen quality. The majority of men wanted to keep their samples stored for a additional 3 years period.
What is known already: The survival rates in adult oncological males have increased due to the success of multimodal therapies and new diagnostic techniques, reason why sperm banking should be offered to all men before cancer treatment due to gonadal damage risk. Some men may return their normal sperm production after termination of cancer treatment.
Study design, size, duration: From 1997 to 2014 December 93 oncological men were advised preserve their fertility. Cancer indications were grouped as testicular (TT), hematological (HT) and other (OT).
Participants/materials, setting, methods: Routine standard sperm or testicular tissue cryopreservation and thawing tecnniques were realized. Phone contact was established to inquire about the destination of sperm samples stored for more than 3 years.
Main results and the role of chance: 93 oncological men (27,2 ± 6,3 years; range 16-43) were referred to sperm banking. 64,5% had TT, 14% HT and 21,5% OT. Only 3 had prior biological children. 6 had known fertility problems. 89 men had their sperm cryopreserved (3 negative testicular biopsy and 1 desistance). The mean sperm concentration was lowest on TT (19,9 ± 0,5x10^6/ml *vs* 54,8 ± 78,8x10^6/ml on HT and 35,1 ± 36,5x10^6/ml on OT; P < 0,005). Only 5 required thawing to ART techniques (5,6%), had realized 11 FIV/ICSI cycles with one pregnancy. From the 52 men contacted after the initial legal period, 2 died and 7 didn't answer. Among the 43 answers, 3 achieved impregnation without banked sperm (7%). When asked about the future of their samples, 4 required destruction (9,3%) and 39 opted for storing maintenance (90,7%).
Limitations, reason for caution: One limitation is the relative low number of men who answered the questionnaire.
Wider implications of the findings: Although the number of patients with a valid sample for cryopreservation was high, only a minority requested the use of their frozen samples.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: cryopreservation, sperm banking, fertility preservation

P-459 Introduction of social network analysis into the field of fertility preservation

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Study question: Can a social network analysis (SNA) help to get insight into professional networks and clinical referral patterns for fertility preservation counselling (FPC)?
Summary answer: A SNA can be performed among healthcare providers in multidisciplinary oncological care with a good response rate (61%) to the questionnaire. Furthermore, this methodology identifies the 'key figures' in a professional network and professional relationships on which healthcare providers could work on to improve fertility preservation (FP) care.
What is known already: To enable good decision-making about FP, clinical guidelines advise referral of female patients for FPC. However, the adherence to this recommendation is poor. To improve the quality of FP care by improving the FPC referral rates, identification of all barriers is necessary. Since quality of multidisciplinary collaboration in oncological care may play a role in these referral rates. Therefore, we aimed to analyse the patterns in multidisciplinary care and professional networks by a SNA.
Study design, size, duration: A cross-sectional study by questionnaire was conducted among oncological healthcare providers in a demarcated region around a university hospital in the Netherlands, where FPC is performed. Healthcare providers answered questions about baseline characteristics, their FP practices, their attitude towards FP and their collaborations with others in the professional network.
Participants/materials, setting, methods: Doctors and nurses (n = 189) involved in the oncological care of the top three cancers in women aged 18-40 years old (haematological/lymphoid cancers, breast cancer, gynaecological cancers) received our questionnaire by e-mail. By conducting a SNA, the oncological healthcare providers' place within the network was calculated using the software program UCINET.
Main results and the role of chance: Eight hospitals (including a university hospital) and one radiotherapeutic centre were included based on their location within 35 km from our university FP centre. A total of 116 healthcare providers (61%) responded to our questionnaire. Visualization of the total network showed that usually oncologists can be seen as key figures in the network. The density of the professional networks for the individual hospitals ranged from 0.49 to 0.91 (on a 0-1 scale). The average minimum distance for the total network to reach one of the gynaecologists involved in FP, expressed as number of steps needed, was 1.55, ranging from 1.24 to 2.00. The two most 'central' gynaecologists were in direct contact with 23% and 36% of all oncological healthcare providers.
Limitations, reason for caution: The selection of hospitals based on the distance, may have caused selection bias. However, these hospitals were responsible for referring 82% of all our patients in the past. Collaboration with other university hospitals for FPC was not taken into account and may have altered the calculations of the total network.

Wider implications of the findings: SNA was helpful to, not only give us insight in the key figures in our multidisciplinary oncological healthcare network, but also to show us which relationships between FP specialists and others need to be improved to possibly increase FPC referral rates. These key figures can be used to spread knowledge from the FP specialist to the other healthcare providers, or they can function as dedicated healthcare providers who can answer questions and give advice.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This work was supported by the Radboud Institute for Health Sciences (research school affiliated to the Radboud university medical center). The authors have declared no conflicts of interest with respect to this work.
Trial registration number: NA.
Keywords: fertility preservation, counselling, female cancer, social network analysis, barriers

P-460 To freeze or not to freeze: analysis of socio-demographic characteristics between women undergoing social freezing and those only asking for information

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Study question: Are there any differences in the socio-demographic characteristics of women who decide to undergo oocyte vitrification to delay motherhood versus those who attend to the clinic for a consultation but do not start the treatment?
Summary answer: We did not find any statistically different socio-demographic characteristic between women who undergo oocyte vitrification and those who only ask for information but do not follow through with treatment.
What is known already: The vitrification of own oocytes in order to delay motherhood (social freezing) is gaining popularity thanks to both technical improvements and an increased awareness among women about this option. Although both individuals and, recently, large corporations express interest in social freezing, the population seeking this option is severely understudied. Limited information is currently available on the decision making factors underlying social freezing versus, for instance, either not freezing or opting to have a child instead.
Study design, size, duration: Cross-sectional study including 120 women who attended a fertility center for a first consultation for oocyte vitrification between July 2012 and May 2014. All women were asked to fill in a 16-item questionnaire at the end of the doctor appointment, and none declined.
Participants/materials, setting, methods: Women were considered 'non-freezers' if they did not start treatment in 6 months from the information appointment. Differences between freezers and non-freezers were analyzed by Chi^2 test and ANOVA. Data were modeled using logistic regression with age, educational level, working status, relationship status and duration, and having children as covariates.
Main results and the role of chance: Women interested in social vitrification were on average 37.4 years old, highly educated (90%), heterosexual (98.3%), and childless (93.3%). The majority either did not have a partner (n = 76, 63%) or were not cohabiting with him at the time of the interview (n = 25, 21%). Sixty-three percent if women indicated the lack of a suitable partner as the main cause of not having children, while 20% reported professional obligations as the main reason. Interestingly, freezers had been consistently longer in their relationship situation than no freezers, whether it was co-habiting with their partner (68.8 vs. 24 months), with a partner but living alone (26.7 vs. 13.4), or being single (21.4 vs. 19.5). None of the characteristics evaluated showed an effect on treatment initiation at multivariate analysis.
Limitations, reason for caution: The main limitation of this study is single center nature; although the women attending the service were from different nationality, caution should be exercised when extending these results to other realities.
Wider implications of the findings: Socio-demographic characteristics of women interested in social freezing does not seem to vary significantly between freezers and non-freezers, although longer permanence in a relationship state might facilitate the decision to freeze. More in depth studies with larger sample base are needed in order to investigate in detail the decision making factors affecting oocyte vitrification for social reasons.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.
Keywords: social freezing, vitrification, oocyte, fertility preservation

P-461 Fertility preservation (fp) and assisted reproductive technology (art) for breast cancer patients (bcp)

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Study question: What are the best methods of FP pre-chemotherapy and infertility treatment for survivors post-chemotherapy in BCP? How many eggs and embryos can be attained pre-chemotherapy and post-chemotherapy, how many days do we need for FP, and what is the percentages of male factors?
Summary answer: In pre-chemotherapy cycles, we can attain more retrieved oocytes, MII oocytes, and embryos compared with post-chemotherapy cycles. Therefore, FP using letrozole within 2 months before chemotherapy is recommended for BCP. After chemotherapy, survivors should receive ART including intracytoplasmic sperm injection (ICSI) as soon as possible for higher chances of pregnancy.
What is known already: At their first visit to the fertility center, BCP tended to be older than other cancer patients (especially with malignant lymphoma and luekemia). More than half of them were likely to have ovarian factor infertility, especially post-chemotherapy. FP should be performed pre-chemotherapy, and low E2 levels are recommended with estrogen receptor (ER) positive patients during controlled ovarian stimulation (COS).
Study design, size, duration: Subjects were BCP who visited Kyono ART Clinic (KAC) or KAC Takanawa from July 2008 to December 2014. Details of breast cancer, including ER and human epidermal growth factor receptor type 2 (HER2), were received in medical referral letters from oncologists.
Participants/materials, setting, methods: 55 BCP [average age at first visit: 37.8 (25-49); diagnosed with breast cancer; 35.1 (23-47); and 78.2% (43/55) married] were divided into two groups [group A (52 cycles): pre-chemotherapy cycles; group B (47 cycles): post-chemotherapy cycles. Average period to FP pre-chemotherapy was 2.0 ± 2.6 months.
Main results and the role of chance: The 55 patients' receptors were [ER; HER2: positive (18; 3), negative (9; 19), no information (28; 33)]. A total of 99 cycles received ART. Number of retrieved oocytes, MII oocytes, and embryos were 5.6 ± 4.2 vs. 2.4 ± 2.5 ($p = < 0.01$), 4.7 ± 3.3 vs. 2.1 ± 2.2 ($p = < 0.01$), and 3.4 ± 4.6 vs. 1.1 ± 1.2 ($p = < 0.01$), in groups A (52 cycles) and B (47 cycles), respectively.
Seven patients (including one after FP pre-chemotherapy and remission) in group A and four in group B became pregnant. 27.3% (15/55) of the patients' partners had male factor. Peak E2 levels in letrozole and non-letrozole cycles were 705.4 ± 886.8 pg/ml and 885.2 ± 777.2 pg/ml (NS).
Limitations, reason for caution: None.
Wider implications of the findings: We recommend BCP to receive ART for ovarian factor (advanced age and/or reduced ovarian reserve) and male factor (especially severe oligozoospermia) as soon as possible. For ovarian factor, more retrieved oocytes, MII oocytes, and embryos can be achieved pre-chemotherapy compared to those in post-chemotherapy treatment. For severe male factor, ICSI is recommended, especially in patients with advanced age.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Kyono ART Clinic, Kyono ART Clinic Takanawa
Trial registration number: NA.
Keywords: breast cancer, fertility preservation, chemotherapy, survivor, assisted reproductive technology

P-462 Effect of local basic fibroblast growth factor and vascular endothelial growth factor on subcutaneously allotransplanted ovarian tissue in ovariectomized mice

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Study question: The role of bFGF and VEGF on ovarian tissue transplantation, especially with regards to the restoration of hormonal function, is not known.
Summary answer: The combination of bFGF and VEGF have beneficial effects on follicle survival, angiogenesis, and resumption of estrous cycles.

What is known already: Strategies to improve and hasten graft vascularization and reduce follicular loss are needed for ovarian tissue transplantation.
Study design, size, duration: Ovarian tissues were isolated from 18-day-old ICR mice. Recipients were adult ICR mice. Female mice underwent bilateral ovariectomy as recipient. Ovarian tissue encapsulated by fibrin hydrogels were transplanted subcutaneously into recipient mice. The fibrinogen solution was mixed with bFGF, VEGF, or a mixture of bFGF and VEGF.
Participants/materials, setting, methods: Follicle morphology and follicle numbers were observed by H&E staining. Angiogenesis were observed tissue by CD31 antibody IHC staining. Daily vaginal cytology was performed to determine estrous cycle and functional restoration of transplanted ovarian tissue. Blood was collected weekly and serum FSH levels were measured.
Main results and the role of chance: The number of primordial follicles and secondary follicles in the bFGF + VEGF group was significantly higher than in the control group. The number of atretic follicles in the bFGF and bFGF + VEGF groups were significantly greater than in the control and VEGF groups. The vascular density in the VEGF group and the bFGF + VEGF groups were significantly higher than in the bFGF group; there was no significant difference between the VEGF and bFGF + VEGF groups. Cyclicity was earlier in the bFGF + VEGF group compared with the control group; all mice in this group restored ovarian function. Serum FSH levels in the bFGF + VEGF group were significantly lower than in the control group by day 14 post-transplantation.
Limitations, reason for caution: However, further investigations are necessary to elucidate the functional mechanism of VEGF and bFGF, in order to ensure the efficacy and safety of exogenous intervention for ovarian tissue transplantation in humans.
Wider implications of the findings: The present study observed the role of bFGF and VEGF in heterotopic transplanted ovarian tissues and showed that combination bFGFand VEGFmight be applied in ovarian tissue transplantation in clinics and might be even benefit for females fertility preservation.
Study funding/competing interest(s): Funding by national/international organization(s) – the Ministry of Science and Technology of China Grants (973 program; 2011CB944504 and 2014CB943203) and the National Natural Science Funds for general program (31371521 and 31230047).
Trial registration number: IRBSZ00000004-2012004.
Keywords: fertility preservation, ovarian tissue transplantation, bFGF, VEGF, angiogenesis

P-463 Short-term hypothermic preservation of human testicular tissue: the effect of storage medium and storage period

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Study question: What is the best condition to preserve human testicular tissue for a short-term period?
Summary answer: Human testicular tissue can be preserved for three days at 4°C in DMEM/F12 without altering tissue morphology, Sertoli cell morphology, number of spermatogonia per tubule or number of apoptotic cells.
What is known already: As of the time that testicular tissue is removed from the patient's body, the tissue has to be kept in optimal conditions until later use. Therefore, the ideal short-term storage conditions until cryopreservation need to be established. Porcine testicular tissue can be preserved up to three days at 4°C in HTS or 20%FBS-Leibovitz medium without tissue morphology deterioration. So far, no studies were done to find the best short-term storage condition for human testicular tissue.
Study design, size, duration: First, human testicular tissue fragments from five patients were kept at refrigerator temperature (RT) for three days in different storage media (DMEM/F12, DMEM/F12 + 20%HSA, DMEM/F12 + 50%HSA and HSA). Secondly, fragments from four patients were kept in the best medium found in the first part for three, five or eight days at RT.
Participants/materials, setting, methods: Viability was measured by TALI® and light microscopy evaluations were performed in order to gain information regarding the general tissue morphology (HE), Sertoli cell morphology (vimentin), number of spermatogonia (MAGE-A4) per tubule and apoptosis (TUNEL). The experimental conditions were compared with fresh control samples.
Main results and the role of chance: DMEM/F12 did not alter any investigated parameter. In all conditions containing HSA, tissue morphology was altered (20% HSA: $P < 0.05$; 50% HSA: $P < 0.0001$; 100% HSA: $P < 0.0001$) as well as Sertoli cell morphology (20% HSA: $P < 0.05$; 50% HSA: $P < 0.0001$; 100% HSA: $P < 0.0001$). The number of spermatogonia was only affected when tissue was stored in 100% HSA ($P < 0.05$). Therefore, DMEM/F12 is suggested as best medium for short-term hypothermic storage. No significant changes were observed in any investigated parameter for the different storage periods apart from tissue morphology as of five days of hypothermic storage (day 5: $P < 0.05$; day 8; $P < 0.0001$). Human testicular tissue should thus not be stored at 4°C for longer than three days.
Limitations, reason for caution: In this study, we have chosen adult human testicular tissue owing to the scarcity of prepubertal material. Functionality tests were not performed because these tests were not yet available for human testicular tissue at the time of this study.
Wider implications of the findings: When human testicular tissue is stored in optimal conditions during transport, the chances of spermatogonial stem cell survival increase which in turn will benefit the patient during future fertility treatment.
Study funding/competing interest(s): Funding by national/international organization(s) – Flemish league against cancer (VLK), Methusalem grant of the Vrije Universiteit Brussel.
Trial registration number: NA.
Keywords: human, testis, short-term, preservation, fertility

P-464 Maturation of human oocytes obtained from small antral follicles during processing of ovarian tissue for cryopreservation

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Study question: Can oocytes from small antral follicles obtained during processing of human ovarian tissue for cryopreservation (non-stimulated) mature in vitro? Does the medium used for the in vitro maturation (IVM) affect the maturation rate? Does bone morphogenetic protein 15 (BMP-15) improve the maturation rate of oocytes from small follicles?
Summary answer: 47% of oocytes from small antral follicles underwent meiotic maturation in vitro. No difference in the meiotic maturation rate was observed under any of the conditions examined.
What is known already: IVM has been successfully applied to cumulus oocyte complexes (COC's) from PCO patients following FSH stimulation (maturation rate > 70%[1]). Whether oocytes from small antral follicles in the non-stimulated human ovary are capable of maturation is unknown. Different culture media have been used for clinical IVM but few comparisons have been reported. Animal studies have shown that oocytes from small follicles benefit from exogenous BMP-15, which is involved in cumulus- oocyte dialog during maturation.
Study design, size, duration: COC's were recovered during tissue processing in approximately one third of patients undergoing cryopreservation of ovarian cortex for fertility preservation. Two IVM media were compared: G2 plus™ with 100mIU FSH/ml and 500mIU hCG/ml[1] and a bovine IVM medium (Vitromat: containing 100mIU FSH/ml) with/without BMP-15 (100ng/ml).
Participants/materials, setting, methods: Ovarian tissue was processed at 37°C in Quinn's Advantage Hepes buffered medium. Small antral follicles (< 5 mm diameter) were punctured and the COC's transferred to IVM medium for 48 hours. The size and Vanderhyden[2] score for cumulus complex (CC) expansion was recorded at 0, 24 and 48 hours.
Main results and the role of chance: 227 COCs were obtained from 28 patients (mean age 23.6, range 18-36 years). Over half of the oocytes (53.7%, 122/227) were enclosed in a large dense mass of CC (> 15 layers) and 26.4% (60/227) had < 5 layers of CC. Of the 227 oocytes, 22.5% (51) remained at the germinal vesicle (GV) stage, 30.4% (69) had undergone GV breakdown and 47.1% (107) had developed to metaphase II. Of the 107 metaphase II oocytes, 8.4% (9) had matured by 24 hours. No difference was observed for the maturation rate in any of the conditions; G2 -44.8% (26/58); Vitromat - 48.1% (26/54); Vitromat + BMP-15 - 47.8% (55/115). The only difference observed was that fewer of the CC demonstrated a spreading appearance (Vanderhyden score 0) at 48 hours in the presence of BMP-15 (12/115) than in Vitromat (13/54; $p < 0.05$).

Limitations, reason for caution: When possible, similar size CC's from the same patient were allocated to all groups but this was not always feasible. Results may be biased by three patients who had > 20 COC's collected. Although meiotic maturation rates were not different, effects on developmental competence cannot be excluded.
Wider implications of the findings: Small antral follicles contain oocytes capable of meiotic maturation although their developmental competence remains to be established. Although this approach suggests that these oocytes may offer an additional source of reproductive potential when cryopreserving ovarian tissue for fertility preservation patients, their potential for achieving an implantation is unknown.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Melbourne IVF.
Trial registration number: NA.
Keywords: in vitro maturation, fertility preservation, ovarian tissue, human oocytes
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P-465 Correlates of women's intentions to use fertility preservation to prevent age-related fertility decline

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Study question: Which factors are associated with the intentions of childless women aged between 28 and 35 to use fertility preservation (FP)?
Summary answer: Higher intentions to use FP were associated with feeling susceptible to infertility, considering FP useful to achieve parenthood, perceiving the implications of infertility (i.e., childlessness) as severe, expecting to have children at a later age and having fewer ethical concerns about FP.
What is known already: Many women consider cryopreserving their oocytes but only a minority actually do it. Usually they are already too old (i.e., older than 36). It is unclear why women are not using FP earlier, as this would increase their chances of achieving parenthood. The Theory of Planned Behaviour (TPB) and the Health Belief Model (HBM) are valid models to understand and predict women's intentions to use FP.
Study design, size, duration: Cross-sectional online survey. Childless women without fertility problems, aged 28-35, who desired to have children, were recruited. We recruited from multiple sources (universities, community panel, social media, online communities, fertility websites, online advertisement and experiment websites). The survey ran from the 9th of May 2014 - 15th of June 2014.
Participants/materials, setting, methods: The final sample consisted of 257 childless women. The online survey included questions about parenthood goals, women's intentions to use FP within 2 years, their fertility knowledge and variables from the TPB and HBM. The data were analysed using Structural Equation Modelling.
Main results and the role of chance: On average women were 30.6 years (SD = 2.3), wanted to have 2 children and expected to have their first and last child at 34.4 and 37.6. Desire for children was high (7.4 ± 2.1 [2-10]), intentions to use FP low (2.5 ± 1.5 [1-7]) and fertility knowledge moderate (3.5 ± 1.4, [0-6]). The HBM showed good fit to the data (X^2 (14, N = 257) = 13.63, r = .477; CFI = 1.000: RMSEA = 00, 90%CI [0.00-0.06], showing that women were more likely to use FP when they felt susceptible to infertility, perceived childlessness as severe, considered FP useful and ethically correct and expected to have children at a later age. The TPB lacked explanatory power (X^2 (6, N = 257) = 94.31, r < .001; CFI = .78; RMSEA = .24, 90%CI [0.20-0.28]).
Limitations, reason for caution: 68.1% of the women were employed and 92.6% had university education, which may result in an overestimation of fertility knowledge and an underestimation of access to FP. This study focused on intentions rather than actual behaviour, previous research suggests that women overestimate their intentions to use FP.
Wider implications of the findings: Women have low intentions to use FP. These seem to be related to a lack of perceived susceptibility to infertility and not appraising its consequences as severe. These perceptions seem misaligned with women's desire to have two children at ages when there is already reproductive decline (i.e., 34-38 years) and their strong desire for children. Healthcare professionals should help women by providing accurate and personally relevant information about fertility and FP to delineate feasible parenthood plans.
Study funding/competing interest(s): Funding by University(ies) – Radboud Honours Academy, Nijmegen, The Netherlands.
Trial registration number: NA.
Keywords: fertility preservation/psychology, fertility, health behaviour/psychology, oocyte cryopreservation

P-466 In vitro maturation: before or after vitrification?

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Study question: The aim of this study was to understand whether it is more or less beneficial in terms of structural integrity (presence of a normal meiotic spindle and correct chromosome alignment), to perform *in vitro* maturation (IVM) of immature oocytes before or after vitrification.
Summary answer: IVM of MI oocytes seems to be more efficient when performed before vitrification. No differences were observed for GV oocytes. However, independently of the moment where IVM were performed, mature oocytes obtained from IVM present structural damages in terms of presence of an abnormal meiotic spindle and/or incorrect chromosome alignment.
What is known already: Advances in cancer treatments allow oncology patients to live for extended periods of time and thus issues such as fertility preservation have become increasingly important for survivors. Within the various female fertility preservation options, oocyte vitrification is the one that most effectively ensures the future reproductive autonomy of women. I*n vitro* maturation is a very important technique when there is a need to cryopreserve immature oocytes. However the success of this technique is questionable.
Study design, size, duration: Cross sectional. 170 immature oocytes obtained after controlled ovarian hyperstimulation from 75 patients were used: 50 GV oocytes - germinal vesicles stage; and 120 MI oocytes - metaphase I stage.
Participants/materials, setting, methods: *In vitro* maturation (IVM) was performed in 37 GV (germinal vesicles stage) and 72 MI (metaphase I stage) followed by vitrification; and was performed after vitrification in 13 GV and 48 MI. For both approaches, IVM success was evaluated. Structural integrity (meiotic spindle and chromosomes) were assessed using immunocytochemistry.
Main results and the role of chance: Our results suggest that IVM is more efficient in MI oocytes (61.11%) than GV oocytes (32.43%, $p < 0.001$). Furthermore, IVM of MI oocytes seems to be more efficient when performed before vitrification (61.11% vs 25.00%, $p = 0.009$). No differences were observed for GV oocytes (32.43% vs 8.30%, $p = 0.098$). More importantly, independently of the moment where IVM was performed (before or after vitrification), mature oocytes obtained from IVM present structural damages in terms of presence of an abnormal meiotic spindle and/or incorrect chromosome alignment.
Limitations, reason for caution: Immature oocytes used were obtained from controlled ovarian hyperstimulation cycles triggered by HCG, and that failed the *in vivo* maturation. The same approach should be used with immature oocytes obtained from controlled ovarian hyperstimulation cycles without the triggering of HCG.
Wider implications of the findings: As it is briefly suggested in the literature, our study demonstrates that although it is possible to obtain mature oocytes using IVM, they present structural damage in terms of meiotic spindle structure and/or chromosome alignment. The use of technologies that allows the visualisation of the meiotic spindle in live oocytes should be considered. These approaches can allow the selection of the good oocytes and clarify if IVM is indeed a viable option.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Funding was from the budget of the University of Coimbra Hospital system, and related to the public oncofertility service provided.
Trial registration number: NA.
Keywords: fertility preservation, oocytes, in vitro maturation, vitrification

P-467 Oocyte cryopreservation: a retrospective analysis of data from Italian ART register 2007-2012

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Study question: To compare the efficacy of mature human oocytes cryopreservation with Slow-Freezing (SF) and Vitrification (VT) in Italy, where these procedures have been most widely used.
Summary answer: VT resulted in a statistically significant higher performance compared with SF based on the higher oocyte survival and pregnancy rates and the lower number of vitrified-warmed oocytes to obtain a live birth.
What is known already: Oocyte cryopreservation is an important option for individuals planning to undergo IVF treatments, because new protocols have greatly improved the applications of this procedure. Two different methods of oocyte cryopreservation are currently performed: Slow-freezing and Vitrification. Vitrification has been shown to be superior, and in some studies its outcomes were similar to those obtained with fresh oocytes. Additional studies are required to confirm these results.
Study design, size, duration: Retrospective analysis of aggregate data collected by the Italian ART Registry (IARTR) from ART centers who have performed at least one frozen cycle in the period from 2007 to 2012. The number of ART centers increased from 93 in 2007 to 116 in 2012.
Participants/materials, setting, methods: Infertile couples who underwent IVF-treatments at Italian ART centers and who have done cryopreservation of supernumerary oocytes. Data were analyzed with IBM SPSS Statistics 21.
Main results and the role of chance: During the 6-year study period ART centers performed 16.517 frozen cycles, of which 57,7% (9.541) with slow-frozen oocytes and 42,3% (6.976) with vitrified oocytes. Following SF and VT, oocyte survival rates were 51,8% vs. 64, 5% and clinical pregnancy rates per cycle and per transfer were 12,2% and 14,9% vs. 14,7% and 18,5%, respectively. On average, 47 oocytes were used to obtain a live birth following SF compared to 36 oocytes utilized with VT.
Limitations, reason for caution: Our data were collected and analyzed in aggregate form only, in compliance with Italian law. This may have limited the variety and depth of our analysis.
Wider implications of the findings: Our findings based on the analysis of a very large number of frozen cycles shows that oocyte cryopreservation is a competitive tool in clinical practice. Because of the good outcomes observed, oocyte cryopreservation represents a viable option for patients declining embryo cryopreservation.
Study funding/competing interest(s): Funding by national/international organization(s) – Minister of Health.
Trial registration number: NA.
Keywords: oocyte cryopreservation, slow-freezing, vitrification, survival rate, pregnancy rate

P-468 In vitro evaluation of an anti-apoptotic drug, Z-VAD-FMK, for further use in ovarian tissue transplantation

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Study question: In a model reproducing early ischemia after ovarian tissue transplantation, does the pan-caspase inhibitor Z-VAD-FMK could prevent granulosa cells apoptosis?
Summary answer: Results obtained with HGL5 granulosa cell line suggest that Z-VAD-FMK is efficient to protect granulosa cells from etoposide or $CoCl_2$ induced apoptosis.
What is known already: Removal, cryopreservation and subsequent graft of ovarian strips after cancer treatment have been successfully used to re-establish female fertility. However, the pregnancy rate after autografting of cryopreserved tissue is about 30%. Indeed, the major problem after transplantation is follicular loss due to ischemic reperfusion injury.
Study design, size, duration: Three human granulosa cell lines (GC1a, HGL5 and COV434) were cultured during 48h with Z-VAD-FMK with or without etoposide to induce apoptosis. To reproduce the ischemic phase of the graft, cells were cultured without serum under reduced O_2 (1%) or with $CoCl_2$ (chemical hypoxia).
Participants/materials, setting, methods: Granulosa cells were used as a model since they are essential for oocyte survival. Metabolic cell activity was evaluated by the WST-1 assay. Cell apoptosis was analyzed by flow cytometry after annexin V-FITC and propidium iodide double staining. The mRNA levels and protein expression of apoptotic markers were evaluated using RT-qPCR and western blot analysis.
Main results and the role of chance: Flow cytometry showed that cells co-treated with etoposide and Z-VAD-FMK displayed a higher percentage of viable cells as compared to etoposide alone. When *in vivo* ischemic stage was mimicked (1% O_2), no beneficial effect of the Z-VAD-FMK was detected. However, a significant decrease of the number of early apoptotic cells was evidenced by flow cytometry for HGL5 cells treated with Z-VAD-FMK. RT-qPCR and western blot analysis revealed that apoptotic molecules were not modulated. Metabolic activity of the 3 cell lines was reduced by $CoCl_2$. For HGL5 cells, this decrease was partially reversed by Z-VAD-FMK. The number of viable cells was reduced by $CoCl_2$ in HGL5 cells but Z-VAD-FMK allowed to preserve a similar number of viable and apoptotic cells than in control condition.
Limitations, reason for caution: In this study we used 3 different cell lines but granulosa cells represent only a part of the cell types present in ovarian tissue biopsies. Experiences on the effect of Z-VAD-FMK on primary culture of granulosa cells have not yet been realized.
Wider implications of the findings: This study suggests that the use of an antiapoptotic drug could be efficient to improve ovarian tissue transplantation outcomes. Ovarian tissue grafting studies using our xenograft murine model will be performed to test the potential efficacy of this drug to improve tissue viability and primordial follicles preservation after transplantation.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – This work was supported by the University of Liège and by grants from the Fonds de la Recherche Scientifique Médicale, the Fonds de la Recherche Scientifique - FNRS (F.R.S.-FNRS, Belgium). The authors declare that they have no competing interests.
Trial registration number: NA.
Keywords: apoptosis, Z-VAD-FMK, granulosa cells, ovarian transplantation, follicles preservation

P-469 Vitrification using dimethyl sulfoxide free cryoprotectant is a promising cryopreservation method for fertility preservation of immature mouse testicular tissue

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Study question: What is the best cryopreservation protocol of immature testicular tissue for fertility preservation?
Summary answer: Vitrification using dimethyl sulfoxide (DMSO) free cryoprotectant appears to be superior to other methods for cryopreservation of immature testicular tissue and in vitro production of functional spermatozoa.
What is known already: Loss of fertility is a major problem for childhood cancer survivors treated with gonadotoxic therapy, and cryopreservation of testicular tissue is an approach to preserve fertility for prepubertal boys. Slow freezing (SF) and vitrification are cryopreservation techniques that were successfully applied in several animal models but need further exploration.
Study design, size, duration: Fragments of testicular tissue from 10 mice were assigned to one of the following cryopreservation procedures: SF using DMSO as cryoprotectants, SF using DMSO free cryoprotectant (Stem Cell Keep®), vitrification using DMSO, vitrification using Stem Cell Keep®.
Participants/materials, setting, methods: Histological and immunohistochemical analyses were performed to evaluate cell viability, intratubular proliferation (Ki-67), apoptosis (caspase-3) after freezing and thawing. Thawed testicular tissue were cultured for in vitro production of mature sperm using previously described organ culture method. The fertility of induced spermatozoa was tested by intracytoplasmic sperm injection (ICSI).
Main results and the role of chance: Seminiferous tubules showed good integrity after cryopreservation and thawing in all groups. Cell viability and their

ability to proliferate was observed by immunohistochemistry particularlly in SF with DMSO group and vitrification with Stem Cell Keep® group, and mature spermatozoa were induced after 35 days of organ culture in these groups. ICSI showed fertility of induced spermatozoa only in vitrification using Stem Cell Keep® group.

Limitations, reason for caution: Supplementary research is required to confirm this study's findings using human prepubertal testicular tissue.

Wider implications of the findings: An optimal cryopreservation protocol enhances the chances for successful fertility restoration.The findings of the present study have potential implications for cryobanking of immature testicular tissue and fertility preservation. Vitrification using DMSO free cryoprotectant would be a preferable cryopreservation method for later induction of fertile spermatozoa.

Study funding/competing interest(s): Funding by University(ies) – The authors declare that no competing interests exist.

Trial registration number: This study is not an RCT.

Keywords: cryopreservation, testicular tissue, slow freezing, vitrification

P-470 Should letrozole-gonadotropin stimulation be the first choice for young cancer patients desiring oocyte cryopreservation?

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Study question: Could the association of letrozole to gonadotropins be beneficial in terms of oocyte yield in young cancer patients?

Summary answer: Letrozole-gonadotropin stimulation in hormonosensitive breast cancer patients produced more mature oocytes than gonadotropin only stimulation in cancer patients and this was not associated with age or the amount of FSH administered.

What is known already: Short antagonist protocols with the association of aromatase inhibitors to gonadotropins are used for oocyte cryopreservation in the context of fertility preservation in hormonosensitive breast cancer patients to avoid the rise in estradiol levels. A synergistic effect with the release of endogenous FSH may result from the negative feedback of estradiol levels during the initial stimulation allowing the recruitment of a higher number of follicles.

Study design, size, duration: A cross sectional study was developed during the last 18 months including 22 female cancer patients (CP), 25 hormonosensitive breast cancer patients (Let) and 40 women with male factor infertility (IP) who underwent ovarian stimulation with short antagonist protocol and FSHr for oocyte collection.

Participants/materials, setting, methods: Stimulation was started on cycle day 3 in IP, any day of follicular phase or after 3 days of antagonist if on the secretory phase in Let and CP groups. Letrozole was initiated in Let the day before FSH start. Triptorrelin or hCG were used for inducing final oocyte maturation.

Main results and the role of chance: The number of mature oocytes was higher in IP (9.00 ± 5.00, $p = 0.015$) and Let (9.52 ± 6.12, $p = 0.011$) than in CP (5.09 ± 3.79), although the total amount of FSH administered was identical in the 3 groups. The Estradiol levels were significantly lower in day 5 in the Let patients (169.54 ± 136.04) versus CP (555.76 ± 426.66, $p = 0.001$) and IP (638.90 ± 393.27, $p < 0.0001$). On the triggering day estradiol was also significantly lower in Let (395.97 ± 205.41) versus CP (1070.68 ± 676.33, $p = 0.011$) and IP (1852.68 ± 890.08, $p < 0.0001$). Patient age was lower in CP (29.59 ± 4.27) than Let (33.80 ± 3.03, $p < 0.0001$) and IP (32.33 ± 3.42, $p = 0.014$). The difference in the starting day of the stimulation seems not to interfere with the outcome confirming the efficacy of random start protocols in fertility preservation patients.

Limitations, reason for caution: Despite the younger age of the CP group it could have a worse prognosis for oocyte collection than the IP group for unknown reasons, although the ovarian reserve was not evaluated. Cancer patients might have a reduced response to ovarian stimulation. The small number of patients was also a limitation.

Wider implications of the findings: Letrozole associated to gonadotropins in a random start protocol can be more effective than regular gonadotropin stimulation in cancer patients, even when hormonal levels are not detrimental. Induction of final oocyte maturation with triptorelin is effective and accelerates luteolysis, enabling regression of estradiol and progesterone to normal levels and the initiation of chemotherapy. If these findings are confirmed in larger studies this can be the first choice protocol for young cancer patient undergoing oocyte cryopreservation.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Centro Hospitalar e Universitário de Coimbra.

Trial registration number: NA.

Keywords: fertility presevation, letrozole, breast cancer, vitrification, protocol

P-471 Slush nitrogen vitrification of human ovarian tissue reduces stromal cells apoptosis and does not affect the expression of genes involved in stress and toxicity pathways

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Study question: Does slush nitrogen (SN2) vitrification protect human ovarian tissue from apoptosis and preserve the expression of genes involved in human stress and toxicity pathway compared with liquid nitrogen (LN2) vitrification?

Summary answer: Results demonstrated that compared with LN2 vitrification, SN2 preserves the ultrastructure of follicles and stromal cells, and their DNA integrity. Moreover, the analysis of 84 genes involved in stress and toxicity pathway demonstrated that the expression of 12 genes was altered by LN2 and not SN2 vitrification.

What is known already: The efficiency of vitrification of human ovarian tissue is currently controversial. SN2 vitrification that increases the cooling rate and avoids the Leidenfrost effect has been shown to reduce cryoinjuries and improve human oocyte vitrification. In a preliminary study we recently showed that SN2 better preserves the ultrastructure of ovarian follicles compared to LN2 vitrification.

Study design, size, duration: We performed a control versus treatment study on human ovarian biopsies collected during laparoscopy from eleven consenting patients. Strips (2×4×1mm) were processed for histology, ultrastructure, TUNEL assay and expression of 84 genes involved stress and toxicity pathway through PCR array, either as fresh controls or after LN2 or SN2 vitrification.

Participants/materials, setting, methods: Ovarian strips were treated at RT in 25%, 50% (5min), and in 100 % (1min) vitrification solution (VS: MEM, HSA 20mg/ml, DMSO 10%, EG 26%, PVP 2.5%, sucrose 1M), plunged in LN2 or SN2 and thawed at 37°C in culture medium with 1M (15 seconds), 0.5M and 0.25M sucrose (5min).

Main results and the role of chance: 1043 follicles (control $n = 240$; LN2 $n = 476$; SN2 $n = 327$) were studied. Ultrastructural data on 27 follicles (control $n = 14$; LN2 $n = 6$; SN2 $n = 7$) showed intact oocytes in all samples. Morphometric analysis reported that SN2 better preserved follicular cells (SN2 = 73.3 vs LN2 = 23.1%) and stromal cells (SN2 = 35.4 vs LN2 = 9%), compared to control (follicular cells = 92%; stromal cells = 59.8%). Tunel assay showed an highly significant increase of stromal cells with fragmented DNA in LN2 samples (control = 0.5, LN2 = 2.3, SN2 = 0.4%). Utilizing RT–PCR array we found that LN2 resulted in an up-regulation in the expression of AQP2, CA9, CCL2, CFTR, CRP, DDB2, EPO, RAD51, TNF, and XPC and a down-regulation of ADM, ATG7 and AQP1 ($P < 0.05$). Conversely, only ATG7 was altered being down-regulated in SN2 samples.

Limitations, reason for caution: The RT-PCR data are preliminary and the study should be validated by in vitro culture and xenotransplantation of thawed ovarian strips.

Wider implications of the findings: As SN2 vitrification better preserves the ultrastructure/DNA integrity of follicles and stromal cells, and does not affect the expression of genes involved in human stress and toxicity pathway, it could improve the resumption of endocrine and reproductive functions in post-oncological patients after ovarian slices transplantation**.**

Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies) – Merck-Serono.

Trial registration number: NA.

Keywords: ovary, cryopreservation, apoptosis, gene expression, ultrastructure

P-472 Female fertility preservation using oocyte vitrification: 7 years experience

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Study question: Is oocyte vitrification a good alternative for female fertility preservation? Can it be used for all indications, i.e., infertility, social and cancer?
Summary answer: Our study shows that Vitrification is an excellent alternative to preserve female fertility and may be indicated for cases of women who need to delay pregnancy for social reasons and/or for women who will undergo treatment that increase the risk of fertility loss.
What is known already: The evolution of assisted reproduction techniques has allowed the development of vitrification that can be applied for oocyte preservation. Cryopreservation of oocytes is one of the most innovative techniques for fertility preservation against the effects of age and for those who are at risk of premature ovarian failure. As it is a relatively new technique, few authors reported their experience with large numbers of patients.
Study design, size, duration: A retrospective study was performed evaluating 182 patients that were submitted to treatment and had their oocytes cryopreserved, between July 2007 and July 2014. Patients were divided in 3 groups – Infertility (85), Social preservation (69) and Cancer (12); 16 were excluded due to lack of data.
Participants/materials, setting, methods: All women were submitted to COS with GnRH agonist protocol, GnRH antagonist protocol or of emergency protocol. Oocyte retrieval, ICSI, embryo culture, vitrification and desvitrification, were the same for all patients.
Main results and the role of chance: The mean age of the patients was 32.13 ± 5.5, 35.78 ± 5.3 and 31.4 ± 1.3 respectively for group Infertility, Social and cancer ($p < 0{,}0001$). The dose of gonadotropins used in the Social group was higher that in the Infertility group (2833.5 ± 695UI x 2301 ± 573UI - $p < 0.0001$). The number of follicles (22,4 ± 14,5 x 10,5 ± 8,79) and MII oocytes (22 ± 12,3 x 9,2 ± 7) were higher in the Infertility group than in the Social preservation group ($p < 0.0001$). A total of 589 MII oocytes from77 patients of the infertility group (86 cycles) were desvitrified. The mean number of oocytes was 6.84 ± 3.9 per cycle (range:1 to 21). The rate of survival was 87%, fertilization 78.6%, embryo development 93.5% and pregnancy 29.8%.
Limitations, reason for caution: This was a retrospective study and some bias may be present. Prospective studies are needed to confirm these results.
Wider implications of the findings: As it is a relatively new technique, few authors reported their experience with large numbers of patients, therefore our results might reassure that oocyte vitrification is an excellent alternative for female fertility preservation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – ORIGEN.
Trial registration number: NA.
Keywords: oocyte vitrification, fertility preservation

P-473 Oocyte freezing for social indications: how do men and women's views differ? internet based survey of knowledge, attitudes and intentions in Denmark and United Kingdom

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Study question: (1) Assess how knowledge and intentions regarding egg freezing differ between men and women. (2) Describe attitudes differences between men and women regarding circumstances which make it acceptable to freeze eggs. (3) Describe differences between men and women regarding circumstances which would make them more likely to freeze eggs.
Summary answer: 20% of women and 21% of men are interested in social egg freezing. Regarding circumstances making it more acceptable to freeze eggs, men were significantly more likely to find it acceptable to allow (1) women to pursue a career (2) for other care commitments, (3) if not ready.
What is known already: Our previous study showed that 89% of women considered reproductive planning to be an acceptable indication for social egg freezing, and around half would consider it for themselves. There is evidence that men's attitudes influence decisions made by women. However, little is known about men's considerations.
Study design, size, duration: A cross-sectional survey was designed to investigate individuals' knowledge and attitudes in Denmark and UK on oocyte freezing and their potential intentions regarding the procedure. Poster and internet based advertising was used to reach respondents in the general population. The questionnaire was accessible online (www.mycompletefamily.co.uk; www.mycompletefamily.co.dk) and completed anonymously.
Participants/materials, setting, methods: From September 2012 to September 2013, 1000 women and 237 men completed the questionnaire. Univariate analyses were used to identify differences between men and women regarding baseline characteristics, attitudes towards social egg freezing, circumstances which make it acceptable to freeze eggs, and circumstances which make freezing eggs more likely.
Main results and the role of chance: 80% men and women have heard of social egg freezing. 20% women were interested; 21% men were interested in their partner having the procedure. Regarding circumstance making it acceptable to freeze eggs, men and women had similar views with regards to social circumstances. However, men were significantly more likely to find it an acceptable option to allow women to pursue a career ($p = 0.023$), care commitments ($p = 0.005$) or not ready ($p = 0.028$). Regarding general circumstances making them more likely to freeze eggs, men and women had similar views, in particular concerning future fertility, safety, cost and percentage chance of having a baby from a cycle. Regarding personal circumstances however, men were significantly more likely to freeze eggs if stopping work would harm their partner's career ($p = 0.001$).
Limitations, reason for caution: The generalizability of the results may be limited due to the internet-based data collection, and may be subject to response bias.
Wider implications of the findings: This study indicates that men and women have similar attitudes regarding the acceptability of social egg freezing for medical reasons. With regards to social reasons however, men found it much more acceptable for women to freeze eggs to allow them to have a career, than women. Similarly, men are significantly more likely to agree to freeze eggs for this reason. Is this a reflection of a positive step towards gender equality? Of economic awareness?
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck Serono.
Trial registration number: Not a trial.
Keywords: social egg freezing, fertility awareness, reproductive life planning, gender equality, men's views

P-474 Preliminary reports of successful pregnancies derived from cryopreserved autologous oocytes of single women for social reasons

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Study question: With more single women seeking oocyte cryopreservation (OC) for fertility preservation, the value of OC for social reasons remains unclear.
Summary answer: This method would be a viable option of fertility preservation for single women with appropriate ages.
What is known already: The utilization of frozen/thawed oocytes for later IVF/ICSI has been proved to have similar fertilization and pregnancy rates to those with fresh oocytes in young women, and it has been used for fertility preservation of cancer patients, an alternative of embryo cryopreservation, or elective delay of childbearing (so-called social freezing). However, data were limited in women who attempt pregnancy purely from social OC.
Study design, size, duration: This is a two-center retrospective cohort study. A total of 240 single women (326 cycles) using OC for fertility preservation due to social reasons were included from January, 2002 to June, 2014.
Participants/materials, setting, methods: Single women underwent ovum pick-up cycles in National Taiwan University Hospital and Stork Fertility Center. OC was performed either by slow freezing or vitrification. The women returned to thaw their oocytes after marriage for pregnancy. The thawed oocytes

were fertilized with intracytoplasmic sperm injection, and embryo transfer was performed.
Main results and the role of chance: In this study, the mean age of OC was 38.1 ± 3.6 years old (y/o) with a mean of 9.7 ± 6.1 oocytes frozen per cycle. There were 11 women whose oocytes were thawed for pregnancy. In 6 women who froze their oocytes before 40 y/o, the mean oocyte survival and normal fertilization rate were 88.9% and 79.2%, respectively. All of them successfully became pregnant. Four women delivered live-born babies beyond 36 gestational weeks (GWs), one woman now was pregnant at 32 GWs, and one woman had blighted ovum but still had five extra-cryopreserved blastocysts. While in the other five women who froze their oocytes after 40 y/o, lower survival and fertilization rates (72.7% and 57.1%, respectively) were noted, and there was no pregnancy being achieved till now.
Limitations, reason for caution: Our limitation is small case number.
Wider implications of the findings: Although the value of OC in cancer or infertility patients has been documented, this remains unclear in social oocyte freezing for fertility preservation. In our preliminary results, we achieved successful pregnancies from oocytes cryopreserved for social reasons with appearing good success rates in women less than 40 y/o. We hope that more cases will be reported and this method will be a practicable option of fertility preservation for single women.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by National Taiwan University Hospital. All authors declare no conflicts of interests.
Trial registration number: NA.
Keywords: fertility preservation, oocyte cryopreservation, social freezing

P-475 The use of *n*-hexyl-2-cyanoacrylate can facilitate the orthotopic ovarian transplantation

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Study question: The use of N-hexyl-2-cyanoacrylate can facilitate the fixation of ovarian pieces in the orthotopic transplantation?
Summary answer: The use of N-hexyl-2- cyanoacrylate facilitates the laparoscopic orthotopic ovarian transplantation without affecting the restoration of their functional activity
What is known already: Two techniques has been successfully used to reimplant frozen-thawed ovarian tissue in an orthotopic site: in a specially created window on the peritoneum or on the remaining ovary. In case of small fragments they can be placed on the decorticated medulla but this is particulary difficult when performed laparoscopically. In this study we examined the usefulness of applying a available cyanoacrylate tissue adhesive in orthotopic transplantation**.**
Study design, size, duration: Observational case-series study. Three patients to whom ovarian tissue transplantation was performed between 2013 and 2014 by laparoscopy were studied.
Participants/materials, setting, methods: Ovarian tissue from 3 patients (Patient 1 with nasopharyngeal cancer, Patients 2 and 3 with breast cancer) was cryopreserved with slow freezing protocol prior to chemo-and/or radiotherapy. After cancer remission, the cryopreserved ovarian tissues were retransplanted orthotopicaly in the ovarian medulla laparoscopically, using *n*-hexyl-2-cyanoacrylate as an absorbable adhesion barrier.Endocrine function was assessed by monthly blood tests and ultrasound after transplantation.
Main results and the role of chance: All 3 patients regained ovarian function between 8 and 24 weeks after transplantation, as shown by follicle development and estrogen production. Ovarian function persists 6 months(patient transplanted in july 2014) and one year after transplantation (2 patients). Pregnancy was not obtained in any of the patients still.
Limitations, reason for caution: Preliminary study wich includes few patients
Wider implications of the findings: The use of N-hexyl-2- cyanocryalate can facilitate the placement of ovarian pieces in the orthotopic transplantation by laparoscopy without affecting the restoration and duration of ovarian activity
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital Clinic.
Trial registration number: NA.
Keywords: fertility preservation, ovarian transplantation

P-476 The negative influence of sperm cryopreservation on the quality and development of the embryo depends on the morphology of the oocyte

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Study question: (i) Is there any effect of sperm cryopreservation on embryo quality and chance of blastocyst formation? (ii) Is there any oocyte quality-dependent negative effect of sperm cryopreservation on embryo quality and the chance of blastocyst formation?
Summary answer: There is an oocyte quality-dependent negative effect of sperm cryopreservation on embryo quality and the chance of blastocyst formation.
What is known already: Sperm cryopreservation represents a valuable therapeutic option in the management of infertility. However, during the process of cooling, freezing and thawing, spermatozoa are subjected to a series of changes, which may cause cell damage. Although the ICSI may circumvent some of the problems with sperm quality, whether ICSI can overcome the effect of the cryodamage of sperm and, therefore, avoid detrimental effects on the quality and development of the embryo, has yet to be elucidated.
Study design, size, duration: This study included 22,186 zygotes, obtained from 2802 patients undergoing ICSI cycles with either fresh (n = 2435) or cryopreserved (n = 367) sperm. The effect of sperm cryopreservation on embryo quality and on blastocyst formation chance was evaluated when the oocyte quality was not considered and when at least one oocyte dimorphism was present.
Participants/materials, setting, methods: To elucidate whether the oocyte quality-dependent influence of the injection of cryopreserved sperm was due to an extracytoplasmic or intracytoplasmic oocyte defect, two more groups were formed: Embryos derived from oocytes with at least one intracytoplasmic defect and embryos derived from oocytes with at least one extracytoplasmic defect.
Main results and the role of chance: The binary regression model showed that the embryo quality and the blastocyst formation chance were not influenced by the origin of the sperm when the quality of the oocyte was not considered. However, when at least one oocyte defect was present, a negative influence of the sperm cryopreservation on the embryo quality (OR = 0.90, CI: 0.85-0.96, $p > 0.001$) and blastocyst formation chance (OR = 0.83, CI: 0.76-0.92, $p > 0.001$) was noted. The injection of cryopreserved sperm into oocytes with extracytoplasmic dimorphisms did not affect the embryo quality, but did affect the blastocyst formation chance (OR = 0.79, CI: 0.59-0.95, $p = 0.015$). Conversely, the embryo quality (OR = 0.58, CI: 0.32-0.76, $p > 0.001$) and the blastocyst formation chance (OR = 0.95, CI: 0.92-0.99, $p = 0.046$) were negatively influenced by the injection of cryopreserved sperm in oocytes with intracytoplasmic defects.
Limitations, reason for caution: The main limitation of this study is its retrospective design.
Wider implications of the findings: Apparently, when at least one morphological defect is present, the oocyte is not able to repair a possible negative effect of sperm cryopreservation; therefore, the embryo quality at the cleavage stage and the blastocyst formation are negatively affected. Considering this, for patients undergoing ICSI cycles, in which oocyte defects are detected, the injection of fresh sperm, if possible, would be a better approach.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fertility - Centro de Fertilização Assistida.
Trial registration number: NA.
Keywords: sperm, cryopreservation, oocyte

P-477 A comparison of methylation levels in HPV16, HPV18 and HPV51 genomes in asymptomatic HPV infection and cervical neoplasia

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Study question: Relationship between methylation status in the L1 gene and the long control region (LCR) of HPV16, HPV18, HPV51 and cervical pathology.
Summary answer: The study revealed that methylation of CpG was more prevalent in the L1 gene as compared to the LCR region of all three HPV types and methylation level correlated with the severity of cervical neoplasia.
What is known already: Human papillomaviruses (HPVs) are common sexually transmitted viruses that can cause cervical cancer and affect female fertility. Epigenetic alterations of HPV genome play an important role in the life cycle of virus and the carcinogenic progression.
Study design, size, duration: A total of 202 cervical specimens were analysed for methylation of HPV DNA: 157 HPV16-positive specimens, 21 HPV18-positive specimens and 24 HPV51-positive specimens.
Participants/materials, setting, methods: The HPV genome was analysed using bisulfite modification, DNA amplification and sequencing.
Main results and the role of chance: The highest methylation frequency of HPV16 was detected at the L1 gene (35.5%) and promoter region (32.3%) in the subgroup of cervical cancer. In other LCR regions it ranged from 0% in asymptomatic HPV16 infection and cervical intraepithelial neoplasia grade 1/2 to 25.7% in cervical cancer. Statistically significant differences after Bonferroni correction were detected between the asymptomatic HPV16 infection and cervical cancer in the L1 region (P = 0.0027). The study also revealed that all HPV18-positive specimens were methylated in the L1 gene in the subgroups of cervical intraepithelial neoplasia grade 3 or carcinoma in situ and cervical cancer. In contrast, the methylation level of the LCR region was very low among all HPV18-positive samples. For the first time, methylation pattern of HPV 51 in cervical cancer has been investigated and compared to that of asymptomatic HPV51 infection and cervical intraepithelial neoplasia grade 1/2. The methylated CpG sites of HPV51 were detected in the L1 gene only in a case of cervical cancer. All other regions of HPV51 were unmethylated in all tested samples.
Limitations, reason for caution: Sample size of the collected speciments of HPV18 and HPV51 was small because of the low prevalence of these genotypes in the population. Most probably this was the reason why there were no significant differences in the methylation frequencies of these genotypes when pathology groups were compared.
Wider implications of the findings: Further investigation of methylation of HPV genome might lead to better understanding and earlier diagnostics of cervical pathology and fertility preservation in women at high risk for cervical cancer.
Study funding/competing interest(s): Funding by University(ies) – Institute of Biotechnology, Vilnius University.
Trial registration number: No trial registration number.
Keywords: human papillomavirus, methylation, cervical pathology

P-478 Prospective study analysing maturation rates of vitrified metaphase 1 stage (M1) oocytes from in vitro maturation and stimulated cycles compared with fresh M1 oocytes

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Study question: Could metaphase 1 (M1) and 2 (M2) stages oocytes from in vitro maturation (IVM) and controlled ovarian hyperstimulation cycles (COH), performed in cancer patients, be frozen in the same time without adverse effect of the vitrification process on further survival (SR) and maturation rates (MR)?
Summary answer: SR of M1 oocytes from IVM or COH are similar with that of M2 oocytes. MR of survived M1 oocytes from COH is similar with that of fresh M1 oocytes. However, MR of M1 oocytes from IVM is significantly decreased compared with that of fresh M1 oocytes.
What is known already: Vitrification of M2 oocytes from COH or IVM may be indicated in the strategy of female fertility preservation. To freeze M1 oocytes at the same time as M2 rather than expecting their eventual further maturation could be a valuable option making the complete procedure easier with only one time of vitrification. To date, nothing is reported concerning this approach.
Study design, size, duration: From November 2013 to October 2014, seventy-two patients suffering from cancer, candidates to oocyte vitrification following IVM (n = 24: 9 studied/15 control IVM group) or after COH (n = 46: 17 studied/31 control COH group) were prospectively included. In addition, 9 egg donors having vitrified M2 oocytes were used as controls.
Participants/materials, setting, methods: Twenty-eight Day-1 and 32 Day-0 M1 oocytes, obtained from studied IVM and COH groups respectively, were frozen using the cryotop device. Warming was immediately performed after freezing and SR were compared with that of 51 M2 oocytes vitrified/warmed from egg donors. MR of survivor oocytes were compared with those of M1 oocytes from control IVM (n = 44) and COH (n = 53) groups, after 24 hours of culture into appropriate media.
Main results and the role of chance: SR of vitrified M1 oocytes after warming were of 87.7 and 76.9%, respectively in the studied IVM and COH groups compared to 89.6% for M2 oocytes, used as controls. No statistical difference of SR was observed between these groups (global p value: p = 0,8). Survived M1 oocytes from the studied COH group reached a 78.9% of MR compared with 81.5% for fresh M1 oocytes from the control COH group ($p > 0.05$). Nevertheless, MR of vitrified/warmed M1 oocytes from the studied IVM group (39.1%) was significantly lower when compared with the control IVM group (74.2%; p = 0.03). Our study showed similar SR of vitrified M1 oocytes following IVM or COH when compared with those of mature oocytes. Furthermore, conversely to the effect observed with M1 oocytes recovered after COH, vitrification procedure may adversely affect the meiotic competence of M1 IVM oocytes having survived thawing.
Limitations, reason for caution: The present findings should be confirmed on a larger series of oocytes in each group.
Wider implications of the findings: In a fertility preservation program, vitrifying M1 and M2 oocytes in the same time could be applied in patients having undergone COH but not IVM.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Jean Verdier University Hospital.
Trial registration number: Not needed.
Keywords: metaphase 1 oocytes, in vitro maturation, controlled ovarian hyperstimulation cycles, vitrification, maturation rate

P-479 Pressure triggered activation of tolerance (PTAT)-preconditioning of spermatozoa has a positive impact on post-thaw recovery rate of cryopreserved human semen

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Study question: Is it possible to adapt preconditioning by the application of pressure triggered activation of tolerance (PTAT) prior to standard slow freezing procedure, in order to successfully increase human sperm cell recovery rate (characterized by post-thaw motility), as it is already shown in animal species like bovine, porcine, sheep, etc.?
Summary answer: Similarly to previous animal studies, optimizing PTAT-treatment protocol (pressure, duration and equilibration time before freezing) to human spermatozoa could significantly increase the recovery rate of human semen samples, compared to the paired controls frozen without conditioning pretreatment.
What is known already: The application of PTAT-treatment prior to freezing or other in vitro procedures has been shown to successfully decrease the adverse effects of intervention and help cell survival in several species (bovine, porcine, sheep, mouse) and cell types (oocytes, sperm cells, embryos, etc.). To our knowledge, no data have been published on the application of PTAT in human sperm cryopreservation; however, this novel technology might successfully contribute to the improvement of cell survival after freezing.
Study design, size, duration: Semen samples of 21 Caucasian men were included in this proof-of-concept study. Fifteen samples were used to optimize PTAT-parameters (magnitude of pressure, duration of the treatment and the

equilibration time between PTAT and freezing); and 6 semen to validate the optimum settings. Samples were evaluated 60 minutes after thawing.
Participants/materials, setting, methods: All patients met the inclusion criteria. PTAT was performed by the programmable GBOX equipment (AppliedCellTechnology, Budapest, Hungary). Freezing was performed by an IceCube 14S controlled rate freezer (Sy-Lab, Neupurkersdorf, Austria), using a standard slow freezing procedure. After thawing, total, progressive and non-progressive motilities were defined by CASA (Microptic, Barcelona, Spain).
Main results and the role of chance: We have set up several PTAT-parameters from 10 to 50 MPa and 30 to 90 minutes, followed by 10 to 90 minutes of equilibration time before starting the slow freezing protocol of the samples. After thawing the straws, each treatment groups were compared to the matched control and each other, in order to find the optimal treatment conditions. The application of the most effective PTAT-parameters resulted in elevated progressive motilities measured 60 minutes after thawing (20.7 ± 4.84 %), compared to the untreated controls (13.0 ± 2.57 %; mean ± SEM; $p < 0.05$; $n = 6$). All results were reproducible: the findings of each experiment days did not show any significant difference. These observations represent a good basis for further, in-depth examinations on a larger population.
Limitations, reason for caution: Whilst these results suggest that PTAT is able to improve human sperm cell survival after slow freezing, further refining of the treatment parameters is needed. Supplementing our findings by flow cytometry measurements (vitality, DNA-integrity) and morphological evaluations are currently in progress to prove the safety and efficacy of the method.
Wider implications of the findings: Semen cryopreservation as part of IVF procedure and fertility preservation (including sperm banking for donation) means a continuously growing demand. The safety and efficacy of cryopreservation procedures are of major importance, and special attention to any methods improving cell survival should be paid. Incorporating PTAT into conventional sperm freezing procedures has brought promising results, and after refining the protocol by further in-depth studies, it will probably represent an impressive evolution in cryobiology.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Applied Cell Technology Ltd.
Trial registration number: NA.
Keywords: pressure triggered activation of tolerance (PTAT), cryopreservation, human sperm, recovery rate

P-480 Prevention of chemotherapy-induced gonadotoxicity in the mouse model: protective effects of natural bioactive compounds

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Study question: This work aims to investigate whether destruction of ovarian reserve by chemotherapy can be attenuated by administration of natural bioactive compounds with antitumoral effects. In particular, we asked whether phytochemicals from *Crocus sativus L*., commonly known as saffron, can prevent gonadotoxic effects of cyclophosphamide (CPM) in the mouse model.
Summary answer: Pre-treatment of mice with saffron phytochemicals (SP) reduces CPM-induced follicle loss and pyknosis. These effects are associated with decreased expression of FOXO3a, a component of the molecular pathway involved in the maintenance of ovarian reserve. Therefore, administration of SPs may be a potential strategy to reduce gonadotoxic effects of chemotherapy.
What is known already: CPM-based chemotherapy severely impacts ovarian reserve. Although mechanisms underlying CPM gonadotoxicity remain poorly understood, CPM was found to cause follicle burnout by activating PI3K/PTEN/AKT pathway involved in suppression/activation of follicle recruitment throughout FOXO3a activation/inhibition. Since FOXO3a activates antioxidant gene expression, oxidative stress (OS) is supposed to be involved in CPM-induced ovarian damage. SPs (mainly represented by carotenoids crocin and crocetin) exhibit their biological activities *in vitro* and *in vivo* as antioxidants and anti-tumour compounds.
Study design, size, duration: Twenty-four CD-1 female mice were divided in four groups and received a single intraperitoneal injection of 100 ml of PBS (CTRL), or an equal volume containing CPM (100 mg/kg) (CPM), saffron extract (300mg/Kg) *per os* for fifteen days (SE), or saffron extract prior to CPM administration (SE + CPM).
Participants/materials, setting, methods: Ovaries were analysed at 24hr post-CPM for FOXO3a expression by Western blotting (WB). At 7 days post-CPM ovaries were monitored for relative abundance of ovarian follicles by hematoxylin-eosin and immunostained for FOXO3a. DNA damage and OS were evaluated by assessing gH2AX-phosphorylation and advanced glycation endproducts (AGEs) accumulation by immunohistochemistry (IHC).
Main results and the role of chance: SE administration prior to CPM-treatment decreased chemo-induced follicles loss, cell pyknosis and molecular damage. The number of primordial and antral follicles was significantly reduced in CPM-mice when compared with control (OneWay ANOVA, Student-Newman-Keuls Multiple Comparison: $p < 0.001$), whereas in the SE + CPM group these follicle classes were similar to control. WB and IHC showed decreased FOXO3a expression in CPM-mice suggesting the activation of CPM-induced follicle recruitment. Consistently, FOXO3a level in the SE + CPM group was similar to control ($p < 0.05$). IHC showed that AGEs increased in CPM and SE + CPM group in comparison to control ($p < 0.05$). Immunostaining revealed increased DNA double strand breaks in CPM, while in SE + CPM they were similar to control ($p < 0.05$). These results indicate that antioxidant compounds may counteract chemo-induced oxidative damage and restore physiological follicle recruitment.
Limitations, reason for caution: Pathways other than antioxidant response could mediate the protective effects of SE. FOXO3a up-and-downstream pathways have not been investigated. Results can be translated to humans with caution.
Wider implications of the findings: Results from this study aims to increase the knowledge of biological and molecular aspects underlying ovarian chemotoxicity and will be helpful to evaluate the possibility to save or rescue fertility in cancer patients. Also, our data may contribute to define new biomolecular markers useful for the evaluation of the level cytotoxicity of innovative pharmacological anticancer treatments and the differential protective potential of molecules in relation to specific anticancer therapies.
Study funding/competing interest(s): Funding by University(ies) – University of L'Aquila.
Trial registration number: Not required.
Keywords: cyclophosphamide, ovarian chemotoxicity, saffron extract, fertility preservation

P-481 Ovarian tissue cryopreservation assisted by computer tomography

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Study question: Ovarian tissue cryopreservation for transplantation is opening a new field in ART. Conventional Slow Freezing is the most used technique, although is not completely satisfactory. Computer Tomography is able to show a 3D map of the concentration of DMSO as well as the eventual presence of ice. Human and animal ovarian tissue cryopreserved by Slow Freezing has been analysed by a nanoCT (BioScan), offering a power tool for the optimization of the cooling and warming protocols.
Summary answer: We have used a nanoCT to measure the amount of cryoprotectant uptaken by ovarian tissue. The high number of electrons of the sulphur atom in the DMSO molecule, makes it visible. This is a unique property of DMSO, not shared with other cryoprotectants.
What is known already: Computer Tomography is an interesting medical tool. It is based mainly in differences of densities, so in principle it is not useful for cryoprotectants based on alcohol groups. However, DMSO can be easily detected by CT.
Study design, size, duration: A set of four ovarian tissues (human and animal origin) were submitted to a slow freezing protocol based on 10% initial

concentration of DMSO. After ice seeding, the tissues were cooled to -40 deg C and then LN2 vapors till -140 deg C. Finally they were stored. The concentration and distribution of cryoprotectant, the presence of ice and the morphology of the tissue were studied by computer tomography.
Participants/materials, setting, methods: Materials: two samples of cow ovarian tissue, two samples of human ovarian tissue and a microCT (BioScan).
Main results and the role of chance: The pattern of distribution of ice and DMSO in a 3D map was obtained. Very explicit images of how the tissue concentrates the DMSO during the cooling protocol showed up. Also the structure of the dendrites of ice in the extracellular media appeared. This information is CRITICAL for the design of a protocol not based in Slow Freezing but in Equilibrium Vitrification (Liquidus Tracking), that is the ultimate goal of our research.
Limitations, reason for caution: The number of samples and the cooling/warming rates should be studied in a parametrical way.
Wider implications of the findings: This study is critical for the improvent of slow freezing protocols of ovarian tissue, as well as whole ovary. Also is critical for the extension of cryopreservation to Equilibrium Vitrification.
Study funding/competing interest(s): Funding by University(ies) – University of Seville.
Trial registration number: 1.
Keywords: ovarian tissue, cryopreservation, computer tomography

P-482 Recellularization of "ghost" testicular matrix using a gas-liquid interphase method

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Study question: Is it possible to reconstitute the tubular seminiferous epithelium in DTM (decellularized testicular matrix)?
Summary answer: A recellularized testicular scaffold could be obtained after inoculation in DTM and short-term culture (36 h) using a gas-liquid interphase method.
What is known already: Full spermatogenesis using three-dimensional or organ culture systems has been reported only in the rodent so far. Recently, a promising step was made towards a human application with the report on the derivation of cytocompatible DTM from human testis with maintenance of important components and characteristics of the native tissue. Nevertheless, the method of cell inoculation still needed further improvement.
Study design, size, duration: DTM was derived by exposing cadaveric testicular tissue fragments to 1% sodium dodecyl sulphate. Afterwards, total adult human testicular cell suspensions were incubated on DTM discs during short-term culture and analysed microscopically for the presence of germ cells and somatic cells.
Participants/materials, setting, methods: Adult cadaveric testes were harvested by the autopsy department of the UZ Brussel. Fresh testicular tissue was obtained from four patients undergoing bilateral orchiectomy as part of a prostate cancer treatment. Picrosirius red-hematoxylin staining was used to study the testicular cell-DTM interaction.
Main results and the role of chance: DTM discs stained positive for picrosirius red indicating the presence of collagen molecules in this natural scaffold and showed a three-dimensional hollow structure devoid of cells. After inoculation and short-term culture using a gas-liquid phase approach, a good retention of testicular cells was observed in DTM. Cells showing distinct morphology typically for germ or somatic cells filled the tubular and interstitial space.
Limitations, reason for caution: More in-depth analyses on the homing of the cells and their gene and protein expression are warranted and will be performed to confirm the current findings. In addition, it is important to evaluate the function of the cells in the scaffold.
Wider implications of the findings: The ability to reconstitute the seminiferous epithelium in-vitro using DTM as scaffold can ultimately help establishing human in-vitro spermatogenesis which has not yet been achieved. Such a system could be used in fundamental studies unrevealing the mechanisms behind spermatogenesis and has an enormous clinical value as it could treat certain male fertility disorders. Also, it would be an interesting tool in toxicology to screen for reprotoxic compounds.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – Agency for Innovation by Science and Technology (IWT), Kom Op Tegen Kanker (KOTK), Scientific Research Foundation Flanders (FWO), Vrije Universiteit Brussel (VUB), Swedish Research Council, Finnish Academy, Emil och Wera Cornells Stiftelse.
Trial registration number: NA.
Keywords: testicular scaffold, human in-vitro spermatogenesis, gas-liquid interphase

P-483 The effect of vitrification on morphology and apoptosis of human ovarian cortex tissue

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Study question: To examine the effect of the operation of vitrification on morphology and apoptosis in human ovarian cortex tissue.
Summary answer: The strategy of vitrification of human ovarian tissue is viable, and still needs more researches.
What is known already: There have been appoximately 26 babies coming from cryopreserved ovarian tissues, most of which were benefiting from the technique of slow freezing, or programmed freezing. In 2005, Silber S.J. has proposed the technique of vitrification could applied in cryopreservation of ovarian tissues, which may also provide a simplified, cheap and reliable method.
Study design, size, duration: Ovarian tissues came from 4 cases of partial ovariectomy, with necessity of diagnosis or treatment, during Nov. 2012 to Feb. 2013. In each case, small pieces of cortex were randomly divided into Fresh Group and Vitrification Group. And then, we conducted vitrification or not according to their groups.
Participants/materials, setting, methods: Inclusion criteria of ovarian tissues were under 40 years old, without any abnormal cells in pathological examination. Morphology of follicles and stromal cells were examined by HE staining, and cell apoptosis in situ was analyzed by TUNEL assay. Furthermore, we quantified the expression of Cleaved Caspase-3 (an indicator of apoptosis).
Main results and the role of chance: The percentage of primordial follicles, primary follicles and secondary follicles in Fresh Group are 53.33%, 35.56% and 11.11% respectively, while the percentage in Vitrification Group are 50.56%, 40.45% and 8.99%. The percentage of primordial follicles, primary follicles and secondary follicles and the apoptosis in situ show no significance between these two groups. Besides, the expression of Cleaved Caspase-3 in ovarian tissue is the same between two groups.
Limitations, reason for caution: Because the eligible ovarian tissues were really rare, the size of study is limited.
Wider implications of the findings: The results are of certain value for providing evidence that vitrification is feasible to preserve fertility of women who suffer from chemotherapy or radiotherapy because of their cancer and desire to have their own babies.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Science Foundation of China(Grant no.81070495), and Natural Science Foundation of Guangdong Province(Grant no.S2013010013404).
Trial registration number: NA.
Keywords: fertility preservation, human ovarian tissue, vitrification, morphology, apoptosis

P-484 Analysis of aquaporins in ovine ovarian tissue after exposure to cryoprotectant agents, followed by vitrification and in vitro culture

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Study question: To evaluate the importance of the membrane transport proteins, aquaporins (AQPs) 3, 7 and 9 during cryoprotectant perfusion, vitrification and *in vitro* culture of ovine ovarian tissue.
Summary answer: Exposure to cryoprotectants ethylene glycol (EG) and dimethylsulfoxide (DMSO) lead to AQP3 up-regulation.
What is known already: AQPs are involved in the transport of diverse solutes including cryoprotectants such as glycerol, EG and DMSO. We found recently the expression of aquaporins in ovine ovarian follicles.
Study design, size, duration: Ovarian tissue from sheep ($n = 9$) were exposed to cryoprotectants (EG, DMSO or both), vitrified and *in vitro* cultured for 48 hours after thawing.
Participants/materials, setting, methods: All experiments were performed using slaughterhouse material from nine sheep. After exposure to cryoprotectants, vitrification and *in vitro* culture, ovarian tissue w submitted to histological analysis as well as mRNA expression of markers for AQP3, 7 and 9 and immunohistochemistry to assess the expression of these proteins.
Main results and the role of chance: Expression of AQP3 was increased after exposure to EG and DMSO, probably due to hyperosmolarity in the medium. AQP3 proteins were located in the granulosa cells of preantral follicles. Down-regulation of AQP9 was observed after exposure to EG and DMSO.
Limitations, reason for caution: Expression of more transporters involved during cryoprotectant perfusion needs to be assessed.
Wider implications of the findings: This study enhance the knowledge about aolute transport, osmotic stress and aquaporins involved during ovarian tissue vitrification.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by CNPq (UNIVERSAL: grant number 475628/2011-0). Antonia Debora Sales is a recipient of a grant from FUNCAP Brazil. In addition, Ana Paula Ribeiro Rodrigues and José Ricardo de Figueiredo are recipients of a grant from CNPq Brazil.
Trial registration number: 475628/2011-0.
Keywords: ovarian, cryopreservation, aquaporins, expression, in vitro culture

P-485 Planned and unplanned fertility in breast cancer survivors: prospective cohort study in breast cancer patients following cancer treatment

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Study question: The aim of this study was to establish the incidence of conception in women of reproductive age group following breast cancer treatment, including those who attempted and those who did not plan a pregnancy
Summary answer: Even though significant proportion of women wished to conceive, only a small proportion actively attempted to do so with half of them being successful. Unexpectedly we identified several unplanned pregnancies that were terminated, causing undue distress for these women.
What is known already: There are numerous studies highlighting the importance of offering fertility preservation to women of reproductive age diagnosed with breast cancer, which is one of the most common cancer in this age group. There are also multiple surveys assessing the attitude of cancer patients and medical professionals towards the option of fertility preservation at the time of diagnosis. However, the pregnancy rate after successful cancer treatment has not been sufficiently studied.
Study design, size, duration: A questionnaire survey regarding fertility intent and pregnancies achieved was prospectively conducted between July 2011 and December 2013 in a tertiary breast cancer centre in South-East London, UK.
Participants/materials, setting, methods: Women between 25 and 42 years of age at the time of cancer diagnosis from 2000-2010 who continued to attend for follow up were identified from the local breast cancer registry. A questionnaire was given to 282 women at one of their outpatient appointments.
Main results and the role of chance: The mean age of patients at the time of cancer diagnosis was 37.2 ± 3.9 years. Systemic chemo and hormonal therapies were given to 212 (75%) and 124 (44%) of responders respectively. 136 (48%) women were childless at the time of cancer diagnosis. At the time of the survey 90 (32%) patients wished to have children. Pregnancy was actively attempted by only 28 (10%) women and live birth achieved by 15 (54%). However, there were also 11 pregnancies reported among those who did not plan to conceive: 7 were terminated, 2 miscarried and 2 resulted in live birth. Among patients who did not wish to conceive 146/198 (74%) reported not using any contraception.
Limitations, reason for caution: Even though this cohort is a large representation from the cancer network of breast cancer patients of reproductive age, there is still a possibility of population bias.
Wider implications of the findings: In spite of high rate of childless women diagnosed with breast cancer, only 10% of women subsequently attempted to conceive 6.9 ± 3.1 years since their treatment with 32% still considering pregnancy. Medical professionals must be increasingly aware of the need to provide contraceptive advice and risk of pregnancy following chemotherapy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Supported by a grant from Guy's and St Thomas's Charity.
Trial registration number: NA.
Keywords: breast cancer, fertility, post chemotherapy, survivors

P-486 Letrozole co-treatment does not jeopardize effectiveness of controlled ovarian hyperstimulation for oocyte vitrification in breast cancer patients

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Study question: To assess letrozole effect on OCH effectiveness for oocyte vitrification in breast cancer patients in terms of number of retrieved oocytes
Summary answer: Letrozole addition to ovarian estimulation protocol in breast cancer patients treated for oocyte vitrification allows to mantain low estradiol levels and does not reduce total and mature oocyte number compared to healthy IVF controls.
What is known already: Letrozole co-treatment during ovarian hyperestimulation for oocyte cryopreservation is generally applied in breast cancer patients with positive estrogen receptor tumours, in order to diminish serum estradiol increase due to gonadotropin effect. This strategy focuses on safety, but it could have a non desirable effect on effectivity of the procedure, in terms of reduced number of retrieved oocytes.
Study design, size, duration: Retrospective cohort study comparing 96 breast cancer patients undergoing controlled ovarian hyperstimulation for oocyte vitrification and 350 IVF healthy controls. Main results considered were numbers of total and mature oocytes. Study was performed between 2010 and 2014. Follow-up was held from starting of stimulation to oocyte retrieval.
Participants/materials, setting, methods: A cohort of 96 breast cancer patients undergoing oocyte vitrification recieved letrozole, low-medium FSH doses and GnRH-antagonist protocol in a tertiaty university center fertility preservation program. This group was compared with a randomly-selected cohort of 350 IVF patients, matched by age and AFC. Median comparisons and relative risk estimation were used.
Main results and the role of chance: Letrozole-exposed patients and IVF controls were comparable in age, antral follicular count, starting FSH dose and total FSH consumption. In breast cancer patients, ovarian stimulation was significantly longer (medians: 9 vs 8 days; $p = 0,04$), final serum estradiol was predictably lower (medians: 223 vs 1327 pg/mL; $p < 0,001$), and number of total retrieved oocytes was significantly higher (medians: 10 vs 8 oocytes; $p = 0,04$), although medians of mature oocytes were comparable (7 vs 6; $p = 0,097$). Letrozole exposition was not associated with an increase of cancellation rate (9/96 vs 38/350; RR:0,86; 95%CI:0,43-1,72; $p = 0,41$). No significant differences were observed in probability of obtaining less than 5 oocytes (17/87 vs 75/312; RR:0,81; 95% CI: 0,5-1,3; $p = 0,23$) or less than 12 oocytes (55/87 vs 226/312; RR:0,87; 95% CI: 0,73-1,03; $p = 0,064$).
Limitations, reason for caution: Controls were matched by age-stratified randomized selection, to ensure control of potential confounding effects.

We have not considered live-birth rate as main outcome because of necessary delaying of pregnancy in breast cancer patients. Even though observed effect of letrozole was not unfavourable, statistical power to assess hypothesized effect was low.
Wider implications of the findings: Randomized clinical trials focused on letrozole co-treatment effect on effectiveness of ovarian controlled hyperstimulation for fertility preservation are lacking. Most of available studies are retrospective cohort designs of undersized samples, with variable degree of bias control and heterogeneous conclusions. Our data, resulting of analysis of a large sample, doesn't support a deleterious effect of letrozole on oocyte availability for vitrification.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital General Universitario Gregorio Marañón.
Trial registration number: Retrospective cohort study.
Keywords: fertility preservation, letrozole co-treatment, breast cancer, effectiveness

P-487 Spermatogonial quantity during prepubertal life

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Study question: Can we determine reference values for spermatogonial quantity in the testis during healthy pre-pubertal life?
Summary answer: Spermatogonial quantity, as defined by the number of spermatogonia per tubular cross section (S/T) and spermatogonial density (S/cm^3), decreases during the first year of life and gradually increases afterwards. The findings can be used as reference for the spermatogonial number in testis biopsies of boys in different age groups.
What is known already: Chemotherapy or radiotherapy can cause damage in spermatogonial stem cells resulting in infertility. Cryopreservation of testicular biopsies to preserve spermatogonial stem cells is offered to prepubertal boys who are at risk of becoming infertile. Studies on S/T and S/cm^3 during prepubertal life only report on a limited number of boys and show contrasting results.
Study design, size, duration: A systematic literature search in MEDLINE and EMBASE through November 2014 focusing on S/T and S/cm^3 in the testes of healthy prepubertal boys. All results from studies using the same method were pooled.
Participants/materials, setting, methods: Literature data on S/T and S/cm^3 from healthy prepubertal boys from 0 to 15 years of age. To estimate S/T and S/cm^3 at different age groups, a polynomial meta-regression analysis was performed combining data described for those age groups.
Main results and the role of chance: From a total of 135 papers, 19 studies were included in our analysis of which four could be used to collect data on S/T and two for S/cm^3. Data revealed a 50% decrease in S/T over the first three years of life. From the age of 3 to 7 years a two-fold increase in cells/T was found with a small decrease at the end of this period. After that, a gradual increase appears, which seemed to accelerate exponential at the age of 10 years to puberty. S/cm^3 showed a similar pattern with a decline during the first three years after birth followed by a gradual increase reaching a maximum at 14-15 years of age.
Limitations, reason for caution: The low number of studies with limited number of boys at different ages. The data in the original papers is reported in age groups spanning 4 to 5 year intervals such that estimates cannot be provided per year of age.
Wider implications of the findings: Our estimates of spermatogonial quantity throughout prepubertal life can be a useful reference in future clinical studies. This is a first step towards determining the required testis volume from a prepubertal testis to obtain sufficient spermatogonia for successful autotransplantation to the adult testis later in life.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by the Dutch foundation Children Cancer-Free Foundation (KiKa86) and the Netherlands Organization for Health Research and Development (ZonMW TAS 116003002).
Trial registration number: NA.
Keywords: fertility preservation, spermatogonial quantity, prepuberty, testis

P-488 Dynamics of follicular growth after xenotransplantation of cryopreserved/thawed human ovarian tissue in SCID mice

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Study question: How does xenotransplantation influence the follicular recruitment and growth in cryopreserved/thawed human ovarian tissue?
Summary answer: The higher proportion of growing follicles compared to resting follicles that was observed after xenotransplantation is most likely not due to apoptosis.
What is known already: Human ovarian tissue xenotransplantation into severe combined immunodeficient (SCID) mice is a good method to assess follicular development after cryopreservation and transplantation in the context of fertility preservation. In animal studies, a rapid decrease of primordial follicles after transplantation was observed. The mechanism behind this phenomenon is still unclear.
Study design, size, duration: A portion of cryopreserved/thawed human ovarian tissue samples donated from female cancer patients (n = 14) was xenotransplanted into 6-week-old SCID mice (n = 46) for 4 and 12 weeks.
Participants/materials, setting, methods: Two 3-mm-pieces of tissue were transplanted into a subcutaneous-neck-pouch of 6-week-old ovariectomized SCID mice for 4 and 12 weeks. By the end of the observation periods, grafts were recovered to be analyzed for follicle number, proliferation (Ki67) and apoptosis (TUNEL). Tissue directly after thawing served as pregraft-control.
Main results and the role of chance: Forty-four out of 46 mice (95.6%) survived through the observation periods. Graft recovery rate was 93.5% as 86 from 92 grafts were found. The recovered grafts were macroscopically comparable to pregraft-controls with some visible vascularization. After 4 weeks, the percentage of primordial follicles was reduced significantly to 43.8% ($P < 0.05$) compared to pregraft-controls (89.7%, $P < 0.05$), while the proportion of growing follicles was significantly increased to 25.2% in 4-week-grafts compared to only 1% in pregraft-controls. The same trend continued within the 12-week-observation period. Antrum formation was observed within 12 weeks. Ki67 immunohistochemistry showed that 75% of the follicles were proliferating after 4 weeks of grafting compared to only 10% in pregraft-controls. No TUNEL-positive follicles were observed in all groups.
Limitations, reason for caution: In this study, the early post-transplantation periods (0-4 weeks) and the molecular mechanisms of the follicular recruitment were not studied. Furthermore, although xenotransplantation is the closest experimental approach, it is also still difficult to translate the results from xenotransplantation to patients' settings.
Wider implications of the findings: The methods used in this study can be applied as a quality control tool for cryopreserved/thawed human ovarian tissue in fertility preservation. This study showed fast follicular growth after xenotransplantation leading to a decrease in primordial follicles and increase in growing follicles. Interestingly, this seems not to be due to apoptosis. Studies on the mechanism of follicular recruitment after grafting are important to improve the grafts quality.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – Tyrolean Research Foundation.
Trial registration number: NA.
Keywords: ovarian tissue cryopreservation, follicular growth, apoptosis, xenotransplantation

P-489 The role of menstrual cycle phase and AMH levels in young breast cancer patients whose ovarian tissue was cryopreserved for fertility preservation

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Study question: Does the phase of menstrual cycle affect the result of combined procedure that means ovarian tissue cryopreservation and oocyte from an excised one side whole ovary for young breast cancer patients who desire to preserve their fertility ?

Summary answer: The phase of menstrual cycle does not significantly affect for the efficacy of combined procedure either the number of collected oocyte, survival and maturation rate. The number of oocytes extracted from cryopreserved ovarian tissue is well correlated with the anti-Müllerian hormone (AMH) levels and age of patients.
What is known already: The researchers reported that the number of immature oocytes that could be retrieved from a partially excised ovary did not depend upon the menstrual cycle when using the "combined procedure".
Study design, size, duration: Data were retrospectively obtained from the clinical records of breast cancer patients who were referred to the Fertility Preservation Outpatients Clinic at the Center for Reproductive Medicine, Department of Obstetrics and Gynecology of our university hospital between February 2010 and September 2014.
Participants/materials, setting, methods: The patients were 35 of breast cancer patients who received ovarian tissue cryopreservation for preserve their fertility. Mostly participants who received ovary tissue cryopreservation had not enough time for oocyte (or embryo) cryopreservation.
Main results and the role of chance: The patients' mean age was 33.7 (± 3.7) years, mean serum AMH concentration was 3.5 (± 2.4) ng/ml, and mean number of extracted oocytes was 7.7 (± 5.9). The phase of menstruation (follicular or luteal) did not affect either the number of oocytes extracted nor oocyte survival or maturation rates. Likewise, the number of oocytes that could be extracted was not affected by the type of laparoscopic procedure (multiple-port or single-incision laparoscopy) or the molecular subtype of breast cancer (either Luminal A or B). Analysis revealed that the number of extracted oocytes was well-correlated with the patient's AMH serum level and age (coefficient of correlation: 0.60 and -0.48, respectively)
Limitations, reason for caution: It is need to clarify the effect of "combined procedure" including assessment of fertility rate and live birth rate based on more large scale study. And also, it is need to assess about the cryopreserved ovarian tissue after thawing and transplantation to patients.
Wider implications of the findings: According to our study that is the most large size retrospective study about "combined procedure" in the young breast cancer patients at present, we conclude that the outcome of the "combined procedure" primarily depends upon the patient's serum AMH level and age. Importantly, the "combined procedure" may be used during any phase of the menstrual cycle to preserve the fertility of breast cancer patients.
Study funding/competing interest(s): Funding by University(ies) – None.
Trial registration number: NA.
Keywords: fertility preservation, ovarian tissue cryopreservation, combined procedure, breast cancer

P-490 Anti mullerian hormone (AMH) co-treatment to prevent chemotherapy-induced follicle activation and loss

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Study question: Chemotherapy induces activation and loss of the ovarian follicle reserve, partly due to the destruction of the growing follicles and the removal of their negative regulation. Could replacement of negative regulator, AMH, at the time of treatment prevent chemotherapy-induced follicle activation and loss of the ovarian reserve?
Summary answer: Chemotherapy induces activation and loss of the ovarian follicle reserve, partly due to the destruction of the growing follicles and the removal of their negative regulation. Could replacement of negative regulator, AMH, at the time of treatment prevent chemotherapy-induced follicle activation and loss of the ovarian reserve?
What is known already: A number of chemotherapy classes have been shown to induce follicle loss by triggering activation of the dormant primordial follicles. This occurs via two mechanisms; upregulation of the PI3K-Akt signaling pathway, and death of growing follicles which are responsible for producing AMH, a negative regulator of follicle activation.
Study design, size, duration: *In vivo* treatment of 12 day Balb/C mice with Cyclophosphamide (Cy)/PBS (n = 10 ovaries, 3 repeat experiments), and *ex vivo* culture of whole mouse ovaries with Cy metabolite Phosphoramide mustard (PM)/Media alone, + /- AMH (n = 16). Ovaries were analyzed 1, 4, and 7 days post treatment.
Participants/materials, setting, methods: AMH mRNA expression levels in mouse ovaries were measured (qRTPCR) following *in vivo* Cy treatment (150mg/kg). *Ex vivo*, ovaries were exposed to 20ug/ml PM for 2 hours, + /- 100ng/ml AMH. PI3K proteins AKT, rpS6 were assessed after 24 hours in culture, and differential follicle counts conducted after 4 and 7 days.
Main results and the role of chance: AMH mRNA expression in the ovary decreases after *in vivo* treatment with Cy, reaching their lowest levels at 12 hours (0.5 relative expression), before rebounding at 24 hours and progressively increasing so that by 7 days levels were two times that in controls. Ovaries exposed *ex vivo* to PM showed a significant reduction in primordial follicles per ovary compared to controls (724 ± 50 vs. 1534 ± 176; $p < 0.01$), which was partially rescued in ovaries co-treated with AMH (1081 ± 131, $p < 0.05$). The ratio of growing:dormant follicles in PM treated ovaries was double that in controls (0.65 vs. 0.3, $p < 0.001$), reflecting the activation of the follicle pool, and this was improved in ovaries co-treated with AMH (0.33, $p < 0.05$). There was no change in expression of PI3K pathway proteins at 24 hours.
Limitations, reason for caution: Protein and RNA measurements were conducted on whole ovary lysates, not isolated follicles, so the specific origin of mRNA expression changes cannot be attributed. We chose not to use isolated follicles since it is model that cannot accurately reflect the *in vivo* state.
Wider implications of the findings: The loss of activation-suppressor AMH in the immediate short term after Cy treatment sheds light on the mechanism behind the follicle activation and 'burn-out'. This proof of principle study suggests that replacement of AMH during chemotherapy treatment may prevent/reduce follicle loss. Following testing in primate/human models, such treatment would provide notable advantages over existing techniques, being non-invasive, suitable for patients of all ages, not dependent on ART, and able to prevent the endocrine related side effects of POF.
Study funding/competing interest(s): Funding by national/international organization(s) – Israel Science Foundation.
Trial registration number: NA.

P-491 Safety of cryopreserved ovarian tissue autotransplantation in leukaemia patients

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Study question: In leukaemia patients ovarian tissue was cryopreserved at remission of disease. May ovarian tissue transplantation be considered safe in this condition?
Summary answer: The risk of finding malignant cells in the ovarian tissue of leukaemia patients cannot be excluded also when the tissue has been cryopreserved at disease remission.
What is known already: An important aspect of cryopreserved ovarian tissue autotransplantation in cured cancer patients is the potential reintroduction of tumour cells with the risk of causing a recurrence of illness. This risk is particularly high in leukaemia patients, for whom the cryopreservation of ovarian tissue might be performed after a few cycles of chemotherapy when testing indicates no evidence of leukaemic cells in the blood.
Study design, size, duration: Cryopreserved ovarian tissue of three patients suffering from different types of leukaemia, Acute Lymphoblastic Leukaemia-ALL, Acute Myeloid Leukaemia-AML, Chronic Myeloid Leukaemia-CML, was checked for the presence of malignant cells by histological and molecular analysis. The presence of specific disease-markers was investigated to allow a highly sensitive analysis of the tissue.
Participants/materials, setting, methods: For AML and CML patients a disease-specific marker (Wilms-Tumor-1 overexpression and chimeric Bcr-Abl gene, respectively) allowed to perform a sensitive evaluation by quantitative Real-Time PCR. In the ALL patient no disease-marker was available at diagnosis so a clonality test for the assessment of T-cell-receptor-rearrangement was evaluated with traditional-capillary-electrophoresis and massive-parallel-sequencing (MPS-Invivoscribe's LymphoTrack).

Main results and the role of chance: At the time of tissue retrieval, the ALL and AML patients were in morphological and molecular disease remission, while the third was only in morphological remission. Histological examination did not reveal malignant cells in ovarian tissues of the patients. As regards molecular investigation, in ALL patient the clonality test (sensitivity10^{-2}) was negative as well as the higher sensitive massive-parallel-sequencing method (sensitivity10^{-5}-10^{-6}). The quantitative Real-Time PCR gave a positive result in both AML and CML patients.
Limitations, reason for caution: Due to the lack of onset material in the ALL patient, only the standard low sensitive clonality test was used although its negativity cannot exclude the presence of malignant cells in cryopreserved samples. Massive-parallel-sequencing, despite a highest sensitivity, is still experimental. This case well embodies how the test-limitations should be amply discussed with patients.
Wider implications of the findings: Only highly sensitive tests allow an accurate monitoring pre-transplantation of cryoprserved ovarian tissue and can minimize the possibility of wrong evaluation (under-estimation) of neoplastic contamination.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Sant'Orsola Malpighi Hospital of Bologna. Competing interest: none.
Trial registration number: Clinical trial 74/2001/O – EM 180/214/O.
Keywords: ovarian tissue cryopreservation, leukaemia, autotransplantation, Real-Time PCR

P-492 The influence of pregnancy on the prognosis of endometrial atypical hyperplasia and adenocarcinoma with assisted reproductive technology after fertility-sparing therapy: a systematic review and meta-analysis

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Study question: Does pregnancy has influence on the prognoses of young women (< 40 years old) with early-stage endometrial cancer (EC) and atypical complex hyperplasia (AH) who were treated by conservative management followed by assisted reproductive technology (ART) ?
Summary answer: Live birth rates were encouraging using ART in young women with EC or AH who desired fertility after conservative management.
What is known already: A total of 5% of endometrial carcinoma is diagnosed under the age of 40 years, and over 70% of patients are nulliparous at diagnosis. The dissection approach is unacceptable for these women who wish to maintain their fertility.
Study design, size, duration: A meta-analysis of observational studies with a random or fixed-effects model was carried out. 527 women from 23 studies included 201 women to assess how prognosis might be influenced by pregnancy.
Participants/materials, setting, methods: This systematic review was conducted in accordance with the PRISMA guidelines and was also in accordance with the a priori protocol agreed upon by all authors. The population of interest in this systematic review included women with AH or early clinical stage EC (1988 International Federation of Gynecology and Obstetrics stage IA). The intervention queried was conservative treatment, and the outcomes were evidence of live births or recurrence. The following electronic databases were searched: PubMed (1950 to 2014) and Web of Science conference proceedings (ISI Proceedings, 1990 to 2014).
Main results and the role of chance: Analysis of no pregnancy studies as the reference group pointed to a statistically significant positive association between pregnancy and decreased risk for recurrence (RR) of 0.56, 95% confidence interval (CI): 0.37-0.84]. Eighteen studies including 104 women enabled determination of live birth rates of women with early-stage EC (79 women) and AH (25 women) following treatment with conservative management followed by ART. For patients with AH, 25 women were treated with ART after pathological remission of disease. Thirteen women achieved intrauterine pregnancies, and nine had 10 live births, with a pooled live birth rate of 0.32 with insignificant heterogeneity (P = 0.925). For those with EC, 79 women were treated by ART, 61 by IVF-ET, 12 by IUI, and 6 by ICSI. Fifty-seven women had intrauterine pregnancies, and 45 had 52 live births, with a pooled live birth rate of 0.60 and insignificant heterogeneity (P = 0.923).
Limitations, reason for caution: Our systematic approach included published literature that had only observational evidence, so prospective randomized trials in larger numbers of patients and with longer follow-up times are warranted to evaluate the effects of fertility-sparing treatments.
Wider implications of the findings: Live birth rates were encouraging using ART in young women with EC or AH who desired fertility after conservative management. Resulting pregnancies could reduce recurrent risk of AH and EC. Additionally, minimizing the time interval between treatment termination and pregnancy was important.
Study funding/competing interest(s): Funding by national/international organization(s) – This project was funded by National Natural Science Foundation Project (81471520), State Scholarship Fund (2011911033), Beijing Natural Science Foundation Project (5122015), and Beijing Project of Training High-level Medical Technical Personnel in Health System. The authors declare no conflict of interest.
Trial registration number: No.
Keywords: fertility-sparing treatment, endometrial cancer, endometrial atypical hyperplasia, live births, systematic review

PARAMEDICAL - LABORATORY

P-493 Comparison between analytical scale and graduated serological pipette for semen volume analysis: a cross sectional study

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Study question: Is there any difference between the measurement of semen volume by weighing the specimen in the container in which it is collected and aspirating the specimen from the container with a serological graduated pipette?
Summary answer: Measuring volume by aspirating the speciment from the container into a pipette underestimate the ejaculate volume. This is due to retention of part of the volume in the container. The magnitude of effect is higher in specimens with high viscosity.
What is known already: Precise measurement of ejaculate volume is essential to determine the total sperm count. Among the semen parameters, total sperm count is one of the most significant as it relates to testicular volume. In addition to the total concentration, viscosity is also a decisive factor when evaluating a semen sample. Highly viscous specimens adheres strongly to the pipette wall and might interfere with final volume determination. In its updated 2010 edition, the World Health Organization manual for the examination and processing of human semen recommends that volume should be measured by weighting the specimen in the vessel in which it is collected, which is more labor-intensive than simply reading the volume from a graduated pipette.
Study design, size, duration: A cross sectional study was performed in an ISO 9001 certified Andrology Laboratory enrolled in external quality control program. Data was obtained from 654 consecutive male patients referred for semen analysis evaluation from September 2013 to December 2014.
Participants/materials, setting, methods: Semen specimens were collected by masturbation after a 2-5 ejaculatory abstinence period into sterile plastic vessels (Fertility, USA) at the collection room adjacent to the Andrology Laboratory. Containers were individually pre-weighed using a calibrated analytical balance (Mettler-Toledo, USA), and weigh was recorded using a label that was attached to the empty container before weighting. Volume was calculated by subtracting the weigh of the container from its weigh with semen in it after liquefaction for 30 minutes at 37°C (v1). Then, volume (v2) was measured by aspirating the specimen into a 10-mL sterile polyestirene graduated pipette (0.1 mL accuracy). Viscosity was determined by assessing the length of any thread, and was considered high when the semen drop formed a thread exceeding 2 cm.
Main results and the role of chance: Semen volume was higher when measurements were carried out by weighting the container with semen in it (3.52 ± 1.68 mL) compared with aspiration of the specimen into a serological pipette (3.24 ± 1.63; $p = 0.002$). Overall, the difference in volume between the two methods was 17%. Of the 654 specimens, 294 (45%) were classified as having high viscosity. The v1 measurements in both hyperviscous and normal viscosity specimens (3.48 ± 1.62 and 3.55 ± 1.72 mL) were higher than v2

(3.17 ± 1.59 and 3.29 ± 1.67 mL; $p = 0.02$). The magnitude of this difference was higher in hyperviscous specimens compared with normal ones (d = 0.20; $p = 0.04$).

Limitations, reason for caution: Results could vary by increasing the liquefaction time. In addition, we have not examined the impact of microscopic semen characteristics, such as sperm count, on the volume determination.

Wider implications of the findings: Measurement of ejaculate volume by weighting the container with semen in it allows the precise determination of total sperm count and the reduction of analytical error in semen analysis. As high viscosity interferes with determination of semen volume, measuring volume by aspirating the specimen container into a pipette is not recommended.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Androfert

Trial registration number: NA.

Keywords: semen quality, WHO 2010, serological pippete, analytical scale

P-494 Relationship between body mass index (BMI) and semen analysis results and effect of World Health Organization criteria for categorization of semen parameters: a cross-sectional study

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Study question: Does body mass index impact on the conventional semen parameters of men referred for infertility evaluation? What is the effect of the new 2010 WHO semen analysis reference values on reclassifcation of previous semen analysis parameters as per WHO 1999 reference values? Does BMI influence the proportion of patients reclassified according to the WHO criteria for semen analyses?

Summary answer: Total sperm count and progressive motility were significantly lower in men with high BMI (> 30 Kg/m2) compared with eutrophic men. The 2010 reference values result in approximately 30% of men with abnormal semen parameters as per the WHO 1999 being reclassified as 'normal', but BMI does not seem to impact on reclassification.

What is known already: Obesity affects about 15-30% of men at reproductive age. It has been suggested that men with high BMI are at risk of subfertility, but studies focusing on semen parameters of such patients are scarce. Semen analysis is still the sole marker for many of the male/couple referrals to infertility clinics, and the World Health Organization criteria is the most used guideline for reference values.

Study design, size, duration: A cross-sectional study was performed in an ISO 9001 certified Andrology Laboratory enrolled in External Quality Control. Data was obtained from 887 men seeking infertility evaluation from 2009 to 2013.

Participants/materials, setting, methods: Men referred for infertility evaluation were included. Patient height and weight were recorded on day of semen collection for BMI calculation. Semen analyses were carried out according to the WHO guidelines, and semen analysis values were compared based on the 2010 versus 1999 reference criteria, both overall and after stratification by BMI, i.e., eutrophic (< 25kg/m^2), overweight (25– < 30kg/m^2) and obese (> 30kg/m^2).

Main results and the role of chance: Median total sperm count and progressive motility were significantly lower in obese men (64.8 M/mL and 54%) compared with eutrophic men (15.7 M/mL and 46.3%; $p = 0.03$). An inverse relationship was observed between BMI and total count (r = -0.11; $p < 0.01$), and BMI and progressive motility (r = -0.09; $p = 0.01$). Overall, 270 patients (30.4%) who had at least one parameter below the reference value according to the WHO 1999 criteria were reclassified to having all parameters at or above the 2010 criteria. Of the men, 9.4%, 6.3%, 22%, 53% and 46% would change classification for volume, count, motility and morphology, respectively. BMI was shown not to play a significant role in these reclassifications.

Limitations, reason for caution: Our findings cannot be extrapolated to the general population because dataset comprised solely men referred for infertility evaluation. Co-founding factors such as age, co-morbidity and other life-style factors such as smoking were not assessed.

Wider implications of the findings: Obesity seems to be associated with decreased semen parameters in men referred for infertility evaluation. The criteria adopted as a reference for semen analysis values impact in the proportion of men classified as normal if status is based on semen analysis alone. However, reclassification as per WHO criteria seems to be independent of BMI. Men facing infertility should be counseled that obesity may pose an additional obstacle for natural fatherhood.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Androfert

Trial registration number: NA.

Keywords: BMI, semen analysis, WHO criteria, subfertility, overweight

P-495 Improving pregnancy rates in IUI procedure

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Study question: Which contributing factors will help improve rates for an IUI program using husbands sperm?

Summary answer: Multiple factors associated with a successful pregnancy were: age of woman, having ≥ 5 million TPMS (Total Progressive Motile Sperm), insemination with at least 50% Grade A sperm, a mean of 1.7 follicles as in 45.8% of successful cohort, while.using hMG protocol with IUI at 29 hour hCG trigger.

What is known already: IUI is universally practiced but the pregnancy rates are not well understood, with diverse protocols and practices, sometimes with higher order multiple births. Based on poor outcomes of about 6%/cycle pregnancy rates using clomid stimulation one RCT labelled IUI as ineffective despite the existence of a much larger HFEA database yielding on average 13%/cycle success rates. IUI has also been blamed for higher order multiples births, and to erroneously recommend IVF instead.

Study design, size, duration: The retrospective cohort study from a large teaching hospital charts the increasing pregnancy rates from 2009 to 2014. Improved outcomes were associated with a shift away from clomid to hMG stimulated cycles, and adding a `consecutive ejaculate' to the first ejaculate in the sperm preparation procedure, and this unique approach has not been reported in clinical application before. The IUI outcomes for 2014 relating to 117 IUI cycles and 73 women will be discussed in detail.

Participants/materials, setting, methods: A qualitative analyses from 2009-2014 charted improving pregnancy outcomes, followed by detailed retrospective analyses from January-September 2014 included 117 cycles, 73 women and 24 clinical pregnancies. The unique clinical use of a `consecutive ejaculate' to enhance the TPMS with a realistic hMG protocol made IUI a viable procedure.

Main results and the role of chance: After IUI the pregnancy rates were 20.5%/cycle and 32.9% of the women became pregnant. The result exceeded the UK average of 13%/cycle, which in turn exceeded the the 6%/cycle from the only published RCT to condemn IUI procedure as ineffective. Our result is probably the best available data, robust, maintainable, and follows the improving trend over the years. The unique use of `consecutive ejaculates' has contributed towards allowing 87.5% of the inseminations reach a threshold of ≥ 5 million TMPS, which has been quoted as a threshold for IUI to be realistic, and 54% of the pregnancies were associated with the `consecutive ejaculate.' Insemination after 29 hour post hCG trigger seems to be positively associated with pregnancies.

Limitations, reason for caution: This is a retrospective study needing to be scaled up and preferably entered in a prospective RCT. Patients serve as their own control.

Wider implications of the findings: This is the best available data for IUI pregnancy rates, which if replicated on a large scale would challenge some IVF results, while allowing for a significant reduction of multiple birth rates. The multiple births and OHSS were none existent in our cohort and inseminating with 2-3 follicles may not present a significant risk as transferring 2-3 embryos as in IVF cycles. Consecutive ejaculate application is unique and will prove beneficial globally.

Study funding/competing interest(s): Funding by hospital/clinic(s) – North Middlesex University Hospital, London, UK

Trial registration number: NA.

Keywords: iui, pregnancy rate, consecutive ejaculate, hMG, hCG

P-496 Oocyte vitrification; case study with fertilization only in vitrified/warmed oocytes and failed fertilization in all un-vitrified oocytes in two consecutive ICSI cycles

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Study question: When validating oocyte vitrification we examined survival rate, fertilization potential and embryo development after vitrification. The aim was to implement standard operation procedures for oocyte vitrification for female fertility preservation, IVF emergency situations and possibility to start donor oocyte bank.
Summary answer: Survival rate were 90 %, fertilization rate 70 % and 42 % of the embryos classified as high quality embryos (HQE). 7 transfers resulted in 4 live births. One participant gave birth to a baby after failed fertilization in all un-vitrified oocytes in two consecutive treatment cycles.
What is known already: Oocyte vitrification technique can be challenging and every clinic needs to validate a protocol for vitrification, warming and fertilization to ensure the outcome. Failed fertilization after ICSI is rare, occurring in 1-3 % of cycles. Failed oocyte activation is the most common cause. Vitrification will induce stress in the oocyte due to mechanical, thermal and chemical stressors. So far, no literature has described failed fertilization counteracted by vitrification.
Study design, size, duration: This was a sibling study performed during autumn 2013. Participants were asked to donate one oocyte if > 8 oocytes were retrieved. One oocyte was randomly selected for vitrification/warming, with the remaining cohort serving as controls. All embryology procedures followed standard operating procedures.
Participants/materials, setting, methods: 29 ICSI patients at the Fertility Clinic at Örebro University Hospital were included in the study. Informed written consent was obtained. Vitrification/warming were done 1 h after OPU using KitaZato media, with ICSI 2 h post-warming. All embryo development culture and analysis was done in EmbryoScope.
Main results and the role of chance: The validation included 30 oocytes from 29 patients. Survival rate was 90 %, fertilization rate 70 %, with 42 % of embryos developing into HQE. For 2013, the control figures for the clinic were 57 % fertilization rate and 44 % HQE. 7 embryos from vitrified oocytes have been transferred, resulting in live births of 4 babies. One female participated twice. First cycle resulted in 16 oocytes, 11 of which were mature. Only the vitrified oocyte fertilized, all control oocytes failed to fertilize. It was transferred as a day 5 morulae, no pregnancy achieved. Second cycle resulted in 11 oocytes, 9 mature. Again, only the vitrified oocyte fertilized. It was transferred as a day 2 four-cell embryo and resulted in the birth of a baby.
Limitations, reason for caution: Study outcome is in line with previous published key performance indicators (KPI) for oocyte vitrification for infertility patients. The high live birth rate indicates good performance and high competency amongst staff members performing oocyte vitrification. Oocyte vitrification KPI will be added for continuous benchmarking.
Wider implications of the findings: The study design is easily adoptable to clinics interested in oocyte vitrification validation. Patient recruitment was easy and participation high. Oocyte vitrification can be applied for fertility preservation, oocyte donor bank, or to resolve IVF emergencies. We found no literature describing failed fertilization after ICSI counteracted by vitrification. The exact same outcome in two consecutive cycles is intriguing and reduces likelihood of chance. A possible mechanism might be artificial oocyte activation by the vitrification procedure.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was supported by the Tissue Establishment of Region Örebro Län, Sweden.
Trial registration number: NA.
Keywords: oocyte vitrification, fertilization only in vitrified/warmed oocytes

P-497 Improved accuracy of embryo scoring using morphokinetic compared with strict morphology

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Study question: Can morphokinetics provide higher objectivity and less reproducibility compared to morphology? Traditional static scoring and selection of embryos using microscopy has reduced reliability and high inter- and intra-observer variability. Morphokinetics might provide a more objective tool. This study compares morphology and morphokinetics on day 5 blastocysts with known implantation data.
Summary answer: Morphokinetic showed improved accuracy compared with morphology. Observers agreed almost perfect on time lapse annotations, whereas for morphology observers showed only fair agreement. Repeated observations by the same observer were more reproducible for morphokinetics. Neither morphokinetics using a previous published model nor morphology correlated with implantation ability for transferred embryos.
What is known already: Morphology suffers from subjectivity and low reproducibility and a reduced potential to predict pregnancy chance. Morphokinetics based on time lapse images takes cleavage patterns into consideration, has been shown to have high objectivity and reproducibility. Changing embryo assessment technique requires validation. In this study, the first to our knowledge, we compare morphology and morphokinetics in terms of intra- and inter-operator variability. Validating morphokinetic parameters is the first step in building prediction models for embryo selection.
Study design, size, duration: This is a retrospective register study from Fertilitetsenheten, Örebro University Hospital, between 2012-2014. 100 transferred blastocysts with known implantation outcome from 100 patients were randomly selected. All embryos were analyzed four times in total, two times each by two experienced embryologist, two months apart, always blinded for previous assessments.
Participants/materials, setting, methods: Blastocysts were scored using Gardner Schoolcraft criteria and examined by detailed time-lapse analysis for pre-selected parameters using EmbryoScope software. ICC for morphokinetics and Cohen´s kappa for morphology was calculated to analyze reproducibility. Morphology and morphokinetics were later correlated with chance of implantation.
Main results and the role of chance: Mean inter-operational variability for morphokinetics were 0.897 (0.753 – 0.996, 95 % CI), which interprets as almost perfect agreement. Highest agreement was found for tPNf, t2, and t5, whereas tPNa and t9 + had slightly lower ICC. ICC for observer A was 0.911 and for observer B 0.901. For morphology, inter-operational agreement had a K value of 0.637, and intra-operational agreement for observer A was 0.477 and for observer B 0.525 (fair agreement). Highest agreement was found for trophodectoderm (0.706), followed by expansion grade (0.669) and inner cell mass (0.541). Ranking the embryos into subcategories using morphology and/or a previous published selection morphokinetic model showed low correlation with implantation.
Limitations, reason for caution: Validation was done on high quality embryos. We repeated the ICC calculations for additional 110 embryos from 20 patients regards of quality. Mean ICC were 0.884 (range 0.701 -0.978) between observer A and B. Therefore, we conclude that ICC for all examined parameters are robust regardless of embryo quality.
Wider implications of the findings: Morphokinetics showed improved accuracy compared with strict morphology. The commonly used morphokinetic parameters were robust and reproducible, therefore suitable in a selection model. However, applying a previously published model to rank embryos did not correlate with chance of implantation. We suggest that each clinic build their own selection model if applying time lapse analysis for embryo selection. The annotation technique per se needs to be validated for each operator to ensure inter- and intra-operational agreement.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Tissue Establishment of Region Örebro Län, Sweden.
Trial registration number: NA.
Keywords: morphokinetics, validation, morphology, embryo assessment

P-498 The blastocoele stage in human blastocysts can be improved by position of the trophectoderm biopsy

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Study question: Although the trophectoderm biopsy within human blastocysts is conducted in preimplantation genetic screening (PGS), can be the blastocoele stage in human blastocysts influenced by position of the trophectoderm biopsy?

Summary answer: The trophectoderm biopsy at near position from inner cell mass (ICM) is the best in order to improve the blastocoele stage in human blastocysts.
What is known already: The best method for the trophectoderm biopsy within human blastocysts in PGS is researched. However, the influence on human blastocysts by position of the trophectoderm biopsy is not yet investigated.
Study design, size, duration: An experimental prospective study was performed from January 2013 to September 2014 on 60 patients (median age 34.3 years) with infertility in our clinic. Furthermore, embryos abrogated with patient's informed consent were cultured to blastocyst stage (Day 5 – Day 7) and were used in the present study.
Participants/materials, setting, methods: Each patient was assigned by position of trophectoderm biopsy as follows. Group A: Near position from ICM ($n = 20$), Group B: Remote position from ICM ($n = 20$), Group C: Position between A and B ($n = 20$). Furthermore, the changes of blastocoele stage between the pre- and post-trophectoderm biopsy were studied.
Main results and the role of chance: The improvement rate of the blastocoele stage in Group A was higher than those in Group B significantly (90% versus 45%, respectively, $p = 0.048$: Fisher's exact test). Furthermore, multivariate analysis showed that the improvement of the blastocoele stage was influenced by position of the trophectoderm biopsy($p = 0.033$) and by the presence or absence of expansion in human blastocysts ($p < 0.001$), regardless of patient's age($p = 0.516$), aspiration pipette's size ($p = 0.781$) and days of human blastocysts in the pre- trophectoderm biopsy($p = 0.051$). Therefore, our findings show that the trophectoderm biopsy at near position from ICM is the best in order to improve the blastocoele stage in human blastocysts.
Limitations, reason for caution: There may be possible biases related to small sample size, although our study is an experimental prospective study.
Wider implications of the findings: This is the first study reporting that the blastocoele stage in human blastocysts can be improved by position of the trophectoderm biopsy. On the other hand, some researches indicate that the improvement of the blastocoele stage in human blastocysts associates with the improvement of clinical pregnancy outcomes. Therefore, a prospective randomized clinical trial is needed to investigate an association between clinical pregnancy outcomes and position of the trophectoderm biopsy in human blastocysts.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Oak Clinic Group.
Trial registration number: NA.
Keywords: human blastocysts, trophectoderm biopsy, blastocoele stage, ICM, preimplantation genetic screening (PGS)

P-499 Benefits of extending routine fresh embryo culture to day 7 on frozen embryo transfer pregnancy rates

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Study question: What effect does extending the routine culture period to day 7 have on frozen embryo transfer (FET) pregnancy rates?
Summary answer: To date, after 5 months of extending routine culture of fresh embryos to day 7 an additional 80 good quality blastocysts have been vitrified and pregnancies have been achieved from subsequent FET cycles.
What is known already: Studies have shown that a reduction of implantation rate in fresh cycles where day 6 embryos are transferred can be attributed to the asynchrony of the endometrial lining at this stage of the cycle, rather than to a slower embryo development rate (Shapiro *et al* 2008). Based on this data and possibly historical poor outcome from slow freezing of blastocysts, embryos were not routinely cultured past day 6.
Study design, size, duration: In this study across 7 laboratories, 905 IVF cycles were completed and grade A or B blastocysts by day 7 were vitrified over a five month period. The number of blastocysts frozen on day 7, subsequent cryosurvival, biochemical and clinical outcomes were measured from FET cycles.
Participants/materials, setting, methods: All embryos were routinely cultured and assessed using the Gardner's Blastocyst Grading System (Gardener 2000). Grade A or B embryos were classified suitable for vitrification. Embryos that were not vitrified on day 5 were reassessed on day 6 and day 7 for vitrification using the same criteria.
Main results and the role of chance: Total number of embryos assessed on day 7 was 1474 and 80 blastocysts (5.4% of day 7 embryos) were suitable for vitrification for 67 patients. Embryos were vitrified on day 7 in 7.4% of total fresh cycles. For 19 patients 24 embryos were frozen on day 7 only. Day 7 vitrified embryos accounted for 6.7% of total number of embryos vitrified (day 5, 6 and 7) over this time period. To date, 7 day 7 embryos have been warmed, 6 embryos survived (86% cryosurvival) resulting in 5 patients receiving embryo transfer, 3 positive pregnancy (60% per FET) with 2 clinical pregnancies (40% per FET).
Limitations, reason for caution: At this stage of our study, we do not have data on the perinatal outcomes for pregnancies resulting from day 7 vitrified blastocysts.
Wider implications of the findings: The introduction of routinely vitrifying embryos that develop into blastocysts on day 7 may increase the cumulative pregnancy rate per stimulated cycle and embryos should not be discarded on day 5 or day 6 as they still have the potential to achieve an ongoing pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – City Fertility Centre, Brisbane, Australia.
Trial registration number: NA.
Keywords: day 7, vitrification

P-500 Pregnancy rates and take-home baby rates are not affected by damage of cells vitrified on day 2 when the loss is less than 25%

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Study question: Does the reduced number of blastomeres caused by cryopreservation and thawing procedures of 4-8 stage embryos affect *in vivo* embryo development and take-home baby rates?
Summary answer: There was no difference between intact embryos and embryos with less than 25% of cell damage on pregnancy rates and birth rates.
What is known already: Some reports demonstrated that similar implantation rates were obtained between intact early stage embryos cryopreserved on day 2-3 and those embryos with less than 25% cellular damage after slow freezing and thawing procedure. It is known to have higher survival rates in vitrification procedure than in slow freezing procedure of early cleavage stage embryos. The birth rates of partially damaged embryos have not been reported yet.
Study design, size, duration: A retrospective study has been conducted between the period of January 2010 and December 2013 on the survival rate of 1549 vitrified and thawed embryos which were cryopreserved on day 2.
Participants/materials, setting, methods: Embryos were divided into four groups (intact embryos, embryos with less than 25% cellular damage, 25%-50% damage and more than 50% damage). The relationship between cellular damage and pregnancy rates was analysed in the 1549 single embryo transfers. Birth rates were assessed for 1491 cases through questionnaires.
Main results and the role of chance: The biochemical pregnancy rates in 0%, ≤ 25%, 25-50% and > 50% of cellular damaged groups were 35.1% (460/1309), 34.4% (43/235), 26.2% (16/61) and 28.6% (4/14) respectively. The rates of gestational sac confirmation in each group were 21.1% (277/1309), 19.2% (24/125), 11.5% (7/61) and 7.1% (1/7). The fetal heartbeat rates in each group were 18.3% (239/1309), 19.2% (24/125), 9.8% (6/61) and 7.1% (1/14). The birth rates in each group were 15.1% (196/1299), 13.4% (16/119), 8.5% (5/59) and 7.1% (1/14). There was no difference between 0% and ≤ 25% groups on each parameter. When the cutoff volume of damage was defined as ≤ 25%, the rates of gestational sac confirmation and fetal heartbeat were significantly higher in embryos with ≤ 25% cellular damage than those with > 25% damage ($p = 0.030$ and $p = 0.047$).
Limitations, reason for caution: A limitation of this study is that the number of embryos in the > 50% group was insufficient to provide enough data for accurate study. To improve the accuracy of the data, it is necessary to perform a long-term study in the future.
Wider implications of the findings: The results suggest that embryos with less than 25% damage have the same potential to result in birth as an intact embryo. This study also demonstrated that a baby could be born from an early cleavage embryo with over 50% blastomere damage.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Hanabusa Women's Clinic.
Trial registration number: NA.
Keywords: vitrification, damaged embryo, pregnancy rate, birth rate

P-501 Times from insemination to three to four cells and to five cells are predictive markers for clinical pregnancy

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Study question: This study aimed to evaluate whether a morphokinetic study using time-lapse imaging can predict clinical pregnancy by comparing the time from insemination to cleavage.
Summary answer: Our study shows that the time from insemination to three to four cells (s2) and that to five cells (t5), as observed by the EmbryoScope (Unisense Fertilitech, Denmark), are predictive of clinical pregnancy, but cc2 is not predictive.
What is known already: Several recent publications have shown morphokinetics of embryos by time-lapse imaging as a new method for selecting good quality embryos or clarifying aneuploidy. These findings are based on the time to certain division of embryos. However, each facility used different instruments and cultures in a different manner. We attempted to verify if the criteria that were used by Meseguer (2011) could be used in a more universal situation in the Japanese laboratory.
Study design, size, duration: This study was performed retrospectively. A total of 48 couples (59 embryos) who underwent ICSI and embryo transfer were enrolled in this study from December 2012 to December 2014. The maternal age of participating couples was younger than 38 years, with less than three times of previous unsuccessful IVF.
Participants/materials, setting, methods: Embryos were observed by the EmbryoScope every 15 minutes. We compared the rate of clinical pregnancy between the in-range division group (w/i group) and the out of range division group (w/o group) at each cell division time. This range was based on the definition by Meseguer *et al*.
Main results and the role of chance: The total rate of pregnancy in all of the participants was 40.1% (24/59). There was no difference in cc2 between the w/i and w/o groups. The s2 w/i group (<0.76 h) had a significantly higher rate of pregnancy (56.5%) than the s2 w/o group (³0.76h, 30.6%, $p = 0.048$). The t5 w/i group (44.8–56.6 h after insemination) had a significantly higher ratio of obtaining clinical pregnancy (51.4%) than the t5 w/o group (25.0%, $p = 0.042$).
Limitations, reason for caution: Our results showed a good clinical pregnancy rate using embryos that were selected by time-lapse imaging. This suggests that the criteria of Meseguer using the EmbryoScope are effective for selecting good embryos. However, further clinical studies are required to determine if this method can be used in different environments.
Wider implications of the findings: This study verified the effectiveness of the criteria that were used by Meseguer *et al.* using a similar instrument and culture conditions. Our study shows that selection of embryos for transfer is possible by time-lapse imaging analysis that was defined by them, even in a different environment.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hanabusa Women's Clinic.
Trial registration number: NA.
Keywords: time-lapse, embryoscope

P-502 A piezo-micromanipulator in intracytoplasmic sperm injection (ICSI) is effective in older women

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Study question: This study aimed to determine if age affects the efficacy of piezo-assisted ICSI compared with conventional ICSI.
Summary answer: On day 6 in women older than 42 years old, the rate of blastocyst formation with piezo-assisted ICSI is significantly higher than that with conventional ICSI.
What is known already: Piezo-ICSI can achieve better fertilization and survival rates of fragile eggs compared with conventional ICSI because injury of oocytes is decreased as much as possible in piezo-ICSI. Generally, the fertilization rate of eggs depends on the age of women because eggs in older women are more fragile than those in younger women. However, there are few studies regarding appropriate indications for this method.
Study design, size, duration: A total of 847 mature oocytes from 98 cycles were used for this study from October 2013 to August 2014. At least four oocytes were retrieved from one cycle. Retrieved oocytes were divided into two groups: half of the oocytes underwent piezo-ICSI and the other half underwent conventional ICSI.
Participants/materials, setting, methods: Confirmation of fertilization in eggs was performed the next day, and then eggs were observed and graded on days 2, 5, and 6. Percentages of fertilization, degeneration after ICSI, good embryos, blastocysts, and good blastocysts were evaluated. Results were analyzed by age using the chi-square method.
Main results and the role of chance: Conventional ICSI and piezo-ICSI were used in 428 eggs and 419 eggs, respectively. The age of women ranged from 38 to 45 years old. When we analyzed the total number of embryos, there was no significant difference in percentages of results between the conventional group and the piezo-ICSI group. However, when we analyzed the results by age (38, 39, 40, 41, and older than 42 years old), women who were aged 42 years old or older had a significantly higher rate of blastocyst formation in the piezo-ICSI group compared with the conventional ICSI group (60.9% vs 25.0%, $p = 0.04$). The rate of blastocyst formation was similar between the two groups for the other ages.
Limitations, reason for caution: A limitation of this study was the inability to adopt our results to all women because of the small number of eggs included for each age, despite the considerable total number of eggs. A further, larger study is required to confirm our results.
Wider implications of the findings: This study partially clarified the indication for piezo-ICSI according to age compared with conventional ICSI. Based on our results, piezo-ICSI is recommended for older women (> 42 years old) rather than younger women. Clinical trials, including clinical pregnancy and birth rates, are needed in the future to obtain more clear indications.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hanabusa Women's Clinic.
Trial registration number: NA.
Keywords: piezo-ICSI, conventional ICSI, age

P-503 Effect of trophectoderm vesicles on blastocyst development in relation to hatching, pregnancy, and spontaneous abortion rates

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Study question: Trophectoderm vesicles (TVs) are sometimes observed at the blastocyst stage. In some areas of the trophectoderm, they can penetrate to the cell exterior through the zona pellucida; however, the morphological and physiological mechanisms underlying this phenomenon remain poorly understood.
Summary answer: We observed that intracytoplasmic sperm injection (ICSI) was associated with a higher prevalence of TVs than conventional in vitro fertilization (c-IVF). However, no significant differences were found between TVs positive or negative groups in the pregnancy rate and the spontaneous abortion rate.
What is known already: It is theorized that TVs are a cell component that induce exhaustion of the trophectoderm when hatching occurs from an opening in the zona pellucida of mice. However, no evidence has been found that this mechanism occurs in humans.
Study design, size, duration: In Study 1, a preliminary study, we used time-lapse cinematography to analyze the presence of TVs vitrified–warmed blastocysts. We then categorized the blastocysts into two groups: Group A, in which TVs were observed, and Group B, in which they were not. We also investigated the prevalence of TVs under two treatment protocols, ICSI and c-IVF. In Study 2, a clinical study, a single vitrified–warmed blastocyst was transferred to

patients in our clinic between September 2012 and August 2014. We evaluated the pregnancy rate and spontaneous abortion rate in regard to the presence of TVs (Group C: present; Group D: not present).
Participants/materials, setting, methods: *Study 1*: For the experiment, 112 vitrified–warmed blastocysts were used. These blastocysts were derived from surplus embryos after obtaining informed consent. The embryos were classified by Gardner's criteria 3AA and 4AA and then observed continuously for 4 days using time-lapse cinematography (EmbryoScope™ [ES]; Unisense FertiliTech, Aarhus, Denmark). We retrospectively evaluated the prevalence of TVs under both treatment protocols (ICSI: 56 embryos; c-IVF: 56 embryos). The hatching rate was then compared based on the presence of TVs. *Study 2*: This clinical study comprised 915 embryo transfer cycles in 723 clinic patients who received a single vitrified–warmed blastocyst. The features of embryonic development were recorded by time-lapse cinematography, and vitrification was then conducted at the blastocyst stage on Day 5 or 6. Next, the blastocysts were categorized based on the presence of TVs (Group C: 169 embryos, TVs present; Group D: 746 embryos, no TVs present). All transfers were conducted under a standard hormone replacement protocol. Finally, the pregnancy and spontaneous abortion rates were compared between the two groups.
Main results and the role of chance: *Study 1*: The prevalence of TVs was significantly higher in the ICSI (51/56; 91%) than in the c-IVF group (25/56; 45%) ($P < 0.01$). The hatching rate was significantly lower in the ICSI (11/56; 20%) than in the c-IVF group (29/56; 52%) ($P < 0.01$). In addition, the hatching rate was significantly lower in TV(+)(14/76; 18%) than in TV(-) embryos (26/36; 72%) ($P < 0.01$). *Study 2*: No significant differences were found between Group C and D patients in regard to age, duration of culture, acquired high quality embryo rate, hormone replacement, and endometrial thickness. In addition, no significant differences were found between groups in the pregnancy rate (Group C: 99/169, 58.6%; Group D: 404/746, 54.2%) or the spontaneous abortion rate (Group C: 13/99. 13.1%; Group D: 57/404; 14.1%).
Limitations, reason for caution: We were unable to confirm the reasons behind either TV production before the hatching stage or the differences in treatment (c-IVF or ICSI)-based prevalence. Furthermore, the composition of TVs remains unclear.
Wider implications of the findings: Further studies are needed on the influence of TVs on culture and quality.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Nishimura Women's Clinic.
Trial registration number: NA.
Keywords: trophectoderm vesicles, blastocyst development, zona pellucida penetration, hatching

P-504 Is assisted hatching a poisoned gift?

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Study question: What is the most effective technique (Hole *vs*. Thinning)? In which cases should each one be applied? Do these techniques enhance the implantation of abnormal embryos? What about monozygotic twins?
Summary answer: Comparing Hole vs. Thinning, the former had better pregnancy rate, despite the increase in monozygotic twins. Three indications were considered for performing the assisted hatching (AH) technique, and in all of them there was an increased pregnancy rate with the procedure. Fetal malformations occurred more frequently in women with increased age (IA) group, but this happens also in spontaneous pregnancies.
What is known already: Failure of hatching is hypothesized as a cause for repeated implantation failure (RIF). This could be due to intrinsic conditions of the embryo and zona pellucida (ZP), or promoted by cell culture and cryopreservation in assisted reproduction techniques, which could lead to ZP hardening.
Study design, size, duration: The study began in 2011 and is still going (147 couples performed 299 cycles, with and without AH). Selection for performing AH techniques was based on 3 indications: IA (> = 37 years) ($n = 49$), dysmorphic zona pellucida (thick, dense) ($n = 51$) and RIF (2 cycles without implantation) ($n = 47$). The choice between the two AH techniques was randomized.
Participants/materials, setting, methods: AH was performed with infrared non-contact diode laser beam fired about 8-10 times during 0.5 ms (wavelength of 1.48 nm). Techniques were performed either by ZP thinning (approximately 50%-80% of initial ZP thickness – $n = 81$) or by creating a hole in ZP ($n = 86$).
Main results and the role of chance: The pregnancy rate with AH was 32% vs. 12% without AH and the abortion rate was 24% with AH and 30% without AH. Considering the kind of AH technique the pregnancy rate was 44% with thinning technique and 6% with the hole technique. In the IA group pregnancy rate was 36% with AH and17% without AH. In dysmorphic ZP group pregnancy rate was 48% with AH and 21% without AH. In RIF group pregnancy rate was 28% with AH and 6% without AH.
Limitations, reason for caution: AH appears to be associated with multiple pregnancy, specially when thinning technique is performed but the evidence of a correlation with monozygotic twins was insufficient.
Wider implications of the findings: AH appears to be beneficial to the three groups included in the study, by increasing pregnancy rates without increasing fetal malformation or abortion rate.
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: assisted hatching, hole, thinning

P-505 Generation of gradients on microfluidic device for high-throughput investigation of spermatozoa chemotaxis

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Study question: This study aimed to establish a stable gradient chip allowing for simultaneous detection of sperm chemotaxis in parallel experiments, which was free from influence of shear stress and could improve efficiency of current sperm research.
Summary answer: Firstly, the microfluidic chip used in our study was capable of maintaining a stable concentration gradient for at least 7 hours. Secondly, three parallel experiments of the same sample were carried out on the same chip to investigate sperm chemotaxis to progesterone. Similar phenomena were observed as previous study described.
What is known already: Sperm chemotaxis plays a vital role in fertilization. Historically, various types of assays have been widely used for in vitro detection of sperm chemotaxis which are, however, typically poor in maintenance of gradient stability, not to mention their low throughput. Microfluidic device offers a new experimental tool for a better control of chemical concentration gradient. Its outstanding advantage in miniaturization and potential for high-throughput analysis makes it more economical and efficient over conventional devices.
Study design, size, duration: Microfluidic chip in our study is featured by a central hexagonal pool surrounded by six channels. Channels were connected with the hexagon by 5mm wide microchannels, through which fluid can flow. Sperm loaded in central pool can sense the gradient progesterone solution formed, thus exhibiting chemotactic behavior.
Participants/materials, setting, methods: We firstly used fluorescent dye to characterize concentration gradient formed in the chip. Then sperm response to two different concentrations (100pM and 1mM, respectively) of progesterone was recorded under phase-contrast microscopy. Fluorescence profile and sperm tracks were analyzed in Image J software. Results were processed in Origin 8.0 software.
Main results and the role of chance: Fluid flow within the device was controlled by adjusting heights of solution in loading reservoirs. Concentration gradient profiles in the central hexagon varied when distinct height differences were applied. Gradient was very weak when height difference (DH) was 0.8 mm, but was stable at a higher DH at 1.0 mm or 1.2 mm. In chemotaxis assays, progesterone solution was doped into every other peripheral channel of each chip. Significant differences in chemotactic parameters were recognized between experimental and control groups ($p < 0.05$). There was no great difference between two experimental groups. In terms of kinematic response, values of parameters in 1 mM group were significantly higher than in other two groups ($p < 0.05$). However, no significant difference was found between 100 pM group and control group.
Limitations, reason for caution: This experiment was only carried out using human sperm samples.
Wider implications of the findings: Simultaneous recording of three replications can greatly reduce possible errors caused by repeated assembly of equipment, and saving time in sample processing. Besides, width of interconnecting

microchannels can be adjusted to a greater size so that chemotactic spermatozoa can swim through into peripheral channels. Thereafter, sperm can be collected from the peripheral channels and be used in in vitro fertilization to improve the performance of assisted reproductive technology.

Study funding/competing interest(s): Funding by national/international organization(s) – The National Science Foundation (No.30973196,81370767, 81101971).

Trial registration number: NA.

Keywords: microfluidic chip, human sperm, chemotaxis, progesterone

P-506 Outcomes of systematic blastocyst vitrification during 2 consecutive years before and after its beginning. A significant increase in delivery rate is reached within the first year

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Study question: Systematic blastocysts slow freezing procedure has been established for 8 years with convincing results in our team. Is the introduction of the vitrification will increase our results when it is proposed for all patients and performed by all members of the team?

Summary answer: After a one year-period of training for all the technical team, significant increase in delivery rate can be obtained with blastocysts vitrified, as soon as the first year when it is compared to slow freezing the year before with the same embryo culture systems and transfer policy.

What is known already: Blastocysts vitrification is an established new freezing technique which can give better results than slow freezing. However a learning curve is described for a technique needing skill and practice for all the members of the team. Therefore it is suggested that this technique cannot be proposed for all patients at the beginning of the procedure. The two freezing techniques might coexist during the time necessary for the habilitation of whole laboratory staff.

Study design, size, duration: This is a retrospective study comparing the outcomes of thawed blastocysts transfer between two consecutive years: 2012 (234 couples) vs. 2013 (291 couples). In addition we compared in 2013 the results of thawed blastocysts transfers according to the procedure of freezing used: slow freezing (150 couples) vs. vitrification (141 couples).

Participants/materials, setting, methods: We used a slow freezing protocol with glycerol and sucrose (Origio) in 2012 and start vitrification for all patients in 2013 with ethylene glycol and dimethylsulfoxide (Irvine) in closed system with high-security straws (CryoBio System). The main outcomes measures were thawed blastocysts survival rates and delivery rates.

Main results and the role of chance: In 2012, 100% of the 292 thawing were realized with slow frozen embryos whereas in 2013, there were 51% of the 369 thawing (others were from vitrified embryos). A significant higher delivery rate per thawing was observed in 2013: 13% vs. 23% ($p < 0.01$). Hence in 2013, 190 and 179 thawing of slow frozen or vitrified blastocysts were realized respectively. A higher survival rate (83 vs. 74% respectively; $p < 0.05$) and a higher delivery rate (27 vs. 18%; $p < 0.05$) were observed for the vitrified blastocysts. Mean number of transferred blastocyts (1.1 and 1.2), and miscarriages rate (8% and 4%) were similar for vitrified and slow frozen embryos respectively ($p > 0.05$). Consequently 55 and 37 healthy babies were born after thawing of vitrified or slow frozen embryos respectively.

Limitations, reason for caution: Confounding factors (remaining frozen embryos per couple, rate of thawed embryos the year of freezing, previous pregnancy rate before thawing) showed similar values between both groups. However, it is obvious that vitrified blastocyts had a shorter storage duration than slow frozen embryos.

Wider implications of the findings: After a training of the whole team, a new procedure can be quickly beneficial to all patients with successful results. Although our slow freezing program of blastocysts gave significant results, the vitrification procedure can still increase them, showing its superiority in our team.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Biomnis laboratory, Lyon, France.

Trial registration number: Retrospective study.

Keywords: blastocyst, vitrification, cryopreservation, slow freezing

P-507 Effect of in vitro-supplementation of myoinositol on DNA fragmentation before sperm preparation for assisted reproductive techniques

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Study question: The aim of this study is to test whether myoinositol in-vitro supplementation of semen samples can reduce the DNA damage that may occur before sperm preparation for assisted reproductive (AR) techniques.

Summary answer: Sperm DNA fragmentation significantly increases after a prolonged incubation of 4 hours from semen production. In these conditions, in-vitro myonositol supplementation can significantly decrease the DNA fragmentation process.

What is known already: During gamete handling for assisted reproductive techniques, a prolonged period of incubation before sperm preparation may have detrimental effects on sperm DNA, decrease the integrity of the sperm nuclei and cause dysfunction of mitochondrial membrane potential (MMP). Myoinositol (MI) is a buffer molecule for MMP and it has already been demonstrated that MI medium supplementation increases spermatozoa motility and MMP.

Study design, size, duration: Cross-sectional study of 10 men presenting at an infertility clinic for semen analysis over a period of 6 months. 500 spermatozoa per DNA test (25,000 total) were analysed for DNA fragmentation by Sperm Chromatin Dispersion method; 2mg/ml MI supplementation was performed by Andrositol Lab (Lo.Li.Pharma, Rome, Italy).

Participants/materials, setting, methods: Samples (basal group) were tested for DNA fragmentation after liquefaction, divided as below, and re-tested after 4 hours

1. seminal plasma alone (SP),
2. SP supplemented with myoinositol (SP + MI),
3. SP diluted 1 + 1 with sperm culture medium (CM),
4. SP diluted 1 + 1 with CM and supplemented with myoinositol (CM + MI).

Main results and the role of chance: Sperm DNA fragmentation rate after a 4-hour incubation significantly increased in all the groups except in the CM + MI group. More specifically, DNA fragmentation rate was: 24.0% (1200/5000) in the basal group; 51.6% (2580/5000) in the SP group; 40.9% (2045/5000) in the SP + MI group; 35.8% (1790/5000) in the CM group; 24.5% (1225/5000) in the CM + MI group; P was < 0.001 between all the groups, except in the case of the basal group versus the CM + MI group (0.576) which showed comparable DNA fragmentation rates.

Limitations, reason for caution: This was a small-scale study on forced stress: lab schedule is usually finalized to avoid unnecessary stress for the spermatozoa, but in AR units with a large number of semen samples to be treated simultaneously, a prolonged period of incubation (> 2 hours) may accidentally occur.

Wider implications of the findings: This study confirms that prolonged incubation before sperm preparation is harmful for DNA integrity but also indicates that dilution of seminal plasma with culture medium and supplementation with myoinositol can successfully protect the spermatozoa. Both culture medium and myoinositol alone reduce sperm DNA fragmentation but their combination seems to block the DNA fragmentation process for at least 4 hours. These findings may lead to a re-evaluation and improvement of routine incubation procedures before sperm preparation.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Cost of DNA kits and Andrositol Lab funded by Lo.Li.Pharma s.r.l., Rome, Italy.

Trial registration number: Not requested. Basic science study.

Keywords: sperm DNA fragmentation, myoinositol, sperm preparation, sperm handling

P-508 Temperature measurements in different brands of IVF culture dishes

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Study question: Do all culture dishes for IVF maintain temperature equally well (or bad) when removed from the incubator or is there a difference between different brands of dishes?

Summary answer: Dishes of all brands fail to maintain the temperature of the culture medium when the lid is removed but the temperature is maintained much better in dishes with direct contact with the heated stage.
What is known already: To maintain temperature in open dishes during laboratory work, heated stage temperature is elevated to 39-40°C and medium is covered with oil. Traditional dishes for handling and culture of oocytes and embryos commonly have an air gap underneath which makes it necessary to increase heated stage temperature above + 37°C to maintain physiological temperature in the medium inside the dish. When the dish lid is removed, temperature drops despite the elevated temperature on the heated stage.
Study design, size, duration: Dishes of three brands (Falcon, Nunc and Vitrolife) were tested. The dishes were Falcon center well dish, Vitrolife center well dish, Nunc 4-well dish and Vitrolife 5-well dish. Falcon and Nunc dishes have an air gap underneath while Vitrolife dishes have a flat bottom without air gap.
Participants/materials, setting, methods: Start temperature was 37°C. Measurements were performed with lids removed and repeated three times. Temperature was measured once per minute for five minutes and heated stage temperature was 39°C. Wells were filled with one ml of medium and moats with 4 ml. Temperature was measured with and without oil cover.
Main results and the role of chance: The temperature of the Falcon center well dish dropped to 31.2°C within 5 minutes after removal from the incubator and continued to go down while the temperature in the Vitrolife center well dish stabilized at 33,2°C. The temperature of the center well dishes covered with oil stayed more stable, the temperature of the Falcon dish went down to 34.5°C and the Vitrolife dish stabilized at 35.8°C. Finally, the temperature of the medium in the Nunc 4-well dish dropped to 31.6°C and the temperature in the Vitrolife 5-well dish stabilized at 34.1°C.
Limitations, reason for caution: The study included a limited number of dish brands and more brands can be studied to give a more complete picture of oocyte and embryo handling temperatures.
Wider implications of the findings: The fact that the temperature in dishes can drop to below 33°C within a few minutes after the lid has been removed during normal IVF laboratory work cause stress to oocytes and embryos resulting in impaired embryo development. All laboratories should check the temperature in open dishes of the brands used in the clinic to minimize a common results reducing factor.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Vitrolife Sweden AB
Trial registration number: NA.
Keywords: IVF culture dishes, temperature, temperature sensitivity, temperature measurement

P-509 Embryo culture in a continuous single medium and selection by morphokinetic analysis: a prospective randomized sibling oocyte trial of the embryo scope and primo vision

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Study question: Is there a difference in the rate of good quality blastocysts per fertilized oocytes, according to the expansion of the blastocoel cavity and the quality of the inner cell mass and trophectoderm, assessed by two different time-lapse systems (EmbryoScope versus Primo Vision) after culture in a continuous single medium.
Summary answer: This ongoing trial showed that culture of embryos using a continuous single medium in the EmbryoScope resulted in a tendency towards an increase in the rate of good quality blastocysts on day 5.
What is known already: It was previously reported that embryos cultured in single medium without renewal during culture, showed an increase in blastocyst development, and that culture and selection of embryos in time lapse monitoring system improves reproductive outcomes. To our knowledge, no randomized controlled trial comparing the two different time-lapse systems, EmbryoScope or Primo Vision has been undertaken.
Study design, size, duration: In this ongoing prospective randomized sibling-split trial, 200 oocytes from 19 women assigned to ICSI were divided randomly to EmbryoScope treatment group (99) or Primo Vision treatment group (101) and cultured to day 5 in a continuous single medium and monitored by time lapse videography.
Participants/materials, setting, methods: Oocytes allocated to EmbryoScope treatment group were cultured singularly in 25 ml (G-TL, Vitrolife, Sweden) in an EmbryoScope culture dish and oocytes allocated to Primo Vision treatment group were cultured in groups in 80 ml (G-TL, Vitrolife, Sweden) in a Primo Vision culture dish. Embryos were cultured and monitored by time lapse videography until day 5.
Main results and the role of chance: Embryo scoring and selection was performed by a time lapse monitoring system (EmbryoScope or Primo Vision) and good quality blastocysts on day 5 were scored according to the degree of expansion > = 2 and Inner Cell Mass (ICM) = {1, 2} and Trophectoderm (TE) = {1, 2}. There was a tendency towards a higher rate of good quality blastocysts in the EmbryoScope treatment group compared to the Primo Vision treatment group (17.2 % versus 14.9 %), however the level did not reach statistical significance.
Limitations, reason for caution: None
Wider implications of the findings: The culture media used (G-TL, Vitrolife, Sweden) achieved good rates of blastocysts development and utilization (embryo transfer and cryopreservation). Thus, embryo culture system that does not require medium renewal during culture provides good condition and greatly reduces external environment stresses on embryos. Time lapse technology combined with a continuous single culture medium may increase the success rate of IVF.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funded by Skane University Hospital, Malmö,Sweden.
Trial registration number: NA.
Keywords: time lapse, primo vision, embryoscope, blastocyst

PARAMEDICAL - NURSING

P-510 Infertility related communication and coping strategies among women affected by fertilitty problems in Sweden

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Study question: The aim was to describe infertility related communication and coping strategies among women affected by fertility problems in Sweden.
Summary answer: Thirty percent of participants had difficulties discussing infertility related subjects with their husband or with close other people. To cope their situation 16 percent of participant avoided socialising with pregnant women, and one third of them had difficulties to find other life goals than children.
What is known already: Involuntary childlessness is a reality for many reproductive aged couples and can be psychologically stressful for many of them. The inability to have children affects couples worldwide and causes psychological and emotional distress on them. Both involuntary childlessness and infertility treatment places pressure on those involved. Difficulty in marital communication and high use of active-avoidance coping are significant predictors of high fertility problem stress.
Study design, size, duration: Cross sectional study design involving 156 Swedish women (mean age 36.3). The participants received a letter regarding information of the study including the questionnaire and a postage-paid reply envelope. Data was collected during 2010-2011. Approval from the Ethical Review Board, Stockholm (EPN 2006/1025-31) was obtained.
Participants/materials, setting, methods: The population consists of women with history of primary infertility, minimum of one year, which sought to a fertility clinic voluntarily or were referred from other clinics. A self-administered semi structured questionnaire (COMPI) was used to collect the data. Descriptive statistical methods were used to analyse the data.
Main results and the role of chance: Response rate was 78 %. Seven percent of the population did not discuss the inability to have a child, and nine percent did not discuss about reasons why they were childless at all with their spouse. Seventy-five percent discussed infertility related subjects only to close other people and 20 % did not discuss about the results of examinations and tests with people outside of the family. Thirty-five percent did not ask other childless individuals, friends or relatives for advice and 15 % were not able to discuss about

how tests and treatments affect them emotionally. Twelve percent reported that they leave the room when the subject children or pregnancies are discussed.
Limitations, reason for caution: Participants were recruited from one infertility clinic from great Stockholm area. The population consists of 156 participants and eighty-four percent of them had postgraduate education.
Wider implications of the findings: By highlighting possible problems with infertility relating communication, physicians, midwifes and nurses at fertility clinics may become aware of this problem and therefore dare to address the question. This knowledge may ensure high quality of care, improve patient wellbeing and possibly higher treatment compliance.
Study funding/competing interest(s): Funding by University(ies) – The study was funded by Sophiahemmet Ideel förening, Patientnära forskning, Sweden. The authors declare that they have no competing interests.
Trial registration number: The study is not a Clinical trial.
Keywords: inferility, communication, coping, women

P-511 Managing a fertility practice using set protocols: clinician-focused or patient-focused?

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Study question: How can set clinical protocols enhance patient experience and practice management in an IVF clinic?
Summary answer: The Set Protocol System provides standardised patient care from all members of staff with benefits to patients, doctors, nurses, laboratory and administration staff. Experience suggests that set protocols do not reduce staff or doctor autonomy, but assures patients of the same level of service across the business.
What is known already: There are multiple ways of managing patient care in IVF clinics. The approach selected is driven by clinical practices designed to suit the practice, its clinicians and staff. In many cases, the practice methodology emerges from the skills and technical experiences of the clinician overlaid by regulatory requirements. It has also been noted that IVF management is influenced by IT systems designed to record practice and patient records. There is no 'one best way'.
Study design, size, duration: The study is based on 14 years of regulated operation of the practice based on the adoption of clinical protocols designed and developed by the senior doctor with assistance from nurses and scientists. The observations have been collected and refined over this time to arrive at set protocols that can be adopted by all doctors in the practice and applied for the benefit of patient outcomes.
Participants/materials, setting, methods: The practice was obliged to establish treatment protocols, whose aim was to standardise treatment regimens and to develop expertise in clinical, nursing and scientific practice. Protocols were matched to international practices through international meetings such as ESHRE and ASPIRE, which remain the source of protocol development. Participants have been doctors, scientists, nurses and administration personnel associated with the practice over 14 years. The research methodology is based on longitudinal observation of set protocols.
Main results and the role of chance: Set protocol start from the moment a patient enters the practice. Patients determine the potential treatment load. All patients have to undertake a set of tests that are obligatory. e.g. rubella, CF, PAP smear for females, semen analysis for males. With additional doctors came standardisation to avoid 'diversity of practice' created by: independent practice habits perhaps because they trained in different years (and universities). Dividing the work to ensure patients received similar treatment was complex, but they could not easily see the same doctor each time they came to the Centre, nor could the same doctor carry out the necessary procedural work. The work had to be divided in fair way that guaranteed appropriate treatment for patients (and agreed remuneration among the doctors).
Limitations, reason for caution: The study relates to one practice that adopted set protocols compared to other practices which did not based on observations collated over 14 years.
Wider implications of the findings: Set protocols create practice standardisation and permit a group of doctors to provide specialised treatment to a diverse and demanding patient group. The protocols assist patients to feel certain about their treatment regimen and the quality of care they are receiving.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Hollywood Fertility Centre.
Trial registration number: NA
Keywords: practice management, protocols

P-512 Elective single embryo transfers (eSET) in Turkey: a need for an educational campaign?

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Study question: Are Turkish patients aware of risks related to multiple pregnancies?
Summary answer: A survey conducted in a private Turkish ART center enrolling 201 infertile couples showed that only 6% are concerned about having a multiple gestation.
What is known already: To decrease the incidence of multiple pregnancies, a legislation regarding eSET was brought into force by the Turkish Ministry of Health in 2010 and modified in 2014. According to this regulation, for women < 35 years of age, with one or no previous ART cycle(s), the number of embryos which can be transferred is limited to one.
Study design, size, duration: A total of 201 infertile females participated in the survey conducted between August and September 2014, just before the embryo transfer procedure. The survey included 25 questions about satisfaction regarding the center, psychological aspects of the treatment and also concerns about multiple gestation.
Participants/materials, setting, methods: The mean age of the population was 34.1 ± 6.0. The educational level was variable: 64% of the patients were graduated from university, 20% from high school and 16% from elementary school. 44% of patients were referred from the same city as the ART center (Istanbul), 48% from the remainder of Turkey and 8% from abroad.
Main results and the role of chance: For some questions multiple answers were possible and analyzed as such. 77% of patients expressed their main concern as failing their ART attempt. Moreover, in the absence of supportive public ART funding, half of the patients are concerned about financial issues (45%). The quarter of the cohort (26%) was concerned about not being able to share their experience about their ART cycle with their family and close friends. But of the 201 patients surveyed only 6% were anxious about having a multiple gestation.
Limitations, reason for caution: The underlying infertility cause could not be taken into consideration because of the relatively small sample size.
Wider implications of the findings: A very low number of patients were concerned about multiple gestation and the accompanying risks. Therefore, educational campaigns should be organized if we really aim to promote of eSET in the near future in Turkey.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Survey was funded by Memorial Sisli Hospital Administration, Istanbul, Turkey.
Trial registration number: Study design and the survey was approved by the Ethics Committee of the Memorial Sisli Hospital, Istanbul, Turkey.
Keywords: ART, eSET, multiple gestation

P-513 Effects of in vitro fertilization on postpartum depression and stress coping

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Study question: This study investigated whether patients becoming pregnant as a result of in vitro fertilization are more likely to suffer from postpartum depression than those becoming pregnant spontaneously.
Summary answer: The patients investigated who became pregnant as a result of in vitro fertilization were more likely to suffer from postpartum depression than those becoming pregnant spontaneously at one month postpartum.
What is known already: The coping scale consists of three subscales: problem-focused, emotion-focused, and avoidance-escape (14 items). Prolonged infertility treatment can lead to a more passive stress-coping strategy (avoidance-escape). In addition, patients receiving infertility treatment are likely to

show a higher score of EPDS, which assesses postpartum depression with a cut off of 8/9(assuming a score of 8 points or lower as negative and 9 or higher as positive), at one month after delivery.
Study design, size, duration: We included 24 patients who delivered a child as a result of spontaneous pregnancy (spontaneous pregnancy group) and 19 who did as a result of in vitro fertilization (IVF group) at our outpatient clinic between January 2012 and December 2014 in the study.
Participants/materials, setting, methods: At one month after delivery, we evaluated the 43 patients using the 30-point Edinburgh postpartum depression scale (EPDS) revised by Okano. Furthermore, we examined which strategy the EPDS-negative versus EPDS-positive patients employed among problem-focused (15-point), emotion-focused (9), or avoidance-escape (18), as defined in the stress-coping scale of Ozeki et al.
Main results and the role of chance: Both spontaneous pregnancy and IVF groups indicated "child health/care" as a primary stressor at one month after delivery. The IVF group showed significantly higher EPDS scores and included a significantly higher proportion of EPDS-positive patients (EPDS of 9 or above) compared to the spontaneous pregnancy group. In addition, when comparing the stress-coping strategies for the IVF group, EPDS of 9 points or higher was correlated with a significantly lower scores for the emotion-focused strategy compared to EPDS-passive (8 points or lower), suggesting that the EPDS-positive patients of the IVF group were unlikely to adapt themselves to active coping, i.e., emotion-focused strategy. Some of these EPDS-positive patients underwent treatment for a second child at our clinic after the birth of their first child.
Limitations, reason for caution: Postpartum depression and stress coping strategies are based on the psychological status at one month after delivery in spontaneous pregnancy and IVF patients. In this study, a smaller sample was used, and stress was assessed based on patient's subjective comments.
Wider implications of the findings: It was suggested that the IVF compared to spontaneous pregnancy group was more likely to suffer from postpartum depression, as demonstrated by higher EPDS scores, at one month after delivery. In addition, when comparing the stress-coping strategies for the IVF group, EPDS-positive compared to EPDS-negative patients showed significantly lower scores for the emotion-focused strategy. Furthermore, some of the EPDS-positive patients again visited our clinic to undergo further infertility treatment for a second child.
Study funding/competing interest(s): Funding by hospital/clinic(s) – CLINIC MAMA.
Trial registration number: NA.
Keywords: IVF, postpartum depression, stress coping

P-514 The 'haves' and the 'have-nots': comparing fertility awareness and attitudes towards infertility between parents and non-parents

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Study question: Is there any difference between parents and non-parents in their fertility-related knowledge and attitudes towards infertility?
Summary answer: Compared to non-parents, parents underestimated to a greater extent the fertility decline, and insisted more strongly on keep trying natural intercourse in the event of infertility.
What is known already: Infertility is a stressful experience and infertile couples often seek social support from their peers, many of whom might be parents. However, it is not known how parents and non-parents perceive fertility issues differently.
Study design, size, duration: An online cross-sectional survey in Hong Kong was conducted in May to August 2013 through a university-wide email invitation.
Participants/materials, setting, methods: A total of 246 participants (parents: $n = 97$, non-parents: $n = 149$) who were aged 30 or above responded to a previously validated questionnaire on fertility knowledge and attitudes towards infertility. The average age was 35.6 (SD = 5.6) for non-parents and 38.8 (SD = 6.3) for parents.
Main results and the role of chance: Although both parents and non-parents overestimated the age at which significant fertility decline occurred (8.2% non-parents and 7.7% parents answered the right age category), parents reported a significantly higher age than non-parents (34.1 and 33.1 respectively, $F = 4.34$, $p < .05$). In the hypothetical event of infertility, parents expressed a stronger wish than non-parents to keep trying natural intercourse ($F = 14.29$, $p < .01$), and less willing to accept childlessness ($F = 20.37$, $p < .01$).
Limitations, reason for caution: Self-selection bias is a limitation of cross-sectional study. This study was conducted in Hong Kong, a Westernized city with an advanced economy where influences of Chinese culture remain strong. Cross-cultural studies should be done in order to generalize the findings.
Wider implications of the findings: This study sheds light for the first time on the discrepancy in perception between parents and non-parents of fertility issues. Since infertile couples are likely to seek social support from peers who are parents, healthcare professionals should make clients more aware of the discrepancy of perception between parents and non-parents and the risk of social pressure in making treatment decisions.
Study funding/competing interest(s): Funding by University(ies) – University of Hong Kong.
Trial registration number: NA.
Keywords: fertility awareness, parenthood, infertility, attitudes towards infertility

P-515 Effects of a partnership support program for couples undergoing fertility treatment

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Study question: The study's purpose was to examine the effects of providing a partnership support program. It was designed to improve Japanese couples' partnership, maintain quality of life, decrease psychological distress, and improve marital relationship satisfaction while they underwent infertility treatment that included the possibility of using assisted reproductive technology.
Summary answer: The intervention group showed significant improvement in the couples' partnership and a significant decrease in women's psychological distress using subgroup analysis.
What is known already: Infertile patients had numerous stresses and anxiety, and their quality of life (QOL) was low. One factor compounding women's psychological distress was a deteriorated marital relationship.
Study design, size, duration: This was a quasi-experimental design using a convenience sample and a nonequivalent age-matched control group with non-random group assignment. An estimated 308 participants must be enrolled to achieve the desired 80% power with an alpha level of.05. The data collection period was from April to November 2013.
Participants/materials, setting, methods: Potential participants were couples undergoing fertility treatment visits at a Japanese fertility clinic. The intervention group patients participated in the partnership support program. The comparison group patients received usual care. The program provided information and used a participatory-interactive approach to enhance understanding and cooperation in couples undergoing fertility treatment.
Main results and the role of chance: Agreeing to participate were 152 patients from the intervention group and 166 patients from the comparison group. Completing the study were 108 patients (71.1%) from the intervention group and 120 patients (72.3%) from comparison group. Missing data and participant dropout were managed using 'baseline observation carried forward analysis' (BOCF) for intention to treat analysis.

1. The intervention group's Partnership scale scores after the intervention were significant higher than the comparison group indicating a stronger partnership.
2. The intervention group's FertiQoL scale scores at post-intervention were not significantly higher than the comparison group.
3. The Distress scale scores for the women in the intervention group were significantly lower than the women's scores in the comparison group meaning the women in the intervention group had less distress.

Limitations, reason for caution: Because this study was not a RCT and the location of data collection was only a single clinic; there is limited generalizability and potential bias. The research design was a nonequivalent control

group designs instead of a RCT therefore the internal validity for the intervention effect was weaker.
Wider implications of the findings: The partnership support program was effective in improving the couples' partnership while undergoing infertility treatment, and that in turn was effective in reducing psychological distress among the participating women; however it had less impact for the men. It's possible that there was less distress for men than women undergoing treatment and therefore the men did not require support from their partners. The program was not effective in improving QOL as a whole for the couples.
Study funding/competing interest(s): Funding by national/international organization(s) – The Japan Academy of Midwifery.
Trial registration number: NA.
Keywords: partnership, QOL, psychological distress

PSYCHOLOGY AND COUNSELLING

P-516 Considerations and actions towards motherhood in 200 single, childless women aged 35 to 43 years

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Study question: Which conditions are of importance and what are the motives for and actions towards motherhood in single, childless women aged 35 and above after fertility assessment and counseling?
Summary answer: The important prerequisites for having children were maturity and the possible combination of work with childbearing. The expected benefits of motherhood were personal development and to give/receive love. The majority feared loss of freedom and less time to job/career. Most expected to have children within two years after the consultation.
What is known already: The reproductive patterns have changed in Europe in recent years. An increasing number of women and men are registered as living alone. In Denmark, 1% of the birth cohort is born by solo-mothers using semen donation and 14% of Danish women never have children. Studies addressing the attitudes towards family formation and fertility awareness have primarily focussed on cohabiting or young women; the knowledge and considerations among older, childless women is limited.
Study design, size, duration: A prospective, population-based, cross-sectional cohort study of 200 single women aged 35-43 years examined at the Fertility Assessment and Counselling (FAC) Clinic at Copenhagen University Hospital from 2011 to 2014. The FAC Clinic was initiated to provide individual fertility assessment and counselling (Hvidman*, et al.*, 2015).
Participants/materials, setting, methods: Eligible participants were heterosexual, single and childless women aged 35 years and above. All completed a web-based questionnaire before and after the consultation including socio-demographic, reproductive, medical, lifestyle and behavioural factors. Consultation by a fertility specialist included transvaginal ultrasound, uptake of a full reproductive history and AMH measurement.
Main results and the role of chance: The 200 single women had a mean (SD) age of 37.5 (2.0) years and 85% had an education length of 3-6 years. The women listed the following aspects as very important/important in relation to childbearing: feeling mature (91%), ability to combine work and children (78%), access to daycare (68%), having a partner (67%) and a stable relationship (67%). The values associated with motherhood were: personal development (90%), to give/receive love (89%), and new perspectives in life (81%). There was a general concern of: loss of freedom (83%), less time to job and career (76%) and an inferior economy (43%). Only half listed children as the meaning of life (55%). Yet, after the consultation, 93% expected to have children within the next two years.
Limitations, reason for caution: Attendance to the FAC Clinic is based on self-referral which could imply a potential selection bias and influence the personal decision-making. The high proportion of women expecting to have children within two years despite their present single status could imply a selected group considering solo-motherhood.
Wider implications of the findings: In contrast to the results of previous studies, having a partner was not the most important prerequisite for having children in our cohort. Personal development, the loving relationship between mother/child, access to day care and the job situation were considered more significant. This may partly explain why an increasing number of heterosexual women of advanced age choose solo-motherhood with donor insemination and emphasizes the importance of social preconditions for family formation among well-educated women.
Study funding/competing interest(s): Funding by national/international organization(s) – The FAC Clinic is a part of the ReproSund and Reprohigh collaboration and was 50% co-financed by EU-regional funding. This study also received funding through the Capital Region Research Fund. The authors have no conflict of interest.
Trial registration number: NA.
Keywords: postponing childbearing, fertility awareness, family intention, single women, fertility assessment and counselling

P-517 Predictors of pain during oocyte retrieval

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Study question: Can medical, procedural or psychosocial factors predict unacceptably high pain levels in women undergoing oocyte retrieval?
Summary answer: Negative gynecological experience, hormonal side-effects, anxiety levels and lack of control were significantly associated with high pain intensity during oocyte retrieval.
What is known already: The experience of pain during procedures such as oocyte retrieval remains a common challenge to endure, for a number of patients, albeit elaborate pain management protocols have been developed. The present study examined the prevalence of pain, as well as possible medical and psychosocial risk factors for experiencing severe pain levels, for a sample of women going through in vitro fertility or intracytoplasmic sperm injection treatment.
Study design, size, duration: Eight hundred and thirty-seven women in IVF/ICSI treatment completed three questionnaires: at their 21st day of the cycle (t1), before the oocyte retrieval surgery (t2), and after the oocyte retrieval when the effect of sedation and analgesics had subsided (t3). Sixty-nine women were lost to follow-up.
Participants/materials, setting, methods: Participants were in their first treatment cycle. Patients undergoing preimplantation genetic diagnosis or acute change of procedure from insemination to IVF due to too many follicles were excluded.
Main results and the role of chance: The pain level was 1.95 (SD 1.434) for the total sample. High pain intensity was reported in a subgroup of 50 (7%) of the women, with the remaining 690 women (93%) reporting moderate to low-pain. Multiple logistic regression indicated that the significant predictors of high pain intensity, measured before the oocyte retrieval, were negative gynecological experiences [adjusted OR: 1.099 95% CI (1.029, 1.173); $P < 0.001$] and hormonal side effects [adjusted OR: 1.619 95% CI (1.123, 2.128); $P < 0.001$]. Significant independent variables measured after the oocyte retrieval were anxiety [adjusted OR: 1.296 95% CI (1.040, 1.615); $P < 0.001$], perceived control [adjusted OR: 0.621 95% (0.492, 0.784); $P < 0.001$] and the duration of the procedure [adjusted OR: 1.084 95% (1.035, 1.136); $P < 0.001$].
Limitations, reason for caution: We obtained no exact information on non-responders as the registration of those who chose to participate and those who declined was incomplete. Any generalization of the results of this clinical population should be made with proper caution.
Wider implications of the findings: The findings of this study may help prospectively to identify those women who are at risk of experiencing unacceptable pain levels during oocyte retrieval procedures. Targeting and understanding the

role of pain catastrophizing and rumination associated with previous negative gynecological examinations may help improve the individual pain management protocol. Also, factors such as anxiety and feelings of lack of control are commonly associated with higher pain levels, which may also be the case for clinical populations awaiting sub-acute, minor surgery. The medical staff is advised to take psychological factors into account.

Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies) – This work was supported by research grants from Merck Sharpe Domer and from The Danish Agency for Science Technology and Innovation. The funders had no influence on the data collection, analysis or conclusions of the study. None of the authors have any conflicts of interest to declare.

Trial registration number: NA.

Keywords: infertility treatment, pain, oocyte retrieval, anxiety, depression

P-518 Psychosocial effects on reproductive outcomes: examining the impact of infertility-related coping strategies and stress on female and male biological responses and on pregnancy

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Study question: Do psychosocial variables play a role on biological responses and on achieving pregnancy in couples engaged in IVF/ICSI/TESE treatments?

Summary answer: Female age and women´s use of active confronting and passive avoidance coping strategies were significant predictors of the number of retrieved oocytes. Men´s use of active confronting coping strategies and female age at the beginning of treatments significantly predicted pregnancy.

What is known already: There is previous evidence showing that psychosocial variables can influence the biological response to infertility treatments, but the link between psychosocial adjustment and the successful outcome of infertility treatments remains unclear. Additionally, studies investigating this link on men are lacking because the vast majority of studies considered only female variables. Moreover, further investigation is needed using couple as unit of analysis.

Study design, size, duration: Sociodemographic, lifestyle, and psychosocial data were collected from 613 subjects seeking infertility treatment between February 2010 and March 2011.Of these, 201 couples referenced for fertility treatment for the first time in a Public fertility center were drawn. In December 2013, this database was linked with the center medical records.

Participants/materials, setting, methods: Participants were 79 couples that underwent IVF/ICSI/TESE. Using two separate multiple regressions, we examined the impact of coping and stress on retrieved oocytes and seminal defect controlling for age and lifestyle habits. Effects on clinical pregnancy were controlled with age, infertility duration and type, sperm motility and referral status.

Main results and the role of chance: Participants had on average 33 (women) and 34.9 (men) years. They were living together for 6.7 years and had been trying to conceive for 4.3 years. In December 2013, 41.8 % of couples had achieved a pregnancy. Female age at treatment (b = -.267; P = .021), active confronting (b = -.281; P = .021) and passive avoidance coping (b = -.252; P = .043) significantly predicted the number of oocytes retrieved. None of the variables were significant on the male model. Female age at treatment (B = -.265; P = .009) and male active confronting coping (B = 1.32; P = .005) were significant predictors of pregnancy. The total model can identify correctly 75.4 % of pregnancies.

Limitations, reason for caution: The number of couples included in the analysis is small. Additionally, it is possible that other non-explored variables could add explained variance. Future research should examine the effects of psychosocial variables with both couples' members in larger samples.

Wider implications of the findings: Coping strategies adopted by infertile patients can affect the biological response to infertility treatments, as well its success. Patients can be informed about this result by the clinical staff and if necessary be referred to an infertility counselor. Future research is needed to better understand the role of infertility-related coping strategies and stress on both female and male biological responses and on pregnancy outcomes.

Study funding/competing interest(s): Funding by national/international organization(s) – This work is supported by European Union Funds (FEDER/COMPETE - Operational Competitiveness Programme) and by national funds (FCT - Portuguese Foundation for Science and Technology) under the projects PTDC/MHC-PSC/4195/2012 and SFRH/BPD/85789/2012.

Trial registration number: NA

Keywords: infertility-related coping, infertility-related stress, oocytes retrieved, seminal defect, pregnancy

P-519 Satisfaction with fertility care during assisted reproduction treatments and its impact in men and women quality of life

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Study question: Does satisfaction with fertility care contributes to quality of life of patients undergoing assisted reproduction treatments?

Summary answer: Satisfaction with fertility care, namely with information on treatment procedures and demands contributes to quality of life of patients undergoing assisted reproduction treatment

What is known already: Infertile patients well being is affected by fertility clinic and staff characteristics. It has been documented that patients value positive attributes of fertility clinics (e.g. accessibility, easiness to access, comfort) and fertility staff positive attributes (e.g. courtesy, sensitivity, being available to patients questions). However, it remains unclear which of all these components of care is more likely to affect the quality of life (QoL) of men and women's undergoing assisted reproductive techniques.

Study design, size, duration: In this cross-sectional, 264 infertile patients (132 infertile couples) undergoing an IVF cycle in a public universitary hospital from January to December 2014 were invited to participate. Both partners received the questionnaire and were asked to fill it separately. Participation was voluntary and anonymous.

Participants/materials, setting, methods: Participants were asked to complete a questionnaire on satisfaction with the clinic assessing characteristics (e.g., clinic facilities) and relational aspects of the fertility clinic staff (e.g. sensitivity to the patient needs) and also the FERTIQOL questionnaire on fertility quality of life – treatment module. Patients received the questionnaires at the day of the oocyte retrieval and were asked to return them prior to the pregnancy test.

Main results and the role of chance: Participants were 88 infertile couples (response rate of 66.7%) mostly undergoing their first treatment (68,6%). Women reported worse tolerability and worse treatment QoL than their partners (Both $p < 0.001$). A regression model was conducted to examine the effects of clinic and staff characteristics in patients' treatment QoL. Easiness to access the clinic (b = -6.79, $p = .009$) and clarity of information from clinic staff (b = -15.39, $p = .019$) significantly predicted women Mind and Body Qol. Regarding QoL in treatment, satisfaction with information on treatment procedures and demands significantly predicted women's and men's environmental QoL (b = 1.29, $p = .001$ and b = 1.98, $p = .004$, respectively), mainly for patients undergoing their first IVF cycle ($p = 0.05$). Patients reported they would rather prefer receive the information at the medical appointment (80%) and by the medical staff (42%).

Limitations, reason for caution: As the study is voluntary, it is possible that there is a self selection sample bias and participants less satisfied with fertility clinic or undergoing higher emotional difficulties did not participate in the study.

Wider implications of the findings: Results show that clarity of information and information on treatment procedures and demands are important predictor of patients QoL, namely of treatment related Qol, especially in patients undergoing their first cycle of assisted reproduction techniques. This study highlights the importance of fertility clinics being aware of the importance of providing their patients with full information on their treatment procedures.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Coimbra Hospital and University Centre.

Trial registration number: The study is not a trial.

Keywords: quality of life, quality of care, IVF

P-520 What do parents say to their children when they have been conceived by embryo donation or double donation treatment?

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Study question: When, how and what do parents tell their children when they have been conceived by embryo donation or double donation treatment?

Summary answer: The majority of parents interviewed have already told their young child something about the embryo donation or double donation treatment. Whilst there are some similarities in what information parents give, exactly what they say to their children and how they decide what to tell varies between, and within families.
What is known already: Little is known about parents' thoughts and feelings about disclosure in cases of embryo donation or double donation, and exactly what parents tell their children. Embryo/double donation parents face unique decisions, including whether and how to tell their child that neither parent has a genetic link to them. Previous evidence suggests that being open about donor conception may be beneficial; however, the majority of existing research focuses solely on egg or sperm donation.
Study design, size, duration: Parents were approached via fertility clinics and a support group, and invited to participate in one-to-one interviews. Interviews began in January 2014 and will continue until April 2015. 21 interviews have been completed, with 25 in total anticipated. Data will be analysed using thematic analysis extracting common themes across interviews.
Participants/materials, setting, methods: Parents with a child aged between 3 and 8 years conceived by embryo donation or double donation were recruited. In-depth semi structured interviews were used to explore thoughts and feelings about disclosure, and to ascertain what parents have told their child. In two-parent families, both parents were interviewed where possible.
Main results and the role of chance: Preliminary findings demonstrate that parents who have already started disclosing to their child, generally begin to do so in the first couple of years of their child's life. The perceived benefits are that it allows them to find the terminology that they wish to use before their child can understand their meaning, and so that the foundation is there ready to develop as their child gets older. The language that parents use varies; however emerging themes include disclosure as a story developing over time, concern and anxiety about the future, and that parents tell their children about the 'kindness' of donors. Parents who have disclosed report that they would benefit from additional support on what to tell their children as their understanding matures.
Limitations, reason for caution: The majority of parents interviewed thus far have started to disclose, however a proportion of parents were recruited through a support group, so may not be representative of all parents in similar circumstances. Findings are based on preliminary data, but all data will be available to present at the conference.
Wider implications of the findings: Findings highlight the importance of understanding this relatively unexplored area of parenting. Conclusions will consider parents' thoughts and feelings on disclosure and how parents decide whether to disclose. Suggestions will be made for how counsellors could help to support parents facing these decisions. In particular, consideration will be given as to whether clinics should give more thought to providing post-treatment counselling to support parents with the implications of disclosure as their child's comprehension develops.
Study funding/competing interest(s): Funding by University(ies) – University of Warwick.
Trial registration number: NA.
Keywords: disclosure, embryo donation, double donation, counselling

P-521 Attitudes towards family formation in single and cohabiting childless women in their mid- to late thirties

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Study question: What are the attitudes and perceptions towards family formation among single and cohabiting women seeking individual fertility counselling?
Summary answer: Well-educated women postpone pregnancy despite their knowledge of the decline in female fecundity with increasing age and their wish of motherhood. We observed an internal conflict of choosing parenthood, as the women described family formation as the meaning of life though they also had considerations related to the negative consequences.
What is known already: During the past 30 years women and men have postponed family formation in Western societies. Several studies have shown that women tend to underestimate the decline in fecundity with age and overestimate the success rate in assisted reproductive technologies. A growing population of women decide to become solo-mothers through assisted reproduction using donor-sperm.
Study design, size, duration: Baseline data from a longitudinal semi-structured qualitative interview study including 20 women aged 34-39 years seeking individual fertility counselling. A total of 25 women were contacted, two were excluded due to pregnancy. In all 10 single and 10 cohabiting women were included. Data was collected between March and September 2014.
Participants/materials, setting, methods: Study participants were single or cohabiting women, residents in the Capital Region of Copenhagen, and seeking individual fertility counselling (Hvidman et al., 2015). Purposeful sampling with maximum variation was used. Data were analysed by qualitative content analysis following the methodology by Graneheim and Lundman.
Main results and the role of chance: The study displayed the conflict towards parenthood both in the single and cohabiting women. The general attitudes and considerations towards family formation were characterised by the fear of negative consequences and lifestyle changes on one hand and the biological clock and the dream of the nuclear family on the other. Finding the right partner was a central point for all of the participants. Despite their advanced age none of the women felt ready to motherhood at the present. Both single and cohabiting women showed an increasing awareness of solo-motherhood as a possible solution to advanced age and the wish for a child.
Limitations, reason for caution: The study participants had all chosen to seek individual fertility counselling. Hence, the results may not be directly transferred to a similar age group in the general population in regards to attitudes towards family formation and concerns of reproductive lifespan.
Wider implications of the findings: Many women postpone childbearing to their late thirties and thereby risk infertility, smaller families than desired, and adverse obstetric outcomes. Our study contributes to the understanding of the personal considerations in relation to childbearing in women (mid- to late thirties). This may be useful in a fertility assessment and counselling setting providing individual guidance to women and men of reproductive age, and it may also be used to enhance pro-fertility initiatives in the general population.
Study funding/competing interest(s): Funding by national/international organization(s) – The study was partly funded through the ReproSund and ReproHigh collaboration receiving EU-regional funding. The authors have no conflicts of interest.
Trial registration number: NA.
Keywords: postponing childbearing, fertility, family formation, women, fertility assessment and counselling

P-522 Family intentions of childless single and cohabiting women aged 35 to 43 years seeking fertility assessment and counselling

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Study question: What characterizes childless women aged 35 years and above seeking fertility assessment and counselling on their reproductive lifespan and are there significant differences between single and cohabiting women?
Summary answer: The women were aware of age related declining fecundity and wished for 1.8 children on average. More than half would consider sperm donation, 45% oocyte vitrification, but only 15% oocyte donation. Despite advanced age, 78% primarily sought fertility assessment and counselling to gain knowledge on the possibility of postponing pregnancy.
What is known already: Recent studies have indicated an increasing demand for ovarian reserve testing in women without any known fertility problem to obtain knowledge on their reproductive lifespan (Hvidman*, et al.*, 2015, Seifer*, et al.*, 2015). Women postpone their first pregnancy and maternal age at first birth

has increased in Western societies over the past two to four decades. Postponed childbearing implies a higher rate of involuntary childlessness, smaller families than desired and declining fertility rates

Study design, size, duration: Baseline data from a prospective population-based cohort study of 340 women aged 35-43 years examined at the Fertility Assessment and Counselling (FAC) Clinic at Copenhagen University Hospital from 2011 to 2014. The FAC Clinic was initiated to provide individual fertility assessment and counselling.

Participants/materials, setting, methods: Eligible women were childless and at least 35 years of age. All completed a web-based questionnaire before and after the consultation including socio-demographic, reproductive, medical, lifestyle and behavioural factors. Consultation by a fertility specialist included a transvaginal ultrasound, full reproductive history and AMH measurement (Hvidman*, et al.*, 2015)

Main results and the role of chance: The study comprised 140 cohabiting and 200 single women. The majority (82%) was well-educated and in employment. Their mean age was 37.4 years. Nonetheless, the main reasons for attending were to get knowledge on their possibility of postponing pregnancy (78%) and a concern about their fecundity (66%). The women averagely wished for 1.8 children and listed their ideal age of first child and last child as 33 (± 4.7) years and 39 (± 3.5) years, respectively. Of the single women, 70% would accept use of sperm donation compared to 25% of the cohabiting women ($p < 0.001$). In general, 45% considered oocyte vitrification, yet only 15% were positive towards oocyte donation. The two groups were comparable regarding lifestyle factors, number of previous sexual partners, pregnancies, and ovarian reserve parameters.

Limitations, reason for caution: The women in the present study were conscious of the risk of infertility with increasing age and attended the FAC Clinic due to a concern about their remaining reproductive lifespan, which in combination with their high educational level could impair the generalizability to the background population.

Wider implications of the findings: Our results display that despite the women´s chronological age of 37.4 years; their proposed ideal age of 33 years at first child, an awareness of declining female fecundity with age and the wish for two children, only few would consider oocyte donation. This paradox could indicate a general overestimation of the women´s own reproductive ability and an underestimation of the risk of future childlessness with the continuous postponement of pregnancies.

Study funding/competing interest(s): Funding by national/international organization(s) – The FAC Clinic is a part of the ReproSund and Reprohigh collaboration that was 50% co-financed by EU-regional funding. This study also received funding through the Capital Region Research Fund. The authors have no conflict of interest.

Trial registration number: NA.

Keywords: postponing childbearing, fecundity, fertility assessment and counselling, sperm donation, oocyte donation

P-523 The role of infertility concerns in predicting the change in men's quality of life during ART treatment in couples with a male infertility factor

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Study question: Does the Quality of Life (QoL) of men, the only cause of a couple's infertility, change during the course of ART treatment, controlling for: duration of infertility, their partners' age, their own and their partners marital satisfaction, need of parenthood and sexual concerns?

Summary answer: Both men and women's QoL increases during the course of ART treatment with men's QoL variation only being influenced by their own marital satisfaction. None of women's variables on commencing ART predict variations in men's QoL.

What is known already: Recent qualitative studies have demonstrated that men's involuntary childlessness constitutes a major life crisis and ART procedures are perceived as burdensome. The psychological profiles of infertile men have a negative impact on their self-image, resulting in relational and psychological distress; there is also evidence that men's psychological distress can predict low levels of QoL. Quantitative data regarding the influence of a couple's relationship (both partners) on men's QoL during ART treatment are scarce.

Study design, size, duration: This longitudinal study comprises two repeated measurements: on commencing treatment (T1) and at the day of Embryo-Transfer (T2). Data were obtained from a restricted group of 56 infertile men and their partners (112 subjects), consecutively referred for ART treatment from February 2013 to December 2014.

Participants/materials, setting, methods: Both members of couples with a male infertility factor were recruited at the ANDROS Clinic in Palermo (Italy). They completed the ENRICH, the Fertility Problem Inventory and the FertiQoL. Structural Equation Modelling was used to analyze the data. Any change in QoL was analyzed by regressing QoL-T1 on QoL-T2.

Main results and the role of chance: Both men's and women's QoL increases during the course of ART procedures ($t = -3.38$, $p < 0.01$ and $t = -4.43$, $p < 0.001$ respectively). The hypothesized model provides an adequate fit to the data ($\chi 2 = 21.54$; $df = 21$; $\chi 2/df = 1.02$; CRI = 0.96; RMSEA = 0.022), showing that a variation in men's QoL is influenced by their own marital satisfaction at T1 ($b = 0.39$; $p < 0.05$). Men with high marital satisfaction on commencing ART treatment, therefore, display an increase in QoL during the course of treatment. Finally, neither men's need for parenthood and sexual concerns nor their partner variables predicts variations in men's QoL.

Limitations, reason for caution: This study has various limitations: firstly, data were obtained from only one clinical site; secondly, the results of the study may be influenced by the small sample size.

Wider implications of the findings: Results of this study show that men's Quality of Life increases during an ART procedure; this could be due to their involvement in how the couples deal with their infertility problem. Due to the influence of men's marital satisfaction on their own change in QoL, couples' relational domains may be identified by health professionals, with a view to providing psychosocial counselling to infertile couples, particularly where men display low marital satisfaction.

Study funding/competing interest(s): Funding by hospital/clinic(s) – ANDROS Day Surgery Clinic, Palermo, Italy. The authors have no conflicts of interests to declare.

Trial registration number: Not necessary.

Keywords: quality of life, marital satisfaction, ART, male factor infertility, longitudinal study

P-524 "A chance to a hope" – opinions of transplanted women of the first uterus transplantation trial

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Study question: What are the opinions and experiences during the first year of the women entering the uterus transplantation trial.

Summary answer: The women ($n = 9$) adjusted well to the new life situation and were properly accustomed in everyday life. Both the seven women with ongoing grafts, as well as the two with graft failure, expressed content with the decision to participate in the trial.

What is known already: No data on the psychology of any patients are available in this field with only 11 cases performed worldwide, and with nine of the women participating in the present study. Within our trial the medical results so far are two cases of early (within 4 months after transplantation) removal of grafts and live births in three cases.

Study design, size, duration: A qualitative interview study one year past the first uterus transplantation. All eligible women $n = 9$, were included in the study. Eight of the participants were lacking the uterus from birth (MRKH syndrome) and one after hysterectomy due to cervical cancer. Age; median 33, range 27-38.

Participants/materials, setting, methods: All women (9) who had undergone uterus transplantation in early 2013 in Sweden were interviewed. The interviews were transcribed verbatim. Data was analysed with a thematic approach. The interviews followed a semi-structured guide focusing on four main domains; Psychological well-being, Relationship with the donor, Follow-up and social aspects.

Main results and the role of chance: The analysis lead to the formation of a master theme, labelled: A chance to a hope. In addition four subthemes were emerged: A body like anyone else's, To be in a transition phase, Getting back to

everyday life, A special relationship. All these subthemes contained underlying categories. This first group of women undergoing uterus transplantation already at baseline showed large adherence in how they reported their psychological wellbeing and commitment to the project. In spite of outcome and adverse events the loyalty of the participants continued during the first postoperative year.

Limitations, reason for caution: This study of the first cohort of women undergoing uterus transplantation is a selected population with possibly greater than average of psychological strength. Even so vulnerability and themes of distress are revealed in this study but need to be explored further and probably expanded in upcoming groups.

Wider implications of the findings: In conclusion, the women of the trial adjusted psychological well to their new lifesituation during the first year after transplantation. When uterus transplantation will enter the clinical arena in a wider perspective the participants will naturally reveal a broader psychological diversity. It will then be of uttermost importance to have gained knowledge of psychological strengths and strains also in a qualitative perspective.

Study funding/competing interest(s): Funding by national/international organization(s) – Jane and Dan Olsson Research Foundation

Trial registration number: NCT01844362.

Keywords: uterus transplantation, psychology, women, qualitative interview

P-525 The mindfulness based program for infertility (MBPI): how does it work in reducing depression?

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Study question: By which mechanism does PBMI help reducing depressive symptoms?

Summary answer: The PBMI contributes to decrease depressive symptoms by increasing self-efficacy in infertile patients through the mindfulness approach.

What is known already: Mindfulness based approaches have been applied and proved efficient to several health problems such as chronic pain, cancer, anxiety disorders, and depression. The MBPI is the first program specifically targeting infertility. This psychological intervention program showed to be effective in reducing depressive symptoms, internal and external shame, entrapment and defeat, in infertile female patients. Inversely it led to a significant improvement in mindfulness skills, self-compassion and in the perception of self-efficacy to deal with infertility.

Study design, size, duration: Longitudinal study. The MBPI is a psychological intervention program that includes 10 weekly sessions, in a group format (10 to 15 participants in each group), with the duration of about two hours each. The MBPI was applied to 5 groups. Data were collect between May 2009 and May 2010.

Participants/materials, setting, methods: Fifty-five women completed the MBPI and 37 women were assigned to a control group. All participants presented a primary infertility diagnosis and were pursuing medical treatment. Standardized measures of depression and infertility self-efficacy were endorsed pre and post MBPI. Participants' recruitment was supported by the Portuguese Fertility Association.

Main results and the role of chance: Significant differences were found on self efficacy ($F_{1,90} = 13.88$, $p < 0.008$) and depressive symptoms ($F_{1,90} = 8.06$, $p = .006$) between intervention and control group from baseline to post intervention. The intervention group reported a significant increase in self efficacy and decrease in depressive symptoms (both $p < 0.001$), while control group reported no significant changes. A mediation analysis was conducted to examine whether changes in self-efficacy mediated the effect of the PBMI intervention in reducing depressive symptoms. Statistical analysis was performed using PROCESS macro in SPSS with bootstrap procedures (5000 samples). Mediation effect was significant (estimate = 1.61, Bootstrap confidence interval:.515; 3.58). Infertile women who received the PBMI intervention increased their perceptions on self efficacy in dealing with infertility, which in turn decreased their depressive symptoms.

Limitations, reason for caution: This was not a blind study given the nature of practical impediments to collecting the sample. Participants' recruitment was conducted through the website of the Portuguese Fertility Association, which means that they have Internet access and may be particularly informed and motivated to some kind of psychological intervention.

Wider implications of the findings: The findings demonstrated the mechanism by which the PBMI is effective in reducing depressive symptoms in infertile patients is by increasing the patients' confidence levels on aspects of cognitive, emotional and behavioral skills related to infertility. The study highlights the importance of promoting self-efficacy, specifically addressing the way infertile patients perceive their abilities to face infertility and the strains of medical treatment when designing psychological intervention programs.

Study funding/competing interest(s): Funding by national/international organization(s) – This research has been supported by the first author Ph.D. Grant (SFRH/BD/68392/2010), sponsored by FCT (Portuguese Foundation for Science and Technology). There is no conflict of interests.

Trial registration number: The study was not a trial.

Keywords: mindfulness based program for infertility (MBPI), infertility self-efficacy, depressive symptoms, mediation analysis

P-526 Patients' educational needs in testicular sperm extraction

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Study question: How do men undergoing Testicular Sperm Extraction (TESE) and their partners experience current patient education and how can this patient education be improved, concerning both the content and the different information channels through which the information can be provided?

Summary answer: Patients were positive about the current patient education, although adjustments could be made regarding the content and different information channels. They wanted more information on 'what's TESE', success rates of the procedure and other patients' experiences. They prefer a physical appointment, but also value a leaflet, website or online application.

What is known already: The fertility patient population consists of young, often high educated men and women, who wish to be well informed about their treatment and frequently search the Internet for information. They consider patient education as one of the most important dimensions of patient-centered fertility care. Although TESE is offered to men with non-obstructive azoospermia for many years, yet little is known about patients' educational needs.

Study design, size, duration: A qualitative study consisting of semi-structured in-depth interviews with 11 couples in May 2014, for which the topic guide was based on a literature review and interviews with an expert panel. In addition, after each interview participants composed a priority list of the five most important items of patient education.

Participants/materials, setting, methods: Eligible couples were couples consecutively visiting the Radboud university medical center for TESE treatment. The number of interviews was determined by data saturation. Data were analyzed in accordance with grounded theory. Items on the priority lists received a score based on their ranking, items were ranked by their sum score.

Main results and the role of chance: Although patients were positive about the current patient education, they wanted more information on several topics. They appreciated information on why to choose for a specific fertility clinic, success rates of the TESE-ICSI treatment, the cryopreservation of TESE semen, other patients' experiences, the psychological impact of the treatment and the possibility to get professional support. Supplementing a physical appointment, patients valued various information channels, such as a leaflet, website or a personalized online application, for which various functionalities were suggested. On priority lists, patients considered 'success rates', 'what's TESE' and 'patients' experiences' as the most important topics of patient education. In these rankings women emphasize on treatment results and other patients' experiences, while men also accent the actual TESE treatment, including risks and recovery.

Limitations, reason for caution: Limitations include the single center design of the study, although it's the largest Dutch center performing TESE. Furthermore there's a shortage of quantitative data. We made a start to provide quantitative data through the priority lists patients composed, but given the small sample size statistic measurements could not be performed.

Wider implications of the findings: In-depth insight in the educational needs of both men undergoing TESE and their partners was obtained. The study center size, together with the fact that patients from across Europe have similar views on patient-centered care, defines the generalizability of our results for other European countries. This supports professionals to customize their patient education and to develop instruments that meet the needs of their patients. In this way, patient-centeredness and quality of care can be improved.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Radboud university medical center.
Trial registration number: NA.
Keywords: patient education, male infertility, TESE, patient-centered care, quality of care

P-527 Reconciling childlessness: comparing the experiences of women who are permanently childless after failed fertility treatments versus delayed childbearing

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Study question: What are the similarities and differences in the psychosocial impact of, and adjustment to, permanent childlessness for women who are childless due to delaying childbearing versus unsuccessful fertility treatments?
Summary answer: Adjustment to childlessness for women who have delayed childbearing is similar in many respects to adjustment to childlessness after unsuccessful fertility treatment. However, there also appear to be important differences that have implications for understanding the psychosocial challenges and support needs of this increasing group of 'unintentionally' childless women.
What is known already: A sizable body of literature has examined the psychosocial consequences of failed infertility treatments and the subsequent transition and adjustment to childlessness for infertile women. However, less is known about the experiences of women who face permanent childlessness, after having delayed childbearing due to the personal, relational, and economic circumstances of their lives.
Study design, size, duration: Results from an interpretive phenomenological qualitative study of the experience of permanent childlessness for women who have delayed childbearing were compared to the existing body of literature on women's adjustment to childlessness after failed fertility treatments.
Participants/materials, setting, methods: Fifteen women who expected to have children but ended up permanently childless after delaying childbearing, took part in two qualitative interviews. Common themes were developed using an interpretive phenomenological analysis (van Manen, 1990). Themes were compared to the existing literature on women's adjustment to childlessness after failed infertility treatments.
Main results and the role of chance: Analysis revealed that women who are childless after delaying childbearing face similar challenges to those who are childless after failed fertility treatments, including: feelings of grief, loss, and isolation; a need to make sense of their childlessness; and a need to rebuild and refocus their lives and identities. In addition, women who are childless after delaying childbearing must also reconcile the reality of their *choice* to delay childbearing based on the personal, social, and economic circumstances of their lives, with feelings of powerlessness to have pursued motherhood when they were likely still fertile. Unlike women who undergo fertility treatments without success, women who expected to become mothers but never actively pursued a pregnancy face more regrets about, and feelings of responsibility for, their childlessness.
Limitations, reason for caution: The findings of this study were based on the experiences of 15 women. More research is needed to increase our understanding of the psychosocial needs and long-term adjustment of the increasing number of women who expect to have children but end up permanently childless after delaying childbearing.
Wider implications of the findings: The findings underscore the importance of educating women about the risks of delayed childbearing, and supporting them in realizing their parenthood goals. They also help to inform mental health practitioners about the psychological support needs of women who end up permanently childless due to delay, and can guide us in our efforts to support this increasing group of women as they reconcile their past choices and construct satisfying and meaningful lives without children.
Study funding/competing interest(s): Funding by national/international organization(s) – Social Sciences and Humanities Research Council Canada #752-2011-2149
Trial registration number: NA.
Keywords: delayed childbearing, childlessness, mental health

P-528 Patient decision-making in female fertility preservation prior to gonadotoxic therapy

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Study question: What do young women indicate as important considerations in their fertility preservation (FP) decision-making at the time of fertility preservation counselling (FPC) and how are these items related to their choices?
Summary answer: The current study revealed that FP decision-making in young women scheduled for gonadotoxic therapy is mainly based on weighing two issues: the intensity of the wish to conceive a child (in the future) and the expected burden of undergoing an FP treatment.
What is known already: Fertility is important for patients whose reproductive function is being threatened by gonadotoxic therapy. To prevent severe psychological effects of infertility and feelings of regret about their FP decision after gonadotoxic treatment, the quality of FPC should be improved. Decisive factors in FP decision-making should be clarified, as they deserve extensive discussion during FPC. These issues have not yet been isolated from the complex interplay of all aspects of FP that women contemplate during decision-making.
Study design, size, duration: By using a mixed methods methodology, a questionnaire developed after qualitative research, was retrospectively sent to eligible patients (n = 143) who had received FPC in the past (1999 - July 2013) and to whom at least one FP option was offered.
Participants/materials, setting, methods: Patients had received FPC at a university hospital in the Netherlands. They were aged ≥ 16 years and were scheduled for gonadotoxic therapy. The relationship between patients' baseline characteristics, their attributed importance to 28 relevant importance items and their FP choices made was investigated.
Main results and the role of chance: After five interviews, 28 importance items for FP decision-making were identified and included in our questionnaire. Of these, 24 items could be clustered into seven importance themes. A total of 87 patients (61%) responded to our questionnaire. After performing a multivariable logistic regression analysis, proceeding with FP was related to higher attributed importance during FP decision-making to the theme 'Wish to conceive (in the future)' (OR 10.8, 95%CI 3.5 – 34.4) and the item 'Having a stable partner relationship' (OR 2.0, 95% CI 1.0 – 4.1), while higher attributed importance to the theme 'Expected burden of FP' during FP decision-making (OR 0.08, 95% CI 0.02 – 0.3) more often resulted in refraining from it.
Limitations, reason for caution: Possible recall, selection bias and the fact that this study was performed in Dutch patients counselled in a single centre possibly limits the representability of our results for a broader European population of patients. Furthermore, we are not able to draw conclusions about the causality of the associations observed.
Wider implications of the findings: The wish to conceive and the expected burden of FP treatment should be discussed carefully with patients during FP decision-making, either by the referring healthcare provider or by reproductive medicine specialist. Prospective research is needed to explore the causality of the associations found. To deliver high quality patient centred care, the development of tools to explore patients' wish to conceive and tools to provide clear information about the burden of FP treatments is needed.
Study funding/competing interest(s): Funding by hospital/clinic(s) – This work was supported by the Radboud Institute for Health Sciences (research school affiliated to the Radboud university medical center). The authors have declared no conflicts of interest with respect to this work.
Trial registration number: NA.
Keywords: fertility preservation, counselling, female, decision-making, gonadotoxic therapy

P-529 Infertile patients' preference for receiving clinical information and participating in decision-making in China

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Study question: To assess the Chinese infertile patients' preference for receiving information and participating in decision–making and to evaluate their satisfaction with information provision.
Summary answer: The infertile patients prefer to receive information concerning their diagnosis and treatment options. The female partners were more anxious to receive information while the male partners dominated in clinical decision-making. The desire for information perception and decision-making was positively related to participants' education.
What is known already: Providing enough and clear information to patients, respecting their right to make treatment decisions is significant for high-quality and patient-centered fertility care. Factors as culture, education, age and gender might play roles in patients' desire for information perception and decision-making. The traditional paternalistic physician- patient relationship in some Asian countries including China also affect the patents' attitude towards decision-making. The patients in these countries to some extent rely on physician's involvement in their decision-making.
Study design, size, duration: Prospective survey was performed in cohort of 200 infertile couples (400 individuals) attending the infertility counseling during July, 2013 to Sept. 2014.
Participants/materials, setting, methods: The couples was interviewed by a physician according to a questionnaire contained questions about patients' attitude toward preference for information and participation in decision-making and satisfaction with information provision. A ten-score scale was used to measure the patients' desire. Necessary clarification for each question was given by the interviewer.
Main results and the role of chance: The mean score for desire to receive information was 8.93 (± 2.1) out of 10. The desire to receive information was significantly greater in female partners than in male (9.14 ± 1.7 vs 8.72 ± 1.9, $p < 0.01$). The mean score for satisfaction with information provision was 8.26 ± 2.4. Male partner was more satisfied with information provision than female (8.53 ± 2.8 vs 8.00 ± 2.7, $p = 0.021$). The mean score for preference to participating in clinical decision-making was 8.69 ± 2.9. The desire to participate in decision-making was greater in male than female partners (9.21 ± 1.7 vs 8.16 ± 2.3, $p < 0.01$). The desire to receive information and participate in decision-making was positively related to education (r = 0.33 and 0.45).
Limitations, reason for caution: Though the patients of our Hospital coming all over the country, the sampling limited to our hospital might not be statistically representative of the Chinese population.
Wider implications of the findings: In China, the infertile couples were highly interested in receiving information about their diagnosis and treatment options and participating in clinical decision-making at present. Further investigation would be performed to clarify the gender gap between their desire to information perception and clinical decision-making.
Study funding/competing interest(s): Funding by University(ies) – Central South University.
Trial registration number: NA.
Keywords: decision making, fertility care, information perception, ethics

P-530 Compliance needs across cultures. A multi-cultural study in European and Latin-American countries

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Study question: The main objective was to investigate intention of discontinuation across countries and cultures in patients receiving fertility treatments in Europe (Spain) and Latin-America (Argentina, Chile and Brazil), regarding how do discontinuation rates vary and if it is related to the patient, the treatment or the clinic.
Summary answer: Of 537 participants, most never considered discontinuing treatments (61.6%), but most who did were from Latin America ($P < 0.01$). The most cited reasons, in both continents, in order, are not related to psychological reasons, but to costs of treatment and poor treatment progress.
What is known already: The field of reproduction produced an alarming view of discontinuation rates (high as 65%) and studies demonstrated that discontinuation has an impact on the estimation of success rates (Gameiro, 2014). But reasons for discontinuation are still not clear (Gameiro, 2014, Boivin 2012). There is no knowledge about intention to drop-out in South America, and in comparison with Europe. Cultural differences are necessary to improve specific knowledge of compliance needs across continents.
Study design, size, duration: A multicentre, cross sectional survey was performed during October 2012- May 2014 in four different countries, by 7 private clinics (Argentina 3: Brazil 1, Chile 1; Spain 2).
Participants/materials, setting, methods: 537 participants answered the questionnaire developed by the Scientific-Committee-of Psychosocial-Area-of-ALMER. 348 participants: Latin-America, 189: Europe. Mean- age, type of family, average of fertility treatments, intention to discontinue, perception of being insufficiently informed by the medical staff, lack of emotional support, feeling stressed about results, relationship with partner, and financial reasons were recorded and compared (chi-squared) between continents. $P < 0.05$ was considered significant.
Main results and the role of chance: -61.6% did not intend to discontinue. -38.4% considered discontinuation. Reasons reported, in order of frequency: **cost, negative evolution of treatment, psychological stress**. All reasons evaluated were: costs of treatment, negative evolution of treatment, abortion, psychological stress, not feeling supported by staff, feeling poor informed about the treatment, treatment-related stress in their relationship, adoption, woman's age, others.
-No differences between continents regarding:

- cited reasons for discontinuing
- feeling emotionally supported by medical staff (79% did)
- type of families (92.3% heterosexual couples)
- treatments (47.6 % homologous IVF)

-Comparisons between continents showed patients in Latin-America scored higher ($P < 0.01$) than Europeans on:

- Possibly discontinuing.
- Number of treatments received.
- Poor or incomplete information on their treatment.
- Worries about the progress of their treatment.
- Concerns about treatment cost.
- Treatment-related stress in their relationship.

Limitations, reason for caution: The Study compared date between Europe and Latin-America. Although a larger and more representational sample would give more evidence, these results can be considered to provide further insight into compliance needs across countries and cultures, and indicate that the most common reasons for discontinuation, are not psychological.
Wider implications of the findings: Although most literature cites psychological factors as the main reason for discontinuation (Olivius, 2004, Domar, 2010), this study in European and Latin-American populations agrees with Boivin (2012), and reports reasons other than psychological as the most common cause for drop-out. Different reasons for discontinuation (with no difference between continents), are economic and poor medical response. Further analysis of clinic- and treatment-associated factors should help reduce drop-out. Compliance needs should give special attention to cultural aspects.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Each participant clinic did it funding.
Trial registration number: No trial.
Keywords: discontinuation, fertility treatments, compliance, cultures

P-531 The ART of communication: results from an international needs assessment in assisted reproductive technologies

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Study question: Given the importance of providing patient-centered care, what are the challenges reported by reproductive physicians and embryologists in discussing the treatment and management of fertility issues in a patient-centric manner?
Summary answer: Challenges were reported pertaining to provider confidence in communicating with patients with fertility issues regarding: 1) Risks associated with treatment; 2) Patient expectations regarding treatment; and 3) Available resources for support. Challenges were reported communicating these topics across countries.
What is known already: ESHRE guidelines for the psychosocial management of infertile patients outline that the provision of patient-centered care is associated with better quality of life and patient wellbeing during treatment. Positive patient-centered care experiences including optimal patient-provider communication are associated with better compliance to fertility treatment and improved chances of a successful pregnancy. Healthcare professionals should be able to communicate effectively with each patient to ensure their individual informational needs and treatment expectations are properly managed.
Study design, size, duration: An IRB-approved, mixed-methods study combining qualitative telephone interviews and a quantitative survey was conducted in two phases between March 2013 and May 2013 in China, Japan, Turkey, Russia, India, and United States and between January 2014 and April 2014 in Canada, Mexico, France, Italy, Brazil, and South Korea.
Participants/materials, setting, methods: Purposive sampling criteria were used to recruit practicing reproductive physicians and embryologists treating infertile couples across 12 countries. The total sample included 373 healthcare professionals involved in the treatment and management of fertility issues, with the majority represented by Reproductive Endocrinologists (63%). More than half of the sample (54%) had 11-20 years of clinical experience.
Main results and the role of chance: Participants reported the importance of discussing sensitive topics with their patients with a few exceptions, but reported low confidence in their ability to discuss these topics. When asked to rate their confidence (1-low, 3-Good, 5-Optimal), 43% of participants reported their confidence was *low-good*. In relation to the management of patient treatment expectations, more than half of participants (54%) reported that their confidence in discussing the potential psychological impact of ART on the patient and/or the couple was *low -good.* Also, 43% of participants reported that their confidence in discussing the failure of a fertility cycle with their patient was *low-good.* When asked about their confidence in discussing references for external social/financial support, 64% of participants reported their confidence as l*ow-good.*
Limitations, reason for caution: Voluntary participation and self-reports may introduce selection and reporting bias. Large variability in the roles and responsibilities of physicians treating infertile couples across countries render generalization difficult. To further validate the findings, this study should be reproduced with larger samples and include other members of the healthcare teams.
Wider implications of the findings: Addressing challenges in patient-centric communication could contribute to better management of patient's expectations, adherence to treatment and ultimately improved treatment outcomes. Findings from this study could be used to design continuing education initiatives with considerations for social, cultural and gender issues in each region, as in the majority of international training systems, communication competencies may not be integrated systemically into graduate or postgraduate education for healthcare providers with direct patient contact
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study was financially supported with education research funds from Merck KGaA, Darmstadt, Germany.
Trial registration number: NA.
Keywords: mixed-methods, assisted reproductive technologies, reproductive physicians, fertility care

P-532 'Because we want a child' - motives and considerations regarding PGD in couples carrying a structural chromosomal abnormality

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Study question: What are motives and considerations of couples carrying a structural chromosomal abnormality deciding on preimplantation genetic diagnosis (PGD)?
Summary answer: Couples carrying a structural chromosomal abnormality consider both PGD and spontaneous conception with or without prenatal diagnosis (PND) as usable reproductive options. Reproductive history is a main motive influencing couples' choice. And couples tend to choose the option, that in their consideration, will lead to a successful pregnancy fastest.
What is known already: PGD can be offered to couples carrying a structural chromosomal abnormality who are at risk of miscarriage or an ongoing pregnancy of an unbalanced fetus resulting in physical or mental disabilities in the child. From our previous study we learned there is no difference in obstetric history between structural chromosomal abnormality couples who opt for or decline PGD. Leading to our question what these couples' motives and considerations are when making a decision on PGD.
Study design, size, duration: A qualitative exploratory study investigating the motives and considerations of couples carrying a structural chromosomal abnormality making a reproductive choice after extensive genetic counselling on PGD. 13 couples were included in 2013 and 2014.
Participants/materials, setting, methods: Semi-structured dyadic interviews were conducted among 13 couples carrying a structural chromosomal abnormality who had an informative consultation in a licensed large PGD centre. Open coding of the data, followed by coding into core themes took place to identify the key aspects of participants motives and considerations regarding PGD.
Main results and the role of chance: A majority of couples indicated that an important reason for them to choose PGD was the wish to increase the chance of a successful pregnancy. All couples considered PGD or natural conception combined with PND in case of an ongoing pregnancy as the only two reproductive options. All couples who opted for PGD had tried to conceive spontaneously at first. They entered the PGD programme because of what they experienced during these attempts (infertility, recurrent miscarriage, termination of pregnancy, affected child). Couples that refrained from PGD express the long trajectory combined with a higher age of the prospective parents as main reasons. However, all couples added that, if conceiving spontaneously does not lead to an ongoing pregnancy, they will eventually try PGD.
Limitations, reason for caution: This is an exploratory study with a qualitative design, results should be confirmed in a quantitative study. Our design excluded couples who do not wish to consider PGD and are therefore not referred to a PGD clinic for genetic counselling.
Wider implications of the findings: This study shows that couples carrying a structural chromosomal abnormality consider both PGD and spontaneous conception with or without PND as usable reproductive options. They are looking for the option that is in their opinion the fastest way to get pregnant. Information on the perceived pros and cons of PGD or spontaneous conception in these couples can help to optimize counselling and psychological support during the decision making process.
Study funding/competing interest(s): Funding by hospital/clinic(s) – G.K. is supported by the 'Fertility Foundation Maastricht' as a junior researcher.
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, structural chromosomal abnormalities, reproductive decision-making, qualitative analysis

P-533 Measuring quality of life in infertile couples using the Italian version of fertility quality of life -FertiQoL- questionnaire

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Study question: The aim of this study was to examine the psychometric properties of the Italian version of the FertiQoL by testing in a national sample the hierarchical factor model previously identified by the authors of the original version and by analyzing the effectof educational level, age, and phase of treatment in which the women are.
Summary answer: CFA confirmed the hierarchical factor model, the internal consistency of the Italian FertiQoL and of its subscales are good or acceplable. The scores are not affected by patients' age and education, while the phase of treatment in which they are has a significant effect on FertiQoL's scores.
What is known already: Infertility and its treatments have significant impact on a individual's Quality of Life (QoL) (Schmidt, 2006; Verhaak et al., 2007; Chachamovich et al., 2010a). The FertiQoL (Boivin et al., 2011) is a fertility specific QoL assessment questionnaires recently developed that has demonstrated good psychometric characteristics (Hsu et al., 2013; Aarts et al., 2011). A recently study (Salerno et al., 2013) tested the Italian version of the questionnaire in a MAP center but the factorial validity was not tested.
Study design, size, duration: Patients in different stages of treatment completed the FertiQoL and other questionnaires within a broader multicenter research on infertile couples attending four centers for assisted reproduction in different Italian cities (Turin, Cattolica, Rome, Catania).
Participants/materials, setting, methods: The sample consisted of 370 infertile women in three different stages of treatment (diagnostic, stimulation, transfer) and with a number of previous treatment cycles ranging from 0 to 9. The FertiQoL raw data were subject to Confirmatory Factor Analysis (CFA), to subscale reliability analysis and the subscales scaled scores to one way ANOVAs.
Main results and the role of chance: The Confirmatory Factor Analysis provided a good fit to the data across the hierarchical model individuated in previous studies with a CFI = .88, RMSEA = .05 and SRMR = .06. All the FertiQoL scales had acceptable internal consistency with values ranging from 0.70 to 0.90. After the deletion of two items, Cronbach's alpha increases from.83 to.87 and from.79 to.81, erasing respectively the item Q4 and T2. Q4, in fact, deal with coping, while the item T2 notice the availability of services. No significant differences among educational level and among age on the FertiQoL scores were found. Interesting effect is observe between the environment subscaled and the previous MAP attempts.
Limitations, reason for caution: The stability of the scores was not investigated.
Wider implications of the findings: The results of this study provide a baseline QoL in infertile couples in Italy and could potentially be used as a reference for future works and clinical QoL counseling.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study was funded by Ferring Pharmaceuticals.
Trial registration number: NA.
Keywords: FertiQuol, infertility, validity, assited reproductive technologies

P-534 Treatment anxiety in assisted reproductive technology (ART): how do women differ from their partners?

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Study question: The aim of the present study was to determine levels of anxiety during the course of IVF treatment and whether gender differences in treatment anxiety exist.
Summary answer: Women show a higher level of anxiety during IVF treatment and hold different concerns. Neither of the sexes appears to be familiar with the risks associated with multiple pregnancies, a matter that should better be addressed.
What is known already: Several studies have shown that IVF treatment causes high levels of emotional and social distress in patients, causing numerous couples to drop out of the treatment before the goal of pregnancy is reached. Less is known about the gender differences and procedures responsible for the distress.
Study design, size, duration: Patients at first, as well as patients at repeat consultations were asked to fill out an eight page survey on their anxieties and worries in the context of their treatment, which consisted of the STAI (Spielberger state – trait anxiety inventory) and a 25 item questionnaire about specific stress factors and possible triggers of treatment anxiety.
Participants/materials, setting, methods: 119 women and 82 men undergoing IVF treatment in a university affiliated, tertiary care IVF program, from November 2012 to June 2013.
Main results and the role of chance: Women and men undergoing IVF score higher on the STAI than the average population in Germany. Overall, female patients show significantly higher values for state and trait anxiety (Mean = 47.4/40.1, SD = 11.0/9.85) than their male partners (Mean = 41.4/35.3, SD = 9.66/8.57, $p < 0.01$). When asked about specific stress factors on a 4-point scale from "not at all" to "very much so", women report as their main anxiety the failure to achieve a successful pregnancy, scoring significantly higher on questions like "disclosure of infertility" (Mean = 2.99, SD = 1.10, $p < 0.001$). Their male partners, however, are highly significant more concerned about the health risks the women have to take such as "occurrence bleeding or infection after the oocyte aspiration" (Mean = 2.58, SD = 0.84, $p = 0.007$). Both genders indicated to be very little worried about multiple pregnancies by IVF.
Limitations, reason for caution: The questionnaire was completed by the patients on a voluntary basis, leaving the possibility of a selection bias by including only certain couples, that were open minded to participation. Furthermore, the sample might not represent the worldwide population, since the data was acquired in just one reproductive medicine centre in Germany.
Wider implications of the findings: The main findings of this study go in agreement with current literature on this topic, especially showing once more the patients' underestimation of multiple birth risks. The generalizability to other populations remains unclear, but literature suggests that for western countries the results are comparable. New insights have been made in terms of significant gender differences of treatment anxiety and specific stress factors within the treatment, opening new perspectives and possibilities for psychological support.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University Hospital Schleswig-Holstein.
Trial registration number: NA.
Keywords: gender differences, treatment anxiety, controlled ovarian stimulation, cost, resource utilization

P-535 How do we relate to each other? Children's, parents' and donors' perspectives in sister-to-sister oocyte donation families

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Study question: How do family members (parents, children, and the donor) experience family relations in sister-to-sister oocyte donation families?
Summary answer: Family members emphasized the strength of their family relationships both as a given – independently of the oocyte donation – and as a condition for oocyte donation. In two families, the donors' status of 'godmother' seemed to capture the special bond between donor and child and made it manageable for all parties.
What is known already: Although sister-to-sister oocyte donation has been practiced for at least 15 years in several countries, little is known about family relations within these families. Literature points at strong and stable sister relations. However, relations between donor and child and between parents and child are relatively underexplored. The current study aimed to offer an in-depth understanding of multiple family relations within these family constellations, based on the perspectives of both parents, children, and the donor.
Study design, size, duration: As part of a larger qualitative research project on family members' perspectives on social and genetic parenthood, semi-structured interviews were conducted with heterosexual couples, their oocyte donors and one of their children. Participants were recruited via the Department of Reproductive Medicine of the Ghent University Hospital.

Participants/materials, setting, methods: Couples eligible for the study were contacted by their counsellor seven to ten years post treatment. Two couples, one mother, three oocyte donors and three children were interviewed separately. Interviews were analysed using Interpretative Phenomenological Analysis, followed by an analysis within families and a comparison across families.
Main results and the role of chance: Family members stressed that their relationships have always been strong, independently of the oocyte donation. What prevailed was thankfulness towards the donor, and a sense of being able to contribute in the donors themselves. Parents and donors put forward that they did not make initial arrangements apart from the decision to disclose to the child, which in all cases was left to the parents. While overall the role of the mother was clearly distinguished from the role of the aunt/godmother, in two families the donor reported increased feelings of responsibility or even primal mother feelings right after the birth of the child. In these families, being a godmother seemed to have a symbolic function, capturing the increased responsibility that was felt towards the donor child.
Limitations, reason for caution: Our analysis was based on a small sample and does not intend to produce generalizable findings. Moreover, it was based on a specific subset of families in which the donor conception was disclosed to the child and parents felt comfortable with their child being interviewed.
Wider implications of the findings: The current study provides a deeper understanding of family relations underlying and stemming from sister-to-sister oocyte donation. In counselling, the necessity of long-term arrangements regarding the rights and responsibilities of the different parties, is often presupposed. The participants in our study however, pictured the donation as a spontaneous undertaking, based on mutual trust. At the same time, counsellors can play a role in exploring the different meanings of genetic links together with all parties.
Study funding/competing interest(s): Funding by University(ies) – The project is funded by the Special Research Fund of Ghent University. Approval by the appropriate Ethics Committee has been obtained. There are no competing interests.
Trial registration number: NA.
Keywords: qualitative research, oocyte donors, family relations

P-536 Longitudinal assessment of patients' willingness to donate frozen embryos for research

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Study question: Which factors are associated with patients' willingness to donate frozen embryos for research over time?
Summary answer: A significant decrease in patients' willingness to donate frozen embryos for research over time was observed. A higher education level, being non-Catholic and considering research with human embryos not to be very important was predictive of participants being less willing to donate embryos for research over time.
What is known already: Patients' willingness to donate frozen embryos for research is subject to change over time. Qualitative studies show that change results from interrelated dynamic dimensions as cognitive appraisals (e.g. success rate, quality of embryos), emotional responses and moral judgments, shaped by sociocultural and organizational aspects. Few quantitative studies focus on the longitudinal assessment of the factors associated with patients' willingness to donate frozen embryos for research.
Study design, size, duration: Prospective longitudinal study. Patients undergoing infertility treatment were consecutively and systematically recruited in one public reproductive medicine centre, Portugal, between August 2011-August 2012 (313 women and 221 men). Participants were reevaluated 12 months later (114 women and 84 men; participation rate = 37%). This analysis is based on 82 heterosexual couples.
Participants/materials, setting, methods: Data on sociodemographic characteristics, reproductive history, psychosocial variables and willingness to donate frozen embryos for research were collected by a self-report structured questionnaire, in both moments. A mixed-effects model with random effects by couple was fitted for the analysis of the willingness to donate on time.
Main results and the role of chance: There was a significant decrease in patients' willingness to donate frozen embryos for research over time (OR = 0.24;95%CI:0.14-0.41). The effect of time was different according to education, religion and importance attributed to embryo research. Patients with > 12 years of education were less willing to donate embryos for research in the second moment ($Or_{education}$ = 2.64;95%CI:0.82-8.48; OR_{time} = 0.65;95%CI:0.35-1.22; $OR_{interaction}$ = 0.06;95%CI:0.02-0.20). Non-Catholics were less frequently willing to donate over time ($Or_{religion}$ = 14.72;95%CI:7.28-29.77; OR_{time} = 0.36;95%CI:0.60-2.35; $OR_{interaction}$ = 0.24;95%CI:0.15-0.41). Participants who considered research with human embryos not to be very important were less frequently willing to donate embryos for research over time than those who considered research very important ($Or_{importance}$ = 0.51;95%CI:0.15-1.70; OR_{time} = 0.36;95%CI:0.20-0.67; $OR_{interaction}$ = 0.21;95%CI:0.06-0.70). Reproductive history, levels of depression and anxiety and the quality of the partner relationship did not influence change in patients' willingness to donate embryos for research.
Limitations, reason for caution: The sample size and the fact that data derives only from one public reproductive medicine centre, located in a university hospital, implies caution regarding data generalizability.
Wider implications of the findings: This study highlights the need of addressing change in patients' willingness to donate embryos for research in order to obtain fully informed consent. Patients' counseling over time should be provided to improve the patientcentredness of care.
Study funding/competing interest(s): Funding by national/international organization(s) – This study was co-financed through FEDER funding from the Operational Programme Factors of Competitiveness – COMPETE and through national funding from the FCT - Foundation for Science and Technology (Portuguese Ministry of Education and Science) within the project "Health, governance and accountability in embryo research: couples' decisions about the fates of embryos" (FCOMP-01-0124-FEDER-014453), and the Grants IF/00956/2013 (to SS), IF/00829/2013 (to HM) and SFRH/BD/75807/2011 (to CS).
Trial registration number: NA.
Keywords: embryo disposition, embryo research, IVF, cryopreserved embryos, decision-making

P-537 Sister-to-sister oocyte donation: couples' experiences with regard to genetic ties

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Study question: How do couples experience the difference in genetic ties to their child?
Summary answer: Couples struggled with the prevailing ideal of genetic connectedness. Couples' ways to deal with the imbalance in genetic ties varied: some couples acknowledged each other's different experiences, others tried to convince one another or pushed away the differences.
What is known already: Few research is available on the experiences of sister-to-sister oocyte donation. The available literature suggests that donors and recipients have good and stable relationships or even strengthened sister relationships. Current study aimed to gather in-depth information on couples' experiences with the difference in genetic ties in the case of known intra-familial oocyte donation, which is frequently practiced in the Belgian context.
Study design, size, duration: Semi-structured couple interviews were conducted with five heterosexual couples (10 participants), recruited via the Department of Reproductive Medicine of the Ghent University Hospital. All participants had at least one child conceived via oocyte donation, ranging from 7 to 10 years old.
Participants/materials, setting, methods: An Interpretative Phenomenological Analysis was performed, with a focus on both individual experiences and the couples' experiences with regard to genetic ties. This inductive method entails a phased process from memo writing to the construction of themes. The

validity and trustworthiness of the analysis was guaranteed through auditing by the co-authors.
Main results and the role of chance: Our analysis revealed that the sister-to-sister donation enabled mothers to equal genetic parenthood. Although the gestational and the indirect genetic connection made mothers feel that the child belonged to them, for some the lack of a full genetic tie remained a meaningful absence and led them to question the legitimacy of their motherhood. They questioned their identity and sense of being the 'real mother'. Couples tried to deal with this imbalance in genetic ties by convincing one another, acknowledging the imbalance or trying to erase it. Couples also managed the family positions by negotiating the closeness in their family relationships.
Limitations, reason for caution: We analyzed a small-scale study in order to provide an understanding of people's lived experiences in a specific context. It should be noted that the context of sister-to-sister donation is different from that of known donation, due to the presence of an indirect genetic link between the recipient mother and her offspring.
Wider implications of the findings: Findings of this study plead for offering post-donation care, besides predonation care, as some families struggled with grieving the loss of genetic parenthood. Caregivers should acknowledge the multiple losses experienced by fertility patients and provide space to express the grieving process. In addition, addressing the couples' coping abilities can strengthen the couples' identity and help to increase confidence in their parental position.
Study funding/competing interest(s): Funding by University(ies) – The project is funded by the Special Research Fund of Ghent University. Approval by the appropriate Ethics Committee has been obtained. There are no competing interests.
Trial registration number: NA.
Keywords: qualitative research, oocyte donors, couple dynamics

P-538 Sexual function in women with polycystyc ovary syndrome: a systematic review

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Study question: The aim of this systematic review was to present a comprehensive summary of the literature on sexual function in women with Polycystic Ovary Syndrome (PCOS). We also assessed the quality of these studies.
Summary answer: We included 19 studies on PCOS and sexual function. Most of them did not meet quality assessment criteria. Results on sexual function were often contradictory.
What is known already: Data on sexual function in women with PCOS are relatively scarce. Sexual function is influenced by both somatic and psychosocial factors. PCOS and its treatment compromises both. Due to elevated androgen levels and comorbidities such as depression and low self-esteem we expect sexual function to be compromised in women with PCOS. Previous research shows that women with PCOS feel less sexual attractive than healthy women but rate a satisfying sex life equally as important.
Study design, size, duration: A systematic review of literature following PRISMA guidelines was done. Nine electronic databases were searched. No restrictions on date, type of publication or language were applied.
Participants/materials, setting, methods: Selection and quality assessment of studies were independently done by two researchers using an adapted version of the Newcastle-Ottawa Quality Assessment Scale and the Cochrane risk of bias assessment tool. Main keywords for the search: polycystic ovary syndrome, sexuality, sexual dysfunction, coitus, libido, arousal, dyspareunia, vaginismus, lubrication, orgasm, masturbation, satisfaction.
Main results and the role of chance: The search identified 1455 original articles. We included 19 studies based on previously formulated criteria. Most studies did not meet quality assessment criteria. Data were summarized consistent with the domains of the sexual response cycle, complemented with frequency of intercourse, dyspareunia, satisfaction, feeling of attractiveness and distress. Some studies reported treatment effects. Results were often contradictory. For example, our preliminary analysis suggests that lubrication problems are more prevalent in PCOS women than in healthy controls but not dyspareunia. Sexual satisfaction seems to be lower than in healthy control women. Sexual distress seems to be prevalent. Treatment with metformine or oral contraceptives may improve sexual function in women with PCOS.
Limitations, reason for caution: Due to the poor quality of previous studies, no definitive statements can be made about sexual function in PCOS women. For example, several studies did not use a control group, or did not exclude PCOS in the control group or did not use valid outcome measures.
Wider implications of the findings: Assessing the quality of a woman's sex life is important particularly in women with PCOS. Hence it should be addressed in a clinical setting and doctors should try to personalize treatment if possible. More research with sound methodologies and validated questionnaires should be performed. For example, the relationship between androgen levels and sexual function is inconclusive and needs further investigation. We also recommend psychophysiological measurements and intervention studies.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Erasmus MC University Hospital, Rotterdam, The Netherlands.
Trial registration number: NA.
Keywords: PCOS, sexuality

P-539 Corticotropin-releasing hormone and postpartum depression: a longitudinal study

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Study question: To assess possible associations between placental CRH (pCRH) at pregnancy week 18 and postpartum depression.
Summary answer: The findings of this study demonstrate that women with depressive symptoms at postpartum week 6 present significantly higher pCRH levels at pregnancy week 18, compared to healthy controls, after the exclusion of study participants with depressive symptoms during pregnancy.
What is known already: Postpartum depression is a common cause of pregnancy and postpartum related morbidity and may have severe consequences for mothers and infants. Most studies support the role of Hypothalamic Pituitary Adrenal (HPA) axis alteration during pregnancy and the subsequent pCRH withdrawal after delivery of placenta in the development of postpartum depressive symptoms. A few studies have assessed possible associations between pCRH levels in pregnancy and postpartum depression.
Study design, size, duration: In the current study, that was designed as a case-control study, a valid pCRH sample along with a completed Edinburgh Postnatal Depression Scale (EPDS) questionnaire was retrieved by 640 women. Study duration was approximately one year.
Participants/materials, setting, methods: pCRH samples were taken at pregnancy week 18, at the Department of Obstetrics and Gynaecology at Uppsala Hospital. EPDS was filled at pregnancy week 17 and 32 and at six weeks and six months postpartum. At postpartum week six, 87.5% (N = 560) of women had EPDS < 12 and 12.5% (N = 80) had EPDS ≥ 12.
Main results and the role of chance: A logistic regression model with EPDS scores at six weeks postpartum (EPDS ≥ 12 points) as the outcome variable and logarithmized pCRH at pregnancy week 18 as the exposure variable showed a positive association between pCRH levels and depressive symptoms (OR = 3.3; 95% CI 1.32 - 8.25, $p = 0.011$). The association remained significant, even after controlling for stressful life events, history of depression and various medical conditions as possible confounders (aOR = 4.84; 95% CI 1.79 - 13.05, $p = 0.002$). The analysis was performed in women with absence of depressive symptoms during pregnancy (EPDS < 12 at pregnancy week 17 and 32).
Limitations, reason for caution: A limitation is the use of a self-reporting psychometric measure instead of a psychiatric interview. The EPDS is a self-reporting instrument, and thus a degree of misclassification may occur. However, this scale is widely used and validated and has a quite high sensitivity and specificity.
Wider implications of the findings: The results of the few existing studies on the association between levels of pCRH during pregnancy and risk of postpartum depressive symptoms are inconclusive. The biological mechanisms underlying postpartum depression have not yet been clearly elucidated. The novel finding of the present study is the positive association between pCRH levels in

pregnancy and genuine postpartum depressive symptoms, after the exclusion of women with depressive symptoms in pregnancy
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Funding by national/international organization(s) – Uppsala University Hospital, Marianne and Marcus Wallenberg Foundation, The Swedish Research Council.
Trial registration number: NA.
Keywords: CRH, cortisol, postpartum depression

P-540 Reaching for paternity: a phenomenological study on the desire to build a family among female-to-male transsexuals undergoing fertility preservation

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Study question: How do female-to-male transsexuals experience life regarding their desire to build a family when undergoing fertility preservation (FP)?
Summary answer: Our results show that female-to-male transsexuals that undergo FP by egg freezing experienced their desire to build a family as highly dependent on surrounding boundaries, such as legislative decisions concerning surrogacy and adoption; FP gave them respite while waiting for societal changes that could open additional possibilities to reach paternity.
What is known already: Contemporary assisted reproduction techniques have extended possibilities beyond the normative heterosexual family, and lesbian, gay, bisexual and transsexual's rights to have children have been recognized. However research in the area is still sparse and no studies have investigated transsexual men's lived experience of wanting children and a family, nor the motivations behind the decision to undergo FP.
Study design, size, duration: Prospective pilot project of FP in individuals with transsexualism. Female-to-male transsexuals undergoing FP by egg freezing were invited to participate in individual interviews with a phenomenological approach. The interviews lasted 68-95 minutes and were digitally recorded and transcribed verbatim. The study inclusion started in May 2014 and is still ongoing.
Participants/materials, setting, methods: By January 2015, seven transsexual men (age 19-33) had participated in the study, shortly after having undergone FP. The interviews focused on individual plans of having children and motives behind undergoing FP. Data was analyzed by using a phenomenological hermeneutic approach in order to interpret lived experience.
Main results and the role of chance: The preliminary analysis resulted in one main-theme and three sub-themes. The main-theme 'Being limited by biological and societal boundaries' describes how the desire to build a family was dependent and limited by different boundaries. This caused distress and bitterness, especially when political decisions hindered them to reach life goals. The sub-theme 'Wanting a child of one's own' concerns how the desire to have children had to be adjusted to available possibilities. The sub-theme 'Having gametes in the freezer' shows how the performance of FP had provided them with resources and opened options concerning future possibilities to have children. The sub-theme 'Planning for building a family' describes how different methods were considered to achieve paternity, such as carrying a pregnancy by them-self, surrogacy or adoption.
Limitations, reason for caution: The results are based on an interpretation of text and the authors strived to be reflective about their pre-understanding of the phenomenon. The men were generally well educated and dedicated to transsexuals' rights. So far only seven men have been interviewed and some caution is advised when interpreting the results.
Wider implications of the findings: Our preliminary results indicate that transsexual men who undergo FP experience limited possibilities to achieve paternity even though they have a strong desire to have children. There is a need to offer specifically developed reproductive counseling directed to transsexual men in order to discuss reproductive possibilities and what might be accessible to them.
Study funding/competing interest(s): Funding by University(ies) – Karolinska Institutet, Stockholm, Sweden.
Trial registration number: NA.
Keywords: transsexualism, fertility preservation, qualitative research, family

P-541 Effects of medical causes, role concepts and treatment stages on quality of life in involuntary childless men

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Study question: Main goal of this study was to investigate differences in quality of life in men contingent upon various fertility treatment stages, infertility causes and adoption of roles.
Summary answer: Participant's perceptions on quality of life differed in enormity according to the cause of infertility, treatment stage and number of adopted roles, with men reporting higher quality of life when having previously experienced severe medical conditions, haven't started treatment yet and resuming several tasks in the treatment process.
What is known already: Involuntary childlessness is seen as major life crisis for men, with distress in fertility treatment rising over time and treatment procedures sensed as high burden; however perceived quality of life seems not to alter significantly. Mixed findings can be found regarding the suffering due to the causer of infertility, though research concerning infertility as consequence of other medical conditions is missing. A high importance of participation for men in the fertility process is suspected.
Study design, size, duration: A quantitative study was devised in three german fertility centers, approved by the Ethics Committee of the Heidelberg University Hospital. The study took place from July to September 2013 with N = 115 participants.
Participants/materials, setting, methods: Participants completed a standardized, fertility-specific questionnaire devised for men (TLMK) and additional sociodemographic and role items. Recruiting took place by direct approach in the fertility clinic. Care was taken to obtain men from different stages in the treatment process. Data were analyzed using correlation, regression, independent t-test analysis and ANOVA.
Main results and the role of chance: Men having experienced severe medical conditions, e.g. cancer, gene dispositions, showed significant higher quality of life scores compared to men with other infertility reasons ($t(56) = -3.57$, $p = .001$). Furthermore, allocating participants into distinctive groups by means of kind of treatment and duration revealed significant group differences in quality of life ($F(2, 111) = 4.94$, $p = .009$), with distress rising with the use of more invasive fertility methods. A positive relationship had been found in men adopting many tasks in the treatment process in terms of reporting more quality of life and perceiving their contribution to the whole process as high.
Limitations, reason for caution: Generalization of findings is limited due to a response rate of 50%, above-average educational background of participants and the fact that not all involuntary childless couples seek infertility treatment in a fertility clinic.
Wider implications of the findings: The high quality of life displayed by men having experienced severe medical conditions contains valuable and far-reaching information about possible resilience factors that need to be researched more in detail. Confirmation of rising distress in treatment for men, independent of treatment duration, with the use of more invasive methods applies for increased psychosocial services in fertility clinics.
Study funding/competing interest(s): Funding by University(ies) – No competing interests.
Trial registration number: NA.
Keywords: men, involuntary childlessness, fertility treatment, cause, quality of life

P-542 Freezing eggs for transsexual men: a qualitative study of transsexual individuals' experiences of fertility preservation and reproductive healthcare in Sweden

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Study question: How do transsexual men experience the procedures required for fertility preservation (FP) by egg freezing and their reproductive care within a pilot program for FP for transsexual individuals initiated at an university-hospital based assisted reproduction center?
Summary answer: Although the processes involved in FP by egg freezing gave rise to negative emotions and symptoms that challenged the patients' perceived masculinity, the men experienced adequate support from the health care team and they appreciated the team's interest on their feelings and needs during the entire course of treatment.
What is known already: In Sweden, one of the most progressive European countries with regards to lesbian, gay, bisexual and transgender rights (LGBT), the previous requirement of sterilization for legalization of gender reassignment was removed in 2013; hence, transsexuals in Sweden may now undergo FP before sex-reassignment surgery. Transsexuals have previously reported negative experiences of general health care but no earlier research was found in the literature on transsexual men's experiences of FP by egg freezing or reproductive health care.
Study design, size, duration: Prospective study of adult transsexual men undergoing hormonal stimulation and egg retrieval for FP. The study was initiated in May 2014 and recruitment is still ongoing. Patients participated in individual qualitative interviews shortly after their FP treatments. The interviews lasted 68- 95 minutes and were digitally recorded and transcribed verbatim.
Participants/materials, setting, methods: By January 2015, seven transsexual men (age 19-33) that underwent FP by egg freezing have been interviewed. Data were analyzed using qualitative content analysis (Krippendorff, 2012). Patient's psychological perceptions and experiences during the processes involved when undergoing FP were assessed and themes were identified.
Main results and the role of chance: The analysis of this pilot investigation of FP in individuals with transsexualism resulted in three themes identified: [1] Bodily changes, [2] Physical intrusion and [3] Health care encounters. In theme [1] hormonal changes in connection with the hormonal stimulation treatment required were described as causing troublesome effects such as swollen breasts and mood swings, which challenged the patients' perceived masculinity. In theme [2] transvaginal ultrasound examinations gave rise to discomfort, as the men were reminded of their female body. In theme [3] the informants described generally positive experiences of the health care. They acknowledged that even if the FP program for transsexuals was novel and the team's experience of transsexuals limited, efforts to improve patient's compliance and alleviate distress during the process were evident.
Limitations, reason for caution: Only seven individuals have so far participated in the study and some caution is advised when interpreting the results. All men were well-educated and most of them dedicated to transsexuals' rights by working within patient societies. Three men had stopped their previous testosterone treatment to perform FP treatment.
Wider implications of the findings: Our preliminary results suggest that transsexual men's experience of FP by egg freezing is essentially contradictory to their perceived gender identity. This knowledge is important in order to provide adequate professional support during FP to transsexual patients. Although the small sample size, we judge our results as transferable to other transsexual men undergoing FP by egg freezing.
Study funding/competing interest(s): Funding by University(ies) – Supported by grants from the local Research, Education and Development council, Department of Obstetrics and Gynecology, Södersjukhuset, Stockholm, and clinical research and ALF grants from Stockholm County Council (KR-W).
Trial registration number: NA.
Keywords: transsexualism, fertility preservation, individuals' experiences, content analysis, qualitative interview study

P-543 Information on websites of fertility clinics in Europe; a matter of decency

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Study question: Are infertile couples well informed on success rates and treatment options on websites of fertility clinics in Europe?
Summary answer: The quality of the websites of fertility clinics in Europe is poor and the recommended success rate 'live births per started treatment cycle' is rarely reported Information on specific treatments such as expectant management, preimplantation genetic screening (PGS) and oocyte vitrification is scarce.
What is known already: Infertile couples have a right to know the proper indications for fertility treatments, and the effectiveness and safety of ART. Accurate reporting of data assists in the building of realistic expectations and promotes patient confidence in the integrity of the ART program. It is unknown whether information provided on expectant management (effective, but not interesting from a commercial perspective), PGS (harmful treatment) and oocyte vitrification (treatment with uncertain efficacy and safety), is accurate.
Study design, size, duration: We performed a sample search from 31 countries by randomly selecting fertility clinics who report to the European IVF Monitoring. We selected the Health on the Net (HON) code to evaluate the quality of the websites. To assess how success rates are presented, adherence to ASRM/SART advertising guideline was measured.
Participants/materials, setting, methods: We assessed the 8 principles of the HON-code, ranging from transparency to financial disclosure. Adherence to the ASRM guideline – recommending reporting success rates as live birth per started cycle – was analysed. Finally we analysed information provided on three strategies: expectant management, PGS and oocyte vitrification to defer childbearing.
Main results and the role of chance: We studied 56 websites. On average, websites adhered to 2,5 HON principles (range 2 - 5,5). None of the websites adhered to all 8 principles of the HON code. Thirty-eight websites reported success rates (68%). Success rates were reported as live birth rate per started treatment cycle on 15 websites (27%). The option of expectant management for unexplained subfertility was described on 8 websites (14%). PGS was not offered. Four websites offered pre implantation genetic diagnosis (PGD) for the indication repeated implantation failure and advanced maternal age, which we interpret as PGS. Oocyte vitrification to defer childbearing was offered on 15 websites (27%).
Limitations, reason for caution: Selection bias might be present since we conducted a sample search. We translated websites that were not available in English with the google toolbar, which may lead to less accurate interpretation.
Wider implications of the findings: The way websites present their information lacks transparency and clarity. In view of the important role of the internet for patients to obtain information, we believe that ESHRE should demand from their members to provide consistent information on their websites. Only then couples are able to make an informed choice and select the clinic which is best suited to them.
Study funding/competing interest(s): Funding by University(ies) – Academic Medical Research Centre.
Trial registration number: NA.
Keywords: websites of fertility clinics, website quality, accuracy of information

P-544 Professionals' knowledge, attitude and referral behaviour regarding preimplantation genetic diagnosis for hereditary breast and ovarian cancer

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Study question: What are the levels of awareness and knowledge, attitudes and referral behaviour of professionals regarding preimplantation genetic diagnosis (PGD) for hereditary breast and ovarian cancer (HBOC) in the Netherlands?
Summary answer: Less than half of the professionals who are in the position to refer patients with HBOC for PGD are aware of the possibility of PGD for HBOC in the Netherlands, whereas 85% thinks offering PGD for HBOC is acceptable.
What is known already: The only quantitative study that investigated professionals' (clinical geneticists not included) knowledge and attitudes towards PGD for hereditary cancer indicated that their knowledge was limited and acceptability of offering PGD was high. Other, mostly qualitative studies showed that physicians are generally in favour of offering PGD for severe life-threatening diseases, though they are less in favour of offering PGD for adult onset diseases and selection of non-medial traits such as sex.
Study design, size, duration: A cross sectional survey was sent to clinical geneticists, genetic counsellors, gynaecologists, oncologists, radiotherapists and fertility specialists in the Netherlands. In collaboration with professional associations for above stated specializations, eligible professionals received an invitational email to participate in the study. Data was collected between August 2013 and May 2014.
Participants/materials, setting, methods: The survey which assessed demographics, awareness, knowledge, attitude and referral behaviour regarding PGD for HBOC was completed by 170 involved health care providers.
Main results and the role of chance: More than half (51%) of the physicians was not aware of the possibility of PGD for HBOC. Of the physicians who were aware of PGD, 41% had a high level of knowledge about PGD. A majority of 85% considered PGD for HBOC acceptable. 28% agreed that the acceptability of offering PGD depends on the patients personal/ family medical history, whereas 32% indicated that it should be the patients autonomy to assess PGD acceptability. Physicians who worked at a university medical centre (UMC) were more likely to be aware of PGD for HBOC and had more knowledge of PGD than physicians who worked at a peripheral hospital. Male physicians and those who did not consider themselves religious were more likely to find PGD for HBOC acceptable.
Limitations, reason for caution: An equal distribution of specialists in this study was not achieved. Gynaecologists are overrepresented and both genetic counsellors and oncologists are minority groups. Except for the oncologists however, these numbers do comply with the general representation of these specialisms in the Netherlands.
Wider implications of the findings: This study suggests in accordance with previous research that awareness and knowledge of PGD are not optimal among involved health care providers. Moreover, a gap remains between intention to refer patients for PGD and actual referral behaviour. Since the health care providers are gatekeepers and sources of information regarding the use of medical technologies such as PGD, their knowledge should be optimized and further research is required to identify physicians' motives regarding their referral behaviour.
Study funding/competing interest(s): Funding by national/international organization(s) – Dutch Breast Cancer Foundation 'Stichting Pink Ribbon.'
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis (PGD), hereditary breast and ovarian cancer (HBOC), BRCA

P-545 The tell me tool: a tool to help professionals to provide and evaluate personalised fertility care

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Study question: Is it feasible to develop and use a tool that facilitates both personalised care and personalised evaluation of care in couples that undergo testicular biopsy (PESA/TESE) and intra-cytoplasmic sperm injection (ICSI)?
Summary answer: It is feasible to develop and use a tool that facilitates personalised care delivery and evaluation in couples that undergo PESA/TESE-ICSI. We developed the Tell Me tool, receiving input of patients and professionals in every step.
What is known already: Delivering personalised care is becoming more and more important, but is difficult to achieve as professionals often misdiagnose the patients' care preferences. Therefore, the individual patients' goals and values should be part of the conversation. In addition, care should be evaluated in a personalised way, combining the individual patients' prior preferences with their experiences after care consumption. We aim to develop a tool that facilitates these two goals.
Study design, size, duration: A user-centred design, involving patients and professionals for optimal effectiveness. We performed a literature search, semi-structured interviews, a consensus meeting and 8 improvement rounds to define the content (important aspects of care) and use of the tool. After a pilot test the tool is implemented in daily practice.
Participants/materials, setting, methods: In total 42 couples undergoing PESA/TESE-ICSI from various steps of the treatment cycle were involved in the development of the tool. Furthermore, physicians, embryologists, nurses, and administrative and laboratory staff were involved.
Main results and the role of chance: We achieved consensus on the list of care aspects important to couples undergoing PESA/TESE-ICSI, which includes for example achieving pregnancy, maintaining a healthy relationship, medical expertise of the staff, and information provision. Before the first consult, the tool asks patients to distribute 10 points over the care aspects that they feel require extra attention. After one treatment cycle, the tool asks patients to rate their experiences on every care aspect. The tool is short, easy to complete, and it identifies and evaluates patients' unique values and wishes, which are very usable for professionals to personalise care. There appears to be a large variation in the patients' answers, indicating the potential value of our tool.
Limitations, reason for caution: We had to balance the tool: making it easier to complete, decreased the usefulness for the professional and vice versa. In addition, these preliminary results are based on the experiences of only 42 couples. More data are available this June, when the tool is implemented for 6 months.
Wider implications of the findings: This tool for personalised delivery and evaluation of care is developed for PESA/TESE-ICSI care, but can easily be adjusted to other areas of fertility care by adapting the list of care aspects. For other fields of care, the Tell Me tool has the greatest potential in fields with intensive treatments, chronic illnesses, or difficult treatment decisions.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This study is supported by Merck, Sharp and Dohme, The Netherlands.
Trial registration number: NA.
Keywords: patient-centeredness, personalised care, user-centred design, patient preferences, TESE

P-546 Relationship between maternal attachment, and oxytocin and cortisol levels during the third trimester

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Study question: Is there a relationship between oxytosin and cortisol levels, and maternal attachment and maternal attachment during the third trimester?
Summary answer: We found that cortisol but not oxytosin level was predictor of maternal attachment.
What is known already: Previous studies showed that during the prenatal period oxytosin was negatively and cortisol was positively related with depression levels. Oxytosin level was found to be associated with attachment between mother and baby during the postnatal period.
Study design, size, duration: This is a cross - sectional study with a sample of 145 pregnant in their 28-36. weeks. The current study was conducted in the Department of Obstetrics and Gynecology in an university hospital between 2012 December and 2013 September.
Participants/materials, setting, methods: We studied the plasma oxytocin and cortisol levels of 145 pregnant. Inclusion criteria were 1- Being between 18 - 35 years old, 2- Having sufficient reading abilities, 3- Having no history of psychiatric disorder, 4- Having a singleton fetus. All participants filled out Prenatal Maternal Attachment Inventory, Edinburg Postpartum Depression Scale, State and Trait Anxiety Inventory. Relationships between variables was assessed by Pearson's correlation analysis. Predictors of the attachment levels and

depression scores were examined by linear regression analysis. Significance at $p < 0.05$ were considered.
Main results and the role of chance: The group was divided into two groups, according to EPDS, Group I (EPDS ≥ 13) was considered as probable depression and Group II (EPDS < 13) was as without depression. There were 28 cases in Group I and 117 in Group II. Oxytosin level in women with depression (Group I) was significantly lower than women without depression (Group I) (108.9 vs 237.4; $p = 0.08$), however there was no difference in cortisol level between two groups (22.3 vs 21.6). Correlation analysis showed that depression score was correlated with maternal - fetal attachment level (r = -.197) and oxytosin level (r = -.164). Attachment score was correlated with cortisol level (r = -.158). State anxiety was correlated with depression (r = .537), attachment (r = -.160) and oxytosin level (r = -.196). Regression analysis revealed that only cortisol level was predictor of mother-child attachment relationship.
Limitations, reason for caution: Although we had a large sample the main limitation was we relied on self-reports instead of structured interviews. We did not consider the psychological features of fathers.
Wider implications of the findings: This study showed that 12% of the women during their trimester had probable depression. Depression had a negative relation on maternal-fetal attachment. Cortisol levels were the predictor of attachment between mother and the fetus. Identifying depressive symptoms during pregnancy and referring them for psychiatric consultation is essential for an optimum attachment.
Study funding/competing interest(s): Funding by University(ies) – This study was founded by Necmenttin Erbakan University Scientific Research Foundation
Trial registration number: 131518006.
Keywords: maternal attachment, oxytocin, cortisol levels

P-547 'He's just like his mom' – understandings of genetics and inheritance by parents of anonymous donor-conceived children

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Study question: How do parents of anonymous donor-conceived offspring perceive the role of genetics and inheritance in the development of their child, given that only one of them is genetically related to the child?
Summary answer: Parents of anonymous donor-conceived children construe understandings of genetics and inheritance that allow them to deal with the unknown genetic influence of the donor; the sensitivities of the non-genetic parent; difficulties during the upbringing; and deviation from the traditional family norm.
What is known already: Many studies have shown that lay people have individual understandings of the concepts of genetics/inheritance and that these do not necessarily conform with scientific knowledge. Various authors suggest that people acquire situated interpretations of genetics through a dynamic, context-dependent process building on their own experiences and relationships. Little is known about how parents of anonymous donor-conceived children interpret genetics within and through their specific context: one in which only one parent is genetically related.
Study design, size, duration: Semi-structured interviews were conducted with three subgroups of parents who conceived through anonymous donation between 2002-2005: heterosexual (9 couples, 1 individual) and lesbian (10 couples) recipients of sperm donation; heterosexual recipients of oocyte donation (7 couples, 2 individuals). Participants were recruited via Ghent University Hospital's Department of Reproductive Medicine.
Participants/materials, setting, methods: Step-by-step inductive thematic analysis was performed with a focus on the couples' perceptions and understandings of genetics and inheritance. This method included a phased process from memo writing to the construction of themes. The validity and trustworthiness of the analysis was guaranteed through auditing by the co-authors.
Main results and the role of chance: Participants described diverse and variable acknowledgement of a determinative role of genetics in the development of the child, with views ranging from the idea that genes express themselves only at the level of the gamete cell, which is small and negligible, to the assumption that the child is the sum of complex genetic traits of one or both genetic forebears. Participants also commonly relativized the genetic basis of the child's development by focusing on their parental input. One common strategy was to oppose the role of genetics to the timing and duration of their gestational and pedagogical 'labor' or 'imprinting'. A few participants waived the role of genetics aside and emphasized the inherent uniqueness and particularity of the child.
Limitations, reason for caution: This qualitative study aimed at a better understanding of the participants' experiences and interpretations and does not intend to produce generalizable results. Only recipients of anonymous donor gametes were included, as with known donation the situated understanding of genetics may be fundamentally different.
Wider implications of the findings: The findings have implications for counseling before donor treatment. Genetic misconceptions are commonly considered to be problematic as they risk undermining effective and informed personal decision-making in a reproductive context. However, these results lend support to the idea that, when discussing one's understanding of genetic and hereditary processes, one should be aware that it is not only the product of information transfer, but also a result of one's perceptions regarding family relations and narratives.
Study funding/competing interest(s): Funding by University(ies) – Special Research Fund of Ghent University.
Trial registration number: NA.
Keywords: qualitative research, gamete donor recipients, genetics, inheritance

P-548 Preparing for shared treatment decision-making: what information do subfertile couples need?

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Study question: Which information do subfertile couples need while being involved in shared treatment decision-making (SDM)?
Summary answer: Couples want information on the options available to them and on the medical reasons justifying the treatments offered. Besides information on effectiveness, safety, burden and costs, information on the following three treatment characteristics is required: 'similarity to natural conception', 'use of advanced technology' and 'patient-empowerment' to deliberate between options.
What is known already: Currently, gynecologists often propose a single treatment and obtain patients' consent after informing them on its effectiveness, safety, cost and burden. Subfertile couples from across Europe have an unmet need for SDM and discussing alternatives for treatment (e.g. adoption). SDM includes team talk (explaining the importance of sharing the decision), option talk (providing evidence based facts on all options) and decision talk (deciding together). Optimal option talk requires identifying the information needed by patients.
Study design, size, duration: In-depth individual or focus group interviews were conducted with fifty-seven subfertile couples between January 2013 and December 2014. Interviews were analyzed with inductive content analysis.
Participants/materials, setting, methods: A convenience sample of subfertile women ($n = 32$) and men ($n = 25$) treated in a Dutch or Belgian fertility clinic participated in individual ($n = 9$) or focus group ($n = 5$) interviews. The interviews were transcribed verbatim and subjected to inductive content analysis with constant comparison by two researchers independently who reached consensus through discussion.
Main results and the role of chance: Subfertile couples want information on all the options available to them. They want to know the medical reasons for offering or not offering each potential treatment. Besides information on effectiveness, safety, burden and costs, information on the following three treatment characteristics is required: 'similarity to natural conception', 'use of advanced technology' and 'patient-empowerment' to deliberate between treatment

options. In general couples want a treatment which is as natural as possible (e.g. with a limited amount of hormonal medication), while at the same time they want treatments to have diagnostic value (e.g. is there fertilization?) and to use the newest and most advanced technology. Treatments empower patients if patients have chosen them and gained expertise (e.g. on subcutaneous injections) and autonomy through them.

Limitations, reason for caution: We are currently continuing data-collection until data saturation is reached. So far, our study mostly included participants who had experience with advanced treatments (e.g. ICSI); efforts are made to develop a more diverse patient sample by additional inclusion of patients with unexplained subfertility.

Wider implications of the findings: A decision aid facilitating SDM should be developed for clinical practice in collaboration with gynecologists and patients. Beside information on efficacy, safety, burden and costs, the decision aid should also provide information on the three newly identified treatment characteristics that were valued by patients. To develop this decision aid, evidence based outcomes for each characteristic and option should be identified by literature review, prognostic models, and patient experience surveys.

Study funding/competing interest(s): Funding by University(ies) – Catholic University Leuven, Belgium

Trial registration number: NA.

Keywords: medically assisted reproduction, shared decision making, patient-empowerment, patient information

P-549 Childless by circumstance: exploring thoughts and feelings using the rational-emotive cognitive behavioural therapy (RECBT) model

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Study question: Do single women experiencing 'childlessness' by circumstance (or 'social infertility') (i.e., for reasons other than physical) experience higher levels of RECBT classified unhealthy negative emotions, than women in *relationships* who are experiencing childlessness by circumstance?

Summary answer: A broad range of unhealthy negative emotions were identified amongst the sample of women who were childless by circumstance. In addition to depression and envy reported in the women in couples, single women who would not consider having a baby on their own, also experienced suicidal ideation, anger and shame.

What is known already: Within the UK, our demographics are changing, with more women having never been married now than in the 1970s and 1 in 5 women remaining childless. Research indicates that women in couples undergoing IVF treatment experience emotions such as anxiety, depression and guilt, yet little is known about the emotions and beliefs of women facing social infertility. This is one of the first studies to look at this topic and the first using RECBT.

Study design, size, duration: Face to face interviews were conducted with 17 women aged 35 to 50 allocated to three groups: single women who would, and would not, consider having a baby on their own, and women in couples. Recruitment was via various routes. Data collection was between June 2014 and August 2014.

Participants/materials, setting, methods: The researcher met with 18 women and conducted 17 semi-structured, face to face interviews exploring thoughts and feelings. Mood/belief and demographic questionnaires were also completed. Interviews were fully transcribed and a thematic analysis conducted with data coded according to RECBT emotions. A second experienced RECBT practitioner checked each identified emotion.

Main results and the role of chance: Single, childless women who would not consider having a child on their own experienced the broadest range of RECBT unhealthy negative emotions of depression, anger, envy and shame. Women in couples experienced depression and envy. Single women who would consider having a child on their own spoke less of unhealthy negative emotions but rated stronger desire for a child. Of the 18 women the researcher met with, 33% had a risk score. Of the single women who wouldn't have a child on their own, 75% had experienced thoughts of suicidal ideation at some point. Further work is required to see whether this is reflected amongst a broader sample. Participants articulated the need for better fertility awareness and greater acceptance for professionals to have children younger.

Limitations, reason for caution: As women were primarily recruited via Gateway Women, Donor Conception Network and BICA, it is not possible to know how the thoughts and feelings of these women represent the broader group of women facing social infertility / childlessness by circumstance. Women in couples and lesbian women were underrepresented.

Wider implications of the findings: It is time to acknowledge our changing demographics and the emotional difficulties those who are childless by circumstance may experience. Professional and patient group support may help them to navigate this and redefine their life goals. If these women are experiencing unhealthy negative emotions triggered by specific beliefs, it is possible that RECBT may be a resource that can help. There was a strong message for further education on fertility awareness supporting previous research.

Study funding/competing interest(s): Funding by University(ies) – Goldsmiths, University of London, London, UK, as part of Masters in Rational-Emotive Cognitive Behavioural Therapy. Approval by Ethics Committees from both Goldsmiths University and the Donor Conception Network has been obtained.

Trial registration number: NA.

Keywords: childlessness, social infertility, psychology

P-550 Two mothers and a donor: exploration of children's family concepts in lesbian households

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Study question: How do children from lesbian families define the concepts of the three actors involved in the building of their families: the biological mother, the social mother and the donor?

Summary answer: Children from lesbian families use hetero-normative concepts to define their family-specific concepts of social mother and donor.

What is known already: Although children from lesbian families appear to make a distinction between a residential father and a donor, defining these two concepts seems to be a challenge. They need to appeal to more familiar concepts such as the hetero-normative concept of 'mother' to give a definition of the unfamiliar concepts they are confronted with.

Study design, size, duration: The study is based on qualitative in-depth interviews with 6 children from lesbian families, all of which have been conceived using anonymous sperm donation. Semi-structured interviews were conducted.

Participants/materials, setting, methods: The participants were recruited via their parents, who took part themselves in our study. After each interview, the parents were asked whether their child (aged 7 to 10 years) would also want to participate in the study and whether the parents themselves would agree to this interview. The authors used inductive thematic analysis as the qualitative methodology for this study.

Main results and the role of chance: Two findings stand out. First, in defining the concepts of biological and social mother, both mothers were described as equal parents. No difference was attached by the children to the mothers' position as a parent. Second, the concepts 'social mother' and 'donor' were defined by looking at the hetero-normative concepts of 'mummy' and 'daddy'. To define the social mother, both a 'mummy' and a 'daddy' were used as a reference. To define the donor concept, often references were made to a daddy. This comparison with a 'daddy' turned out to be complex due to the conflict between the role as a progenitor and the lack of a social relationship. The lack of language surrounding this concept turned out to be difficult.

Limitations, reason for caution: This is a qualitative study; the authors present a thematic analysis of the views and experiences of 6 children. The data gathered are not representative for all children from lesbian couples in Belgium.

Wider implications of the findings: This study illustrates the complexity and ambiguity of children's experiences and perceptions when dealing with issues related to genetic and social parenthood.

Study funding/competing interest(s): Funding by University(ies) – Special Research Fund of Ghent University.

Trial registration number: NA.

Keywords: child interviews, gamete donation, lesbian families, family concepts, qualitative

P-551 Emotional state and coping potential of Hungarian infertile couples undergoing *in vitro* fertilization

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Study question: What are the Hungarian infertile couples' emotional responses to *in vitro* fertilization (IVF), and how does their emotional state change in the course of the treatment? What coping potential do they have?
Summary answer: Hungarian infertile women experience significantly lower positive affectivity than men. The emotional state of couples does not change significantly in the course of IVF. Getting into a stress situation, women apply seeking social support, seeking emotional balance and withdrawal coping more frequently than men.
What is known already: Previous studies have shown that infertility and infertility treatments are associated with increased levels of anxiety, depression and stress. Most of these studies have examined psychological reactions before and after IVF, and compared them on the basis of IVF outcome; few investigations have focused on these factors during the treatment, especially in Hungary. Besides, most studies have focused exclusively on women, but only a few studies have assessed both partners' psychological reactions.
Study design, size, duration: This preliminary study used a longitudinal design in which fifty-two infertile couples were followed during IVF at the following stages: at the beginning of the treatment (T1), before embryo transfer (T2), and before pregnancy test (T3). Couples were recruited from a Hungarian fertility clinic between September 2013 and September 2014.
Participants/materials, setting, methods: Fifty-two couples (104 participants) completed the following questionnaires: Positive and Negative Affectivity Schedule, the short form of Beck Depression Inventory, the State and Trait Anxiety Inventory, the Hungarian shortened version of the Ways of Coping Inventory, the Psychological Immune Competence Inventory, and a demographic and infertility-specific questionnaire.
Main results and the role of chance: According to the results, we have found no significant differences concerning emotional state between occasions neither among women, nor among men. Besides, we have found no relationship between emotional state and number of previous IVF cycles. Women score significantly lower than men on positive affectivity at T1 and T2 ($p < 0.05$). Compared with a representative sample of the Hungarian adult population, more favourable results have been observed among IVF couples as regards depression, negative and positive affectivity. Getting into a stress situation, women apply seeking social support, seeking emotional balance and withdrawal coping more frequently than men ($p < 0.01$). Men have achieved significantly higher 'Problem-solving capacity,' 'Self-efficacy,' 'Social creating capacity' and 'Emotional control' than their partners ($p < 0.05$). Women have achieved significantly higher 'Social mobilizing capacity' than men ($p < 0.001$).
Limitations, reason for caution: These are preliminary results of an ongoing study, and further longitudinal studies with larger sample sizes are needed for better understanding of the Hungarian infertile couples' emotional reactions to infertility treatment, coping mechanisms and personal resilience resources.
Wider implications of the findings: Our research confirms that consideration and management of psychological factors in treating infertility have an important role to play. Psychological counselling has not been incorporated into the infertile couples' health care yet. Furthermore, most of the studies on this subject are foreign; therefore our study is essential in Hungary. Further studies need to be performed to investigate the effects of psychological factors on IVF outcome and the cultural differences.
Study funding/competing interest(s): Funding by University(ies) – Funded partially by the University of Debrecen.
Trial registration number: Nil.
Keywords: infertility treatment, emotional state, coping strategies, personal resilience resources

P-552 Helping beyond skills and ethics: cultivating compassion satisfaction among healthcare professionals working on ART in Hong Kong

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Study question: How effective is the professional training course on infertility counseling for healthcare professionals working in ART settings in cultivating their professional quality of life in terms of compassion satisfaction and compassion fatigue?
Summary answer: The course could effectively enhance the professional quality of life in terms of Compassion Satisfaction (CS). For healthcare professionals working in ART where patients face much uncertainty and uncontrollability, it is important to cultivate CS in order to guarantee both parties' interest, quality of service and life satisfaction.
What is known already: Professional quality of life incorporates two aspects, the positive (Compassion Satisfaction) and the negative (Compassion Fatigue). While abundance of research focused on the job burnout and compassion fatigue among healthcare professional, less attention has been put about compassion satisfaction. CS refers to the positive feeling of job satisfaction derived from doing helping work effectively. People with higher CS will feel contentment, experience positive thoughts and enjoy the work they do.
Study design, size, duration: This is a quasi-experimental design. Two to three healthcare professionals from 11 licensed ART clinics from public or private hospitals were nominated to attend. The course consisted of nine 3-h weekly sessions spanning two months, with self-reflective exercises like mindfulness training, compassionate meditation, and reflections on meaning of life.
Participants/materials, setting, methods: 25 Healthcare professionals (doctors, nurses, and embryologists) working in ART settings were invited to complete a self-administered questionnaire before and after the course. The questionnaire was comprised of Professional Quality of Life (ProQol) measuring Compassion Satisfaction and Compassion Fatigue (Burnout and Secondary Trauma) and measures related to psychological wellbeing.
Main results and the role of chance: It was found that the participants showed significant improvement in CS after attending the course (Pre-course: 33.94 + 4.4; Post-course: 36.35 + 3.82, $t = -3.29$, $p < 0.005$). However, no significant difference was found in Compassionate Fatigue (Secondary Trauma and Burnout) under ProQol. This showed that regardless of no improvement in Compassionate Fatigue, it is essential to uphold their CS. Healthcare professionals who were younger in age and with lower education level showed greater improvement in CS ($p < 0.05$). Moreover, those who have religious beliefs showed significant improvement in CS as well ($p < 0.05$). Another interesting area is, those who work longer in general medical settings, but shorter in ART field showed significant improvement as well.
Limitations, reason for caution: This is a professional training course with limited number of participants. No random assignment could be performed. In order to evaluate the course in greater details, qualitative analysis such as focus group and interviews should be conducted to obtain better understanding about their compassion satisfaction in working in ART.
Wider implications of the findings: There are a lot of studies focusing on the alleviation of compassion fatigue, while very few on cultivating healthcare professionals' compassion satisfaction. From a positive perspective in enhancing a positive feeling and satisfaction among helping professionals, it is believed that both themselves and service recipients can benefit from better service quality and prevent burnout. This component should thus be taken into consideration in the professional training programme in infertility counseling.
Study funding/competing interest(s): Funding by University(ies).
Trial registration number: Nil.
Keywords: healthcare professionals, ART, compassion satisfaction, infertility counseling, training courses

P-553 Do cultural beliefs play a role in fertility-related quality of life? A study of Chinese women after unsuccessful first IVF cycle

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Study question: What is the quality of life (QoL) of Chinese women after their unsuccessful first IVF cycle, and do cultural beliefs play a role?
Summary answer: Comparing FertiQoL scores with an existing Western sample of people with fertility issues, Chinese women in Hong Kong who had unsuccessful first IVF cycle reported better fertility-related QoL but similar treatment-related QoL. Traditional cultural beliefs about childbearing were associated with worse QoL scores.
What is known already: Past studies have found IVF and its negative outcome could be stressful and affect QoL among infertile women. Boivin and team (2011) developed a measure of fertility-related quality of life (FertiQoL) and obtained mean scores in a Western sample of people experiencing fertility problems. Little is known about the QoL of Chinese infertile women, especially those after unsuccessful treatment.
Study design, size, duration: A cross-sectional survey consisting of FertiQoL, questions about Chinese cultural beliefs of childbearing, and demographic characteristics was conducted from February to November 2014 in a university-affiliated hospital. Participants were recruited through individual invitation at the assisted reproduction clinic.
Participants/materials, setting, methods: A total of 465 patients were approached after their first IVF cycle was found unsuccessful. Of them, 198 (response rate: 42.58%) have completed the self-administered questionnaires.
Main results and the role of chance: The mean age of participants was 37.0 (SD = 3.5), duration of marriage was 7.4 years (3.7), and duration of infertility was 4.1 years. The majority of them received tertiary education and had full-time job. Mean scores of Total FertiQoL, Core FertiQoL, and Treatment FertiQoL were 63.4 (12.5), 64.1 (14.5), and 61.9 (13.2). Both Total and Core scores were higher than that from a Western sample reported by Boivin et al. (2011) ($t = 8.10$ and 8.36, $p < 0.01$) while no difference was found in Treatment score ($t = 1.41$, *n*.s.). Total and Core FertiQoL were found to be negatively associated with identification with Chinese cultural beliefs about childbearing ($r = -0.446$, $p < 0.05$).
Limitations, reason for caution: Self-selection bias was inevitable in questionnaire survey, and the cross-sectional nature of the study did not permit causal inferences. Only infertile women who failed their IVF cycles were recruited, so the fertility quality of life for those in other stages of IVF is yet to be investigated.
Wider implications of the findings: This study sheds light on the quality of life of Chinese infertile women after unsuccessful IVF treatment. Identification with Chinese beliefs of childbearing, which emphasizes the patrilineal culture, was associated with worse QoL. The findings underline the importance of cultural sensitivity in addressing fertility-related issues in order to better facilitate psychosocial support at the clinic.
Study funding/competing interest(s): Funding by University(ies) – The University of Hong Kong.
Trial registration number: Nil.
Keywords: IVF, unsuccessful cycle, FertiQoL, Chinese women, cultural beliefs

P-554 Psychosocial counselling in treatment with donor sperm: exploring expectations of non-anonymous sperm donors

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Study question: What did men expect when they received psychosocial counselling on becoming a non-anonymous sperm donor and how did they experience being a sperm donor?
Summary answer: Sperm donors valued implication counselling before registration and that the counsellor addressed important issues like disclosure and future contact with donor-offspring. They expected that psychosocial counselling would be available to them when donor-offspring would actually seek contact in the future.
What is known already: Most studies on sperm donors focus on medical screening and motivations of sperm donors. Women/couples being treated with donor sperm value psychosocial counseling before treatment commences. There is limited knowledge on the expectations of sperm donors on psychosocial counselling, as well as their experience of being a sperm donor.
Study design, size, duration: We performed a qualitative study from March 2014 until June 2014 in 25 Dutch non-anonymous sperm donors, who had been a donor at the Centre for Reproductive Medicine of the Academic Medical Centre in Amsterdam any time between 1989 and 2014.
Participants/materials, setting, methods: We held semi-structured in-depth interviews based on literature and counselling experience with non-anonymous sperm donors. The interviews were fully transcribed and analysed using the constant comparative method of grounded theory.
Main results and the role of chance: The average age of the 25 sperm donors was 45 years. Sperm donors found it important to talk about issues as disclosure to family and friends, future contact with donor-offspring and rules and regulations and these issues had been addressed during pre-registration counselling. In the years following the donation, most donors just wanted to know how many offspring had been born and had no need for further counseling. Sperm donors frequently mentioned that they were concerned whether the donor-offspring were doing well. In this light they valued the availability of psychosocial counselling at the time when donor-offspring would seek contact in the future.
Limitations, reason for caution: A limitation of the results is the generalizability since only non-anonymous donors at a single center were studied. Potentially, variation in how donors are counselled upon intake affects how donors value psychosocial counselling.
Wider implications of the findings: This study reports the issues donors want to be addressed during pre-registration counselling and at time of actual contact seeking of donor-offspring. These findings can be used to achieve a higher quality of care for sperm donors and may be the starting point for developing guidelines on psychosocial counselling of sperm donors.
Study funding/competing interest(s): Funding by University(ies) – none.
Trial registration number: NA.
Keywords: sperm donor, non-anonymity, counselling, donor-offspring

P-555 The experience of women undergoing fertility preservation while having breast cancer: a qualitative study

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Study question: How do women experience fertility preservation while being newly diagnosed with breast cancer?
Summary answer: The intertwining of cancer and looming infertility was challenging. Fertility preservation was experienced as a welcome way of taking control and focus on survival. It triggered questions about women's own identity as a fertility and cancer patient, and offered them hope for the future.
What is known already: To circumvent the risk of cancer therapy-induced infertility, women can cryopreserve oocytes or embryos. This consists of controlled ovarian stimulation, a treatment known to cause stress. This takes place in the already stressful period of being diagnosed with cancer. How women experience going through fertility preservation whilst being newly diagnosed with breast cancer has not yet been investigated.
Study design, size, duration: We used a descriptive phenomenological approach to study the lived experience of being diagnosed with breast cancer and cryopreserving oocytes or embryos. Twenty-one women were interviewed once between March and July 2014, which was sufficient to reach data-saturation.
Participants/materials, setting, methods: Women with breast cancer who cryopreserved oocytes or embryos between January 2013 and July 2014 in two university based fertility clinics were eligible for inclusion. In-depth, face-to-face interviews were guided by open questions and a topic list. The transcribed interviews were analysed by two researchers.
Main results and the role of chance: It was challenging for women to simultaneously cope with life-threatening cancer, the threat to their fertility and treatments for both. The threats especially collided in case of hormone-sensitive

breast cancer. The two new identities of a cancer patient and of a fertility patient were unpleasant and resulted in shame. Women explained that their cancer survival-mode helped them to experience fertility preservation as a welcome action plan. Whereas breast cancer confronted women with a doom scenario for their future, fertility preservation gave them a new and hopeful perspective. The intertwining cancer and fertility preservation treatment trajectories resulted in time-pressure and confusion, as women did not know whether they should attribute stress and emotions to their hormonal treatment or to emotions about having cancer.

Limitations, reason for caution: The threat of memory bias due to the retrospective nature of the interviews, seemed limited as experiences did not differ depending on how long ago women cryopreserved their oocytes or embryos.

Wider implications of the findings: This study provides an in-depth understanding in how women with breast cancer deal with fertility preservation. This insight in patient's experience can increase health care providers' empathy and ability to support their patients.

Study funding/competing interest(s): Funding by University(ies) – No funding, researchers were allied to Academic Medical Centre (University of Amsterdam) and Utrecht Medical Centre (University of Utrecht).

Trial registration number: NA.

Keywords: fertility preservation, breast cancer, experience, oocytes, embryos

P-556 Do couples' view on disclosure of donor conception change over time? A comparison of disclosure intentions at time of counselling with disclosure decisions during childhood

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Study question: Do disclosure intentions at the moment of donor counselling in the fertility centre (Time 1) predict disclosure decisions of mode of conception during childhood (Time 2)? Do parents' view on disclosure change?

Summary answer: In parents of anonymous donor-conceived children an accordance is shown between their disclosure intentions at the moment of pretreatment counselling and their disclosure decisions when their first child is between 7 and 10 years old. If parents change their mind, it is towards secrecy.

What is known already: Literature indicates that intentions to disclose are not interchangeable with the actually assessed disclosure, suggesting that intentions at the time of treatment may differ from what parents actually do later on (Klock, 1997, Daniels et al., 2007, Paul and Berger, 2007). To our best knowledge the presented data is the first comparison of disclosure intentions at the time of donor counselling with disclosure decisions during childhood.

Study design, size, duration: This study is embedded in a research project on genetic and social parenthood. The recruitment of couples treated with anonymous donor sperm or donor oocytes via the Ghent University Hospital was performed between June 2013 and February 2014. Forty-one couples having a child born between 2002 and 2005 were contacted.

Participants/materials, setting, methods: All couples were contacted by phone by the counsellor of the Department. At the time of this contact their disclosure decisions were queried. A comparison with the documented disclosure intentions at counselling was performed. In thirty-five of the forty-one couples all information was available at the two points in time.

Main results and the role of chance: Fourteen couples (40%) had the intention to disclose at the moment of counselling. At time 2, having children between 7 and 10 years old, three of the fourteen families (21%) had informed the child and five (36%) still had the intention to do so. In total a majority (57%) was in accordance with the initial intention. Five out of 14 parents (36%) changed their mind towards secrecy. When the initial intention of couples was not to disclose (40%), the majority of families stayed in accordance with their intention (79%). Seven families were unsure at moment of counselling (20%). At time 2, the majority of them had changed their mind to keeping the mode of conception a secret (57%). Two couples were still unsure and one couple had told the child.

Limitations, reason for caution: The sample size is small and this limits the generalizability of the obtained results.

Wider implications of the findings: The findings have implications for counselling before donor treatment, showing that the moment of counselling is the first moment of reflection on disclosure and an important moment in the further decision-making process of parents.

Study funding/competing interest(s): Funding by University(ies) – The project is funded by the Special Research Fund of Ghent University. Approval by the appropriate Ethics Committee has been obtained. There are no competing interests.

Trial registration number: NA.

Keywords: disclosure intentions, disclosure decisions, donor conception

QUALITY AND SAFETY OF ART THERAPIES

P-557 Adverse effects in pregnancy after treatment with preimplantation genetic diagnosis – a Danish national multicenter follow-up study

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Study question: Do women conceiving by preimplantation genetic diagnosis (PGD) and their children have greater risk of adverse pregnancy and birth outcomes compared to spontaneously conceived children and children born after intrauterine insemination (IUI) or *in vitro* fertilization (IVF) with or without intra-cytoplasmatic sperm injection (ICSI).

Summary answer: Compared to spontaneously conceived pregnancies PGD-pregnancies were at significantly increased risk of placenta praevia, caesarian section, preterm birth, shorter gestation, heavier placenta, and longer neonatal admission. PGD-pregnancies carried systematically higher risks than IVF-pregnancies, although this was significant only for caesarean section and longer neonatal admission.

What is known already: The ESHRE PGD consortium has continuously collected data from a total of 54 PGD centres since 1997. Results from the first 10 years of data collection showed a risk of malformations of 3.9%, and 10% of newborns presented with neonatal complications. Data are generally reassuring, but the validity is difficult to evaluate. Also, it is unknown if any increased risk is directly caused by the invasive procedure or by the underlying known genetic disorder.

Study design, size, duration: The ESHRE PGD consortium has continuously collected data from a total of 54 PGD centres since 1997. Results from the first 10 years of data collection showed a risk of malformations of 3.9%, and 10% of newborns presented with neonatal complications. Data are generally reassuring, but the validity is difficult to evaluate. Also, it is unknown if any increased risk is directly caused by the invasive procedure or by the underlying known genetic disorder.

Participants/materials, setting, methods: We obtained information on type of fertility treatment from the IVF-Registry. PGD cycles were validated against lists of all PGD treatments in relevant clinics. Information on spontaneous conceptions and adverse pregnancy and birth outcomes was obtained from the national Medical Birth Register and linked by the unique personal identification number.

Main results and the role of chance: The indications for PGD-treatments were monogenic diseases (32%), X-linked diseases (21%), and chromosomal translocations (47%). Women undergoing PGD were older, more often nulliparous, and non-smokers compared with women conceiving spontaneously but did not differ from women undergoing IVF/ICSI- or IUI-treatment. Compared to spontaneously conceived pregnancies PGD-pregnancies were at significantly increased risk of placenta praevia (adjusted OR (ORa) = 6.2 (95% CI: 1.5; 25.4)), caesarian section (ORa = 1.7 (1.1; 2.7)), preterm birth (ORa = 1.8 (1.0; 3.1)), shorter gestation (-3.4 days (-6.0; -0.7)), heavier placenta (37 g (7; 68)), and longer neonatal admission (24 days (17; 30)). PGD pregnancies were at significantly increased risk of caesarean section (ORa = 1.6 (1.0; 2.5)) and longer

neonatal admission (23 days (15; 32)) compared with IVF/ICSI-pregnancies, and risks were systematically higher after PGD-treatment compared to IVF/ICSI.
Limitations, reason for caution: The main limitation of the study is the small sample size. However, all PGD-treatments were validated against medical records. Danish national registries have generally been shown to contain valid information.
Wider implications of the findings: There are two immediate implications of our findings. First, the information can be used directly in the communication with couples undergoing PGD-treatment. Second, since we observed systematically higher risk estimates for obstetrical and neonatal outcomes after PGD-treatment compared with IVF/ICSI, where only some suffer chronic diseases, this would suggest that at least part of the observed risks associated with IVF/ICSI-treatment in general may be caused by chronic disease rather than the treatment itself.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Aarhus University Hospital.
Trial registration number: NA.
Keywords: PGD, safety, IVF, adverse effects

P-558 No excess risk of preterm birth for singletons following blastocyst transfers compared to cleavage stage embryo transfers in Australia and New Zealand

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Study question: Do singleton and twin pregnancies resulting from blastocyst transfers have higher odds of preterm birth than those resulting from cleavage stage embryo transfers?
Summary answer: During the study period, one in eight births were born preterm. Compared to cleavage stage embryo transfer, blastocyst transfer was not associated with excess risk of preterm birth for singletons, and was associated with 20% lower odds of preterm birth for twins.
What is known already: Some recent studies from Canada, Sweden and the United States using population ART registers found that singletons following blastocyst transfers had higher rate of preterm birth than singletons following cleavage stage embryo transfers. Given the marked differences in ART practices in Australia and New Zealand compared to Canada, Sweden and the United States, the excess risk of preterm birth following blastocyst transfers may not be present in Australia and New Zealand.
Study design, size, duration: A population-based study used Australian and New Zealand Assisted Reproduction Database (ANZARD). The study included 47438 live births following transfers of blastocyst or cleavage stage embryos during 2009–2012 in Australia and New Zealand.
Participants/materials, setting, methods: The rate of preterm birth (<37 weeks gestation) was compared by parental demographics and ART treatment factors. Univariate and multivariate logistic regressions stratified for singletons (43952) and twins (3418) were used to assess the likelihood of preterm birth. Odds ratio and adjusted odds ratio (AOR) were computed.
Main results and the role of chance: The overall rate of preterm birth was 13.1%, with 14.2% for cleavage stage embryo transfers, 12.6% for blastocyst transfers, 9.2% for singletons and 61.5% for twins. Of singletons, there was no significant difference in preterm birth between blastocyst and cleavage stage embryo transfers (AOR 1.00 95% CI 0.94–1.08). Of twins, blastocyst transfer was associated with 20% lower odds of preterm birth compared to cleavage stage embryo transfer (AOR 0.80 95% 0.70–0.93). The number of embryos transferred significantly modified the relationship between preterm birth and blastocyst transfer for twins, with AOR 0.74 (95% CI 0.63–0.88) for double blastocyst transfers compared to double cleavage stage embryo transfers and AOR 1.12 (95% CI 0.76–1.65) for single blastocyst transfers compared to single cleavage stage embryo transfers.
Limitations, reason for caution: The difference in cancellations before transfer between blastocyst and cleavage stage embryo cannot be assessed because the data was not available in ANZARD. Not all potential confounders were adjusted in the study due to availability.
Wider implications of the findings: In contrast with the findings from Canada, Sweden and the United States, blastocyst culture in Australia and New Zealand was not associated with excess risk of preterm birth for singletons, but was associated with a reduction in the risk of preterm birth for twins following double embryo transfers. Further studies assessing infant and child morbidity and mortality are needed to evaluate the risk related to blastocysts and cleavage stage embryos.
Study funding/competing interest(s): Funding by University(ies) – University of Technology Sydney. University of New South Wales.
Trial registration number: NA.
Keywords: preterm birth, blastocyst, cleavage stage embryo

P-559 Maternal age and the risk of major birth defects after *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)

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Study question: Does older maternal age explain the observed excess risk of major birth defects *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)?
Summary answer: Advanced maternal age did not explain the excess of birth defects in the ART population as it is robustly protective for birth defects compared to young women.
What is known already: It has been claimed that advanced maternal age in the ART population underlies the associations between ART and birth defects. However, this is untested and assumes that the relationship between maternal factors and birth defects observed in the fertile population generalizes to ART conceptions.
Study design, size, duration: This was a whole-of population retrospective cohort study consisting of a census of all births within the State of South Australia for the period January 1986–December 2002 including 2226 IVF and 1395 ICSI births. Over 99.5% of ART births were successfully linked to perinatal outcomes.
Participants/materials, setting, methods: All treatment cycles of assisted reproductive technology (ART) were linked to population registries for births, terminations of pregnancy, birth defects coded to IC9-BPA notified to a child's 5th birthday, and cerebral palsy. Odds ratios (OR) for birth defects were calculated separately for births from IVF and ICSI for exposures of maternal age, parity, pre-pregnancy BMI, smoking, pre-existing diseases and conditions in pregnancy. Analyses controlled for treatment factors, multiplicity and infertility etiology.
Main results and the role of chance: The unadjusted prevalence of any birth defect was 7.1% and 9.9% in the IVF and ICSI groups. For IVF the unadjusted prevalence of birth defects was 9.3% in women ≤29 years compared to 3.4% for women 40+ years. The significant adjusted odds ratios for birth defects after IVF included young age ≤29 years (aOR = 1.59, 95% Confidence Intervals (CI) 1.01–2.49), smoking (aOR = 1.54, 95% CI 1.00–2.36), and being overweight (aOR = 1.63, 95% CI 1.04–2.55) or obese (aOR = 2.16, 95% CI 1.28–3.63). For ICSI, the unadjusted prevalence of birth defects was 10.0% for women aged ≤29 compared to 6.1% for women aged 40+. The significant adjusted odds ratios for birth defects after ICSI were nulliparity (aOR = 2.20, 95% CI 1.32–3.67), pre-existing hypertension (aOR = 3.08, 95% CI 1.00–9.45), anemia (aOR = 1.72, 95% CI 1.06–2.81), and urinary tract infection (aOR = 2.29, 95% CI 1.23–4.26).
Limitations, reason for caution: The relatively sparse data by maternal factors will reduce the capacity to discern types of defect and may reduce statistical power. The study does not preclude the possibility of currently unknown patient factors contributing to the findings.
Wider implications of the findings: Advanced maternal age did not explain the excess of birth defects in the ART population as it is robustly protective for birth defects, which implicates treatment related factors in the etiology of birth defects, particularly in younger women. Variation in risk profiles between IVF and ICSI also require further investigation. The present study should be replicated in other datasets to ensure generalisability to other patient populations.
Study funding/competing interest(s): Funding by national/international organization(s). Supported by grants from the National Health and Medical Research Council (349475, 349548, 453556, and 465455) and the Australian Research Council (FT100101018).
Trial registration number: NA.
Keywords: maternal age, IVF, ICSI, birth defects

P-560 Early embryo cleavage and multiple pregnancies after ICSI

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Study question: Whether Number of early cleavage embryos can be a predictor for multiple pregnancies after ICSI.
Summary answer: Early cleavage could be an additional factor for selecting embryos with a higher potential of implantation and the number of EC embryos could be a useful parameter for the prediction of multiple pregnancies.
What is known already: One of the greatest problems in ART technology today relates to the selection of the optimal embryos for transfer, to achieve high pregnancy rates without increasing multiple pregnancy rates (Tomari et al., 2011). Much effort has been devoted to refining existing embryo scoring systems and finding simple, non-invasive parameters that could improve the embryo selection procedure (Senn et al., 2006; Brezinova et al., 2009). Early cleavage is one of the most promising new selection parameters.
Study design, size, duration: This was cohort prospective study conducted in the period from December 2010 to September 2012. A total of 193 infertile couples were included in this study.
Participants/materials, setting, methods: 193 infertile couples. Controlled ovarian hyperstimulation was performed. Embryos were assessed at 25–27 h after ICSI for early cleavage and classified as Early Cleavage (EC) embryos or Non Early Cleavage (NEC) embryos. The patients were subdivided into two subgroups; one transfer (EC) and the other transfer (NEC).
Main results and the role of chance: Transfer of early cleavage embryos (EC) led to significantly higher pregnancy rates as compared to non early cleavage embryos (NEC) (43.30% versus 21.88%; $P = 0.005$), and also higher implantation rates (25.58% versus 11.35%; $P = 0.000$). Multiple pregnancy rate were significantly increased up to 26% when the number of EC embryos was two or more compared to 15.38% when less than two.
Limitations, reason for caution: No limitation.
Wider implications of the findings: Early cleavage could be an additional factor for selecting embryos with a higher potential of implantation and the number of EC embryos could be a useful parameter for the prediction of multiple pregnancies.
Study funding/competing interest(s): Funding by University(ies) – Al-Azhar University.
Trial registration number: Cohort study.
Keywords: ICSI, multiple pregnancy, early cleavage

P-561 Estimating the risk of monozygotic twins in IVF-ICSI pregnancies using the perspective of a prenatal diagnosis unit

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Study question: Is the risk of monozygotic twin (MZT) pregnancies truly increased in IVF-ICSI cycles?
Summary answer: IVF-ICSI pregnancies have a three-four folds increased risk of MZT.
What is known already: MZT is accepted to be increased in IVF pregnancies. However, available evidence is not consistent and estimates of the magnitude of this risk vary widely. These discrepancies may be explained by differences in population studied and methodological pitfalls. In fact, a definitive conclusion on the risk of MZT in IVF-ICSI pregnancies is still lacking. In this study, we suggest to approach the issue from a different perspective, i.e., using data from a tertiary care prenatal diagnosis unit.
Study design, size, duration: Retrospective evaluation of 145 MZT pregnancies. This sample size was calculated based on an expected rate of IVF-ICSI pregnancies in our population of 2.0%, setting type 1 and 2 errors at 0.05 and 0.20 respectively and stating as clinically relevant a three-folds increase in the risk of MZT.
Participants/materials, setting, methods: Data was obtained from outpatient and inpatient charts and phone contact. We included twin pregnancies with a sonographic diagnosis of monochorionic MZT that progressed beyond 12 weeks' gestation. Collected data included baseline clinical characteristics, mode of conception and pregnancy outcome.
Main results and the role of chance: Ten out of the 145 MZT pregnancies were achieved using IVF-ICSI, corresponding to a rate of 6.9% (95% CI: 3.5–11.8%), thus significantly higher than the expected 2.0%. The Odds Ratio (OR) of MZT in IVF-ICSI pregnancies is 3.6 (95% CI: 1.8–6.6). When considering exclusively MZT pregnancies achieving delivery of two vital newborns ($n = 132$), the number of IVF-ICSI pregnancies was nine (6.8%, 95% CI: 3.7–12.5%). The corresponding OR is 3.3 (95% CI: 1.9–7.0). Baseline pre-pregnancy characteristics did not differ between IVF-ICSI and natural pregnancies (data not shown). Moreover, we failed to document significant differences in pregnancy outcome. The rates of delivery of the two alive twins (90% and 91%), twin-to-twin transfusion syndrome (10% and 18%), delivery before 34 weeks' gestation (22% and 25%) and SGA (11% and 23%) were indeed similar ($p = 1.00$, $p = 1.00$, $p = 1.00$ and $p = 0.38$, respectively).
Limitations, reason for caution: Firstly, the antenatal perspective consents to prevent several but not all confounders. Secondly, the small sample size does not allow us to investigate the potentially detrimental independent role of ICSI, assisted hatching or blastocyst transfer. Thirdly, the small sample size exposes our results on pregnancy outcome comparisons to a significant type II error.
Wider implications of the findings: Women undergoing IVF-ICSI should be informed that they have an increased risk of MZT. Largest studies are warranted to assess whether the prognosis of IVF-ICSI and natural MZT pregnancies differs.
Study funding/competing interest(s): Funding by University(ies) – none.
Trial registration number: NA.
Keywords: IVF, monochorionic pregnancies

P-562 The comparsion of live-birth defects following luteal phase ovarian stimulation with conventional/mild ovarian stimulation for *in vitro* fertilization and vitrified embryo transfer cycles

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Study question: Our previous study showed that luteal phase ovarian stimulation (LPS) can produce competent oocytes/embryos and optimal pregnancy in women undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatments. Are there any differences in the live-birth defects following the LPS compared with conventional/mild ovarian stimulation?
Summary answer: Until now, this unique approach has resulted in hundreds of live born infants following frozen-thawed embryo transfer (FET) in our center, while the rates of live-birth defects following LPS were similar to those following conventional/mild ovarian stimulation.
What is known already: So far, there are no reports of this aspect.
Study design, size, duration: A retrospective cohort study was conducted at our center; IVF/ICSI-FET cycles following LPS and conventional/mild ovarian stimulation started between March 1, 2012 and July 1, 2013 are collected.
Participants/materials, setting, methods: Patients who underwent IVF/ICSI-FET treatment leading to births between January 1, 2013 and May 1, 2014 were completed telephone interviews. The final data involved 2060 live-born infants were stratified into groups: LPS ($n = 587$ births), the standard GnRH agonist short protocol ($n = 1257$ births) and mild ovarian stimulation ($n = 216$ births).
Main results and the role of chance: Birth characteristics regarding gestational age, birth weight and length, multiple delivery and early neonatal death were comparable in all groups. The incidence of live birth defects among the LPS group (1.02%) and the short GnRH agonist protocol group (0.64%) were all slightly higher than in the mild ovarian stimulation group (0.46%). However, none of these differences reached statistical significance ($P = 0.624$). For congenital malformations, the risk significantly increased for the infertility-duration factor and multiple births; the adjusted odds ratios (OR) were 1.161 (95% confidence interval [CI]: 1.009–1.335, $P < 0.037$) and 3.899 (95% CI: 1.179–12.896, $P < 0.026$), respectively. No associations were found between congenital birth defects and different ovarian stimulation regimens, maternal age and BMI, parity, insemination method and infant sex.
Limitations, reason for caution: Our data source was limited to extracting neonatal information from parent questionnaires rather than direct access to their medical records. Therefore, it is likely that minor problems may have

escaped detection and birth defect rates may be underestimated, although it is unlikely that this would have altered infant birth characteristics.
Wider implications of the findings: There is no evidence of detrimental effects of LPS on live-born infants at birth, and continuous surveillance will be needed to explore its long-term safety. However, infertility itself poses a risk factor for congenital malformation. A higher likelihood of birth defects in multiple births may influence couples to favor elective single-embryo transfer, and couples undertaking ART should be aware of the known increased birth defects associated with a twin birth.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Foundation of China. Natural Science Foundation of Shanghai, China.
Trial registration number: NA.
Keywords: luteal phase ovarian stimulation, *in vitro* fertilization, intracytoplasmic sperm injection, neonatal outcome, congenital malformation

P-563 Failure mode and effects analysis of witnessing protocols for ensuring traceability of gametes and embryos during *in vitro* fertilization: a single centre analysis

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Study question: Is failure mode and effects analysis (FMEA) helpful to enhance safety in the IVF laboratory by identifying critical steps where miss-matches may occur and supporting the introduction of new strategies to minimize risks?
Summary answer: FMEA performed by a multidisciplinary team is effective identifying critical procedures during traceability and witnessing of patients and samples. Although double-checking approach and permanent recording of critical data were in place, the analysis allowed to identify weaknesses and redundancy in the protocols and to set-up a more effective system.
What is known already: Traceability of cells during IVF is a fundamental aspect of the treatment that involves witnessing protocols integrated in an effective Quality Management System. Although it is a mandatory requirement according to specific European Directives, miss-matches have been recently reported with dramatic consequences for both patients and health care professionals. FMEA is a method for identifying real or potential breakdowns in processes and to develop strategies to mitigate risks.
Study design, size, duration: To examine the risks associated with traceability and witnessing protocols for patients and cells identification, to identify possible causes of failures and their potential effects, we performed a proactive risk-assessment analysis (FMEA) in a large IVF centre (>1.000 cycles/year), prior and after the implementation of electronic witnessing systems.
Participants/materials, setting, methods: A multidisciplinary team was formed, moderated by a human factors specialist, to calculate a risk-priority-number (RPN) for each element most likely to contribute to failures by multiplying 3 factors: severity-occurrence-detection (graded 1–5). A second analysis was performed after the implementation of electronic witnessing systems (IVF witness, RI).
Main results and the role of chance: The IVF team identified 7 main process phases (oocyte collection, sperm donation, gamete processing, insemination, embryo culture, embryo transfer, cryopreservation), 19 associated process steps and 32 possible failures modes. The highest RPN was 30 (moderate risk) confirming the relative low risk that miss-matches may occur in IVF when a manual witnessing system (based on double-checking approach and permanent recording of critical data) is used. Possible failures modes were mainly associated with: heavy clinical workload and distraction, communication failures between the team, automaticity during witnessing, ambiguous responsibility of the single tasks or redundancy of the protocols. The introduction of the electronic witnessing system allowed reducing the moderate-risk failure mode by 2/3 (highest RPN = 10).
Limitations, reason for caution: The study was performed in a single large centre, with complex protocols for traceability and witnessing. The results are thus only applicable to our setting. FMEA analysis has been shown to be a useful prospective tool however its absolute validity is questionable due to the subjectivity of the judgments.
Wider implications of the findings: The FMEA is effective to support multidisciplinary IVF groups in understanding traceability and witnessing process and identifying critical steps where miss-matches may occur. Due to the irreversible and dramatic consequences of miss-matches in IVF, it is suggested to enhance safety by performing proactive risk-assessment analysis and to consider the implementation of electronic witnessing systems that has been shown in our setting to prevent potential risks.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Funding by national/international organization(s). None.
Trial registration number: NA.
Keywords: FMEA, miss-match, proactive risk-assessment analysis, IVF

P-564 Reduction of multiple pregnancy rate in advanced maternal age population after the introduction of an elective single embryo transfer policy: a pre- and post-intervention study

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Study question: Is elective single-embryo transfer (eSET) policy efficient and effective in women aged >35 years?
Summary answer: Elective SET coupled with enhanced embryo selection policy in women older than 35 years reduce the multiple pregnancy rate while maintaining the cumulative success rate of the IVF programme.
What is known already: SET policy is recommended in cases of good prognosis patients. No general consensus has been reached for SET application in the advanced maternal age population (AMA) defined as women older than 35 years. Our objective was to evaluate the results in terms of efficacy, efficiency and safety of eSET policy coupled with routine application of blastocyst culture and pre-implantation genetic screening (PGS) for this population of patients in our IVF program.
Study design, size, duration: In January 2013, a multidisciplinary intervention involving optimization of embryo selection procedure and introduction of an eSET policy in the AMA population was implemented. This is a retrospective 4-year (January 2010–December 2013) pre- and post-intervention analysis.
Participants/materials, setting, methods: Surplus oocytes and/or embryos were vitrified during the entire study period. In the post-intervention period, all couples with good quality embryos were offered eSET. Embryo selection was enhanced by blastocyst culture and PGS (blastocyst stage biopsy and 24-chromosomal screening) was routinely offered. eSET was also applied in cryopreservation cycles.
Main results and the role of chance: Patient and cycle characteristics were similar in the pre- and post-intervention groups (mean age 39,5 + 2,1 and 39,4 + 2,2; range 36–44) as assessed by logistic regression. 1609, 937, 138 and 574, 350 and 27 oocytes retrievals, embryo and oocyte warming cycles were performed, respectively. These resulted in 1854 and 508 total embryo transfers in the pre- and post-intervention period. A mean number of 2,1 + 1,1 and 1,4 + 0,8 embryos were transferred, respectively ($P < 0,01$). Similar cumulative clinical pregnancy rates per transfer and per cycle were obtained: 26,8%, 30,9% and 29,7%, 26,3%. The total efficacy (delivery/oocyte retrieval cycle) was not affected by the intervention (21,1% and 20,4%; OR = 1.17,95% CI: 0.88–1.55;$p = 0.26$) However, significant increased efficiency (life birth/transferred embryo) was observed in the post-intervention group 17,0% vs 10,6% ($P < 0,01$). Multiple pregnancy rate decreased from 20,8% to 6,9% (OR = 0.32 95% CI 0.14–0.71; $P < 0,01$).
Limitations, reason for caution: In this study, the suitability of SET was individually assessed on the basis of both clinical and embryological prognostic factors and was not standardized. For the described eSET strategy coupled with enhanced embryo selection policy an efficient culture system and cryopreservation program is necessary.
Wider implications of the findings: Due to the increased maternal morbidity and mortality and perinatal complications related to multiple pregnancy it is generally recommended to extend the eSET policy also to the AMA population. As shown in this study, in this population of patients enhanced embryo selection procedures allow to reduce the number of embryo transferred at a time without affecting the total efficacy of the treatment but increasing efficiency and safety.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.

Trial registration number: NA.
Keywords: SET, multiple pregnancy, embryo selection, ART safety

P-565 The effect of initiation of strict embryo transfer limits on neonatal complications with in-vitro fertilization (IVF)

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Study question: What is the effect of the initiation of a government funded in-vitro fertilization (IVF) program with strict limits on numbers of embryos which can be transferred on neonatal complication rates and incidence?
Summary answer: This first North American publicly funded IVF program has decreased the multiple birth rates related to IVF. However, the absolute numbers of IVF babies born prematurely or requiring admission have slightly increased. Also, the average admission cost per IVF baby has seen a substantial increase.
What is known already: Multiple pregnancies carry with them risks to both mothers and fetuses. Several international jurisdictions have demonstrated that publicly funded fertility programs with a single embryo transfer (SET) policy decrease multiple pregnancy rates. Also, IVF pregnancies are generally associated with higher rates of complications than spontaneous pregnancies, attributed partially to multiples. In August 2010, Quebec started funding of IVF with SET, with a goal of decreasing neonatal complications and their costs.
Study design, size, duration: This a retrospective study. Data compares outcomes of all IVF cycles performed in Quebec from the 2009 to 2010 (last complete pre-coverage) to 2012–2013 (first complete post-coverage) fiscal years. This study is based on 168 602 spontaneous and IVF deliveries. In 2009–2010, 906 women conceived with IVF, while in 2012–2013, 1746 conceived.
Participants/materials, setting, methods: Data was extracted from two reports by the Health and Welfare Commissioner as well as the Ministry of Health and Social Services published in June 2014 and October 2013, respectively. This data was collected from all assisted reproduction centers in Quebec providing IVF services. Data was compared using chi-squared tests.
Main results and the role of chance: The number of babies born from IVF increased 63% from 2009–2010 to 2012–2013 (1057–1723). Multiple pregnancy rates decreased from 24.06% in 2009–2010 to 9.45% in 2012–2013 ($p < 0.0001$). The proportions of IVF babies that were the result of multiple births, were premature, or required intensive-care unit (ICU) admission, decreased by 55% ($p < 0.0001$), 35.5% ($p < 0.0001$), and 37% ($p < 0.0001$), respectively, from 2009–2010 to 2012–2013. These changes in absolute numbers were a decrease from 407 to 297, an increase from 313 to 329 and an increase from 199 to 204 babies, respectively. The average ICU admission costs for a baby conceived through IVF and spontaneously was $19,990 and $14,563 in 2009–2010, respectively, and $28,418 and $17,155 in 2011–2012, respectively.
Limitations, reason for caution: Retrospective data concerning IVF cycles and clinical outcomes was gathered from several sources. However this is a robust study on data collected from more than 160,000 women who underwent conceptions either spontaneously or through IVF.
Wider implications of the findings: Publicly funded IVF programs substantially decrease multiple pregnancy rates. However, due to substantially increased usage, neonatal complications increase. Interestingly, the cost per IVF neonatal-ICU admission skyrocketed when the cost of caring for multiples was reduced. This suggests that the singleton IVF pregnancies which require neonatal-ICU care are much sicker than IVF twins which end up in the ICU. Further research should be directed into decreasing the rate of ICU admissions for singleton IVF conceptions.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding was provided.
Trial registration number: NA.
Keywords: ART, elective single embryo transfer, neonatal complications

P-566 Advanced maternal age and ART: a Nordic cohort study from the CoNARTaS

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Study question: What is the effect of advanced maternal age on maternal and perinatal outcomes after assisted reproductive technology (ART)?
Summary answer: Advanced maternal age seems not to be a further risk factor for adverse maternal and perinatal outcomes in addition to the increased risks associated with ART.
What is known already: Advanced maternal age is associated with increased risk for pregnancy complications and adverse neonatal outcomes in spontaneously conceived (SC) pregnancies. ART compared to spontaneous conception is associated with increased risk for pregnancy complications and adverse neonatal outcomes also in singleton pregnancies.
Study design, size, duration: Population based cohort study performed in three Nordic countries (Denmark, Sweden and Norway) from 1988 to 2007.
Participants/materials, setting, methods: 39922 singletons conceived after ART and 266907 SC singletons. Main outcomes were hypertensive disorders in pregnancy (HDP), placental abruption, placenta praevia, preterm birth (PTB, <37 weeks), low birth weight (LBW, <2500 g), small for gestational age (SGA). Crude and adjusted odds ratios (AOR) with 95% confidence interval were calculated.
Main results and the role of chance: Compared to SC pregnancies in women at age 18–34 years, pregnancies in women ≥40 years had a significantly higher risk of both HDP and placental abruption, regardless of conception method (AOR ART 1.5 and 1.7, SC 1.8 and 1.6, respectively). The risk for placenta praevia was generally increased in ART women (AOR 8–11). The risk for PTB, LBW and SGA were significantly higher in both ART and SC pregnancies for women ≥40 years, compared to SC pregnancies for women 18–34 years. In ART pregnancies, risk was similar across age groups for maternal and neonatal outcomes, while risk increased with increasing age for SC pregnancies.
Limitations, reason for caution: It was only possible to adjust for parity, year of birth, offspring sex and country, thus there may be residual confounders. Women who conceive through ART at an advanced age may represent a selected group.
Wider implications of the findings: The results are reassuring for a large group of women treated with ART at an advanced age.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). The study was supported by grants from the University of Gothenburg/Sahlgrenska University Hospital (LUA/ALF 70940) and the Nordic Federation of Obstetrics and Gynaecology (NFOG).
Trial registration number: Not relevant.
Keywords: advanced maternal age, ART, risk, maternal outcome, neonatal outcome

P-567 Risk of preeclampsia and hypertensive disorders of pregnancy (HDP) in singleton and twin oocyte donation (OD) pregnancies – a systematic review and meta-analysis

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Study question: How much is the risk of HDP and preeclampsia increased in OD singleton and twin pregnancies compared to *in vitro* fertilization (IVF) singleton and twin pregnancies?
Summary answer: The risk of HDP and preeclampsia is almost three-fold increased in OD singleton and twin pregnancies compared to IVF singleton and twin pregnancies.
What is known already: Several mainly smaller studies including multiple pregnancies have shown that the risk of HDP and preeclampsia is increased in OD pregnancies compared to IVF pregnancies. This may be caused by immunological factors as the fetus in OD pregnancies is genetically unknown to the mother. No previous meta-analyses have been performed.
Study design, size, duration: In a systematic review we included 13 studies out of which 6 studies present odds ratios of HDP in OD singleton pregnancies compared to IVF singleton pregnancies and 7 studies present odds ratios of preeclampsia in OD singleton pregnancies compared to IVF singleton pregnancies. Two meta-analyses were conducted on HDP and preeclampsia respectively. Hypertensive disorders of pregnancy include chronic hypertension, pregnancy induced hypertension, preeclampsia/eclampsia and HELLP syndrome.
Participants/materials, setting, methods: We identified 13 original studies presenting data on occurrence of HDP and preeclampsia in OD pregnancies in PubMed and Embase. Only studies in English language and from the last two decades were included. Only studies presenting separate data on singletons or adjusting for plurality were included in the meta-analyses.
Main results and the role of chance: In the meta-analyses the pooled odds ratio of HDP was 2.71 (95% CI 2.10–3.51) and the pooled odds ratio of preeclampsia was 2.92 (95% CI 2.00–4.29) in 869 and 835 OD singleton pregnancies, respectively, compared to IVF singleton pregnancies. In twin pregnancies, 36% (73 out of 200) developed HDP and 21% (27 out of 130) developed preeclampsia. These numbers were too small to perform meta-analyses.
Limitations, reason for caution: Only a limited number of small studies could be included in the meta-analyses as most existing studies pool data on singleton and multiple pregnancies without adjustments. Studies were heterogeneous and up to 20 years old.
Wider implications of the findings: The risk of HDP and preeclampsia in OD pregnancies is noticeably increased and extended antenatal care including extra blood pressure controls and induction of labor at term should be offered. Further women with OD pregnancies may benefit from low dose aspirin treatment.
Study funding/competing interest(s): Funding by University(ies) – no external Funding was obtained for this project.
Trial registration number: No trial registration.
Keywords: oocyte donation pregnancy, preeclampsia

P-568 Systemic oxidative stress could predict assisted reproductive technique outcome

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Study question: Main aim of this research was to detect oxidative stress (OxS) levels in couples attending assisted reproductive technique (ART) procedure.
Summary answer: The study uncovered negative effect of both partners' oxidative stress on maintenance and outcome of pregnancy. A strong positive correlation between the partners' OxS levels was seen.
What is known already: Previous studies have indicated that OxS may appear as a possible reason for poor ART outcome. High seminal ROS (reactive oxygen species) level is associated with impaired sperm fertilizing ability and lower pregnancy rates after IVF. To date, one of the most common marker to assess systemic OxS is measurement 8-isoprostanes (8-EPI), the byproducts of lipid peroxidation.
Study design, size, duration: Prospective laboratory-clinical cohort study of 79 couples attending ART procedure in 2011–2012.
Participants/materials, setting, methods: Altogether 79 couples attending ART procedure (58 IVF and 21 ICSI) were recruited at Nova Vita Clinic (Tallinn, Estonia). Oxidative DNA damage (8-OHdG) and lipid peroxidation (8-EPI) were detected in urine, vaginal fluid and seminal plasma by ELISA method. Clinical background and ART outcome were also recorded in both partners.
Main results and the role of chance: Both OxS markers were significantly associated with clinical conditions of the subjects, the biggest effectors being genital tract infections, endometriosis, uterine myoma and smoking. Furthermore, men whose partners had genital tract infections, showed significantly higher OxS levels compared to men, whose partners were without known diseases (a strong positive correlation among couples $r = 0.42$, $p < 0.001$). The most significant effectors of male OxS were bacterial vaginosis and salpingo-oophoritis. No pregnancies after ART procedures occurred among 63% of patients, 9% of pregnancies were detectable only by pregnancy test and 28% of pregnancies were detectable also by ultrasound. The highest 8-EPI levels were detected in both partners (women 97,8 (±16,7) vs 72.9 (±22,9), $p = 0.007$; men, 89.6 (±20,4) vs 72,1 (±22,6), $p = 0.049$) when biochemically detectable pregnancy did not develop into clinically detectable pregnancy.
Limitations, reason for caution: The weakness of current study was the absence of fertile control group and small study groups. As there are large number of markers and a lot of different technologies available for detecting OxS level in different body fluids, the confirmatory studies with bigger study group are necessary.
Wider implications of the findings: High grade systemix OxS in both partners may negatively affect the maintenance and outcome of pregnancy. Applying detection of OxS in ART patients may select the patients with higher success rate and/or those who need antioxidant therapy. This would lead to improvement of ART outcome as well as natural fertility.
Study funding/competing interest(s): Funding by University(ies) – Funding by commercial/corporate company(ies). The present study was supported by Enterprise Estonia (Grant no. EU 30020), Estonian Ministry of Education and Research (Target Financing SF0180132s08, Institutional Research Fundings IUT 20–42 and IUT 15–19) and University of Tartu (Grant no. SARMBARENG). No competing interests.
Trial registration number: NA.
Keywords: 8-EPI (8-iso prostaglandin F2a), 8-OHdG (8-hydroxy-2¢-deoxyguanosine), ART (assisted reproductive technique), OxS (oxidative stress)

P-569 Coaching an efficient quality management system for small IVF laboratories

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Study question: Are small fertility units – performing less than 50 oocyte retrievals per year – able to achieve success rates significantly better than the mean success rates published by their national IVF registers?
Summary answer: Coaching based on continuous education, survey mainly via the internet and alternated by quarterly on site visits results in the establishment of small autonomous IVF laboratories having success rates per treatment cycle upto 15% above the national mean.
What is known already: The Swiss National IVF Register data reveal that in 2012, from the 25 centers 20 did less than 1 oocyte puncture a day. The pregnancy rate per initiated cycle ranged from 8.8% to 31.4% in 2012. This large difference remained constant since: 2010 (6%–37%) resp. 2011 (7.7%–42.9%). Such data fuel the believe that smaller units produce less good results because of the availability of an embryologist and/or a discontinuity in their activity, lack of adequate survey and quality management or the difficulty of obtaining adequate technical support.
Study design, size, duration: A laboratory coaching system was setup from the middle of 2013 on in 2 laboratories: one situated in Basel (CH) and one in Constanta (RO) and is still ongoing.
Participants/materials, setting, methods: The coaching consisted in regular on site visits with control of all physical and chemical parameters which are known to influence the outcome of assisted reproductive technologies: temperature logging, infrared thermography, pH logging, magnetic field survey and volatile organic compound analysis by passive chromatographic absorption and active logging. All used instruments for the surveys were calibrated by accredited organizations on a yearly base. All laboratory procedures were reviewed, standardized, skills of all laboratory personal was assessed and continuously monitored via their enrollment in monthly Online Quality Survey Schedules (FERTAID) all personal participates in a continuous education program based on ABB accredited webinars (life interactive internet seminars).

Main results and the role of chance: For the laboratory in Basel the clinical pregnancy rates per oocyte retrieval was: in 2012 13/50 (26%), in 2013 15/54 (28%) and in 2014 19/43 (44%) for the unit in Constanta the results were: in 2012 8/35 (23%), in 2013 10/20 (50%) and 7/20 (35%) in 2014. The Z-test for proportions for dependent groups in 2013 and 2014 was significant at the 95% confidence interval level in the unit located in Basel and significant at the 80% confidence interval level for the unit in Constanta.
Limitations, reason for caution: The median age in both centers for the 3 periods was 35 and ranged between 26 and 42 years.
Wider implications of the findings: Small IVF units are able to provide constant good treatment outcome under continuous external coaching.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Marc Van den Bergh is CEO of Quartec GmbH.
Trial registration number: NA.
Keywords: quality, management, laboratory, internet, accreditation

P-570 The effect of transferring one or two blastocysts in women 40–42 years of age on pregnancy, clinical pregnancy, live birth and multiple pregnancy rates

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Study question: Does transferring 2 blastocysts in women at least 40 years old increase the pregnancy, clinical pregnancy, live birth or multiple pregnancy rates as compared to 1 blastocyst transfer?
Summary answer: Transferring two blastocysts does not improve the clinical pregnancy rate or the live birth rate in this age group, but trends to increase the multiple pregnancy rate.
What is known already: In women less than 36-years old transferring two-blastocysts increases the multiple pregnancy rate without necessarily increasing the clinical pregnancy or live birth rates. Few studies have also demonstrated similar findings in women 36–39 years of age. Quebec limits the transfer to a maximum of two blastocysts in women ≥40 years of age. Development to blastocyst does not translate in to euploidy in women of this age. No studies have investigated transferring one or two blastocysts in this population.
Study design, size, duration: A retrospective study was performed on all blastocyst transfers done in women at least 40 years of age ($n = 126$). Data was collected from August 2010 till June 2012. August 2010 was selected as a start date because this is when Quebec limited number of embryos transferred.
Participants/materials, setting, methods: Data was collected at a university center. Research ethics board approval was obtained (13-053-SDR). Data was analyzed using multivariate analysis to control for confounding effects of female age, ovarian reserve, stimulation parameters, and embryo quality. A total of 38 single blastocyst and 88 double blastocyst transfers were performed in women 40–42 years of age.
Main results and the role of chance: When comparing one or two blastocysts transferred, pregnancy rates (45% vs. 38%, $p = 0.54$), clinical pregnancy rates (34% vs. 27%, $p = 0.60$), and live birth rates (21% vs. 19%, $p = 0.82$) did not differ. Best blastocyst transferred grade seemed to trend better among women with a pregnancy ($p = 0.08$), but not those with a clinical pregnancy ($p = 0.43$) or live birth ($p = 0.12$). Best blastocyst grade did not differ for one or two blastocysts transferred ($p = 0.83$). There were no multiple pregnancies among women with single blastocyst transfer. When comparing single vs. double blastocyst transfer, the rate of two intrauterine gestational sacs (0% vs. 20%, $p = 0.06$), two foetal heart beats (0% vs. 25%, $p = 0.05$) and live birth of twins (0% vs. 24%, $p = 0.06$) trended higher in the two embryos transferred group.
Limitations, reason for caution: Retrospective study. Interestingly the pregnancy, clinical pregnancy and live birth rates were higher although not statistically significant in the women with single blastocyst transfer, compared to double blastocyst transfer.
Wider implications of the findings: More data is needed to confirm these findings. However, initially it appears that transferring two blastocysts in women 40–42 years of age does not improve clinical outcomes, only increases the multiples rates, when compared to single blastocyst transfer. Given that the pregnancy rate does have room to increase, it seems likely that transferring 3 or more blastocysts in this age group would improve clinical results. Concurrently, likely further increasing the multiple pregnancy rates.
Study funding/competing interest(s): Funding by University(ies) – none.
Trial registration number: NA.
Keywords: IVF, multiple pregnancy, blastocyst, advanced maternal age

P-571 The role of cyclin D1 in the development of borderline ovarian tumors after ovarian hyperstimulation

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Study question: Concerns have been raised that ovarian stimulation may increase the risk of ovarian malignancies, especially Borderline Ovarian Tumors (BTOs). To understand if repeated cycles of gonadotropin stimulation could modulate intracellular localization and content of proteins controlling cell cycle progression in mouse ovaries and fallopian tubes (FT).
Summary answer: Repetitive gonadotropin stimulations did not induce changes in a set of proteins directly involved in cell cycle progression and usually altered in ovarian cancer. By contrast, cyclin D1 level increased significantly after the fourth cycle of stimulation in FT of treated mice.
What is known already: Epidemiological data evidenced an increased risk for BTOs in infertile women treated with IVF and about two thirds of serous borderline ovarian tumors are characterized by KRAS mutations that determines a significant increase of cyclin D1 expression.
Study design, size, duration: Ovaries and FT of naturally-ovulating mice and of mice undergoing 2–4 rounds of gonadotropin stimulations were analyzed to detect localization and expression levels of Oct-3/4, Sox-2, p53, β-catenin, pAKT and cyclin D1. Ovulated oocytes were analyzed to detect meiotic spindles and chromosome alignment.
Participants/materials, setting, methods: Two to four rounds of stimulations were performed with intervals of 1 week between each. Repetitive cycles of ovarian stimulation were performer according to the protocol of Van Blerkom and Davis. For each experiment, control (Ctr; $N = 4$) and hyperstimulated ($N = 12$; 4/round) mice were sacrificed. The experiment was replicated four times.
Main results and the role of chance: After round 4, ovaries and FT of control and treatment groups showed no differences in Oct-3/4, Sox-2, p53, b-catenin intracellular localization nor in Oct-3/4, Sox-2, p53, b-catenin and pAKT contents. By contrast, cyclin D1 level increased significantly in FT of treated mice. Number and quality of oocytes decreased meanwhile frequency of abnormal meiotic spindles increased with treatments.
Limitations, reason for caution: The significant increase of cyclin D1 detected in the FT needs to be further investigated.
Wider implications of the findings: We cannot reject the possibility that in a small percentage of susceptible women also a relatively low increase of cyclin D1, as that recorded in mice, could sensitize epithelial cells towards malignant transformation. As a consequence, even if repetitive hormonal stimulation is not "per se" cause of OC, it remains ethically proper to inform women at risk, as those with a family story of solid cancers, about the potential consequences of infertility treatments.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). This work has been funded by the Italian Ministry of Education, University and Research to S.C. and G.C. (ex 60%), and by FARI 2012, "Sapienza" University of Rome to R.C. The study has been performed in the framework of the "Research Centre for Molecular Diagnostics and Advanced Therapies." The authors wish to thank the "Abruzzo earthquake relief fund" (Toronto, Ontario) that supported in part this research with the purchase of confocal microscope Leica TCS SP5 II (Leica, Germany).
Trial registration number: Not needed.
Keywords: cyclin D1, borderline ovarian tumors, ovarian hyperstimulation

P-572 The effects of regular QC on embryo transfer rates per MD: a 10 year experience

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Study question: At our clinic analysis of pregnancy rate per MD is analysed tri-annually and deviations from the average are assessed. Any MD below 10% from the average is retrained. This study assesses if there is any evidence that the continual QC process causes range between MD results to narrow over time.
Summary answer: There is a clear pattern towards a narrowing in the range of results for the original partners in the clinic but as new partners have joined it takes some time for them to align with the other partners. Fellows demonstrate stable results over their 2 year fellowship.

What is known already: Previous studies have shown differing results in terms of the impact of the physician on the pregnancy rate (van Weering, 2005, Hearns-Stokes, 2000, Desparoir, 2011) however these studies analysed the direct impact of the MD on the pregnancy rate not the ability to narrow the range of pregnancy rates between physicians or the impact of adding new partners with different previous experience to the team.
Study design, size, duration: A retrospective data analysis was conducted for 12649 embryo transfers (ET) between 2004 and 2014. The progress of the original 5 MD partners was analysed along with the changes as 8 new MD partners joined the practise between 2006 and 2010. Furthermore the results of REI fellows was analysed independently.
Participants/materials, setting, methods: The range of pregnancy rates per year for the five original MD was analysed. Additionally 8 MD joined the team; one in 2006, 2007, 2008, 2009 and three in 2010. One was an experienced ET practitioner, one had very little experience and the other five joined from their fellowship.
Main results and the role of chance: The results demonstrate a reduction in the range between the highest and lowest performing original MD for the study period (17, 17, 32, 22, 11, 14, 13, 10, 12, 7, 10) however when the 8 additional MD are added to the analysis the narrowing between practitioners demonstrates more of a plateau suggesting that there is a time element required to reduce the differences. Of special interest is the impact in 2010 of a law change in Quebec forcing the use of eSET: the new, less experienced MD had more difficulty adapting this change and their range increased from 13 (2009) to 25 (2010) returning to 12 (2011), where as the more experienced original MD showed no impact on their range of difference (14, 13, 10).
Limitations, reason for caution: These data involve 12649 cycles over a 10 year period and appear to demonstrate that regular QC can reduce the range in PR between MD however to confirm this it would be necessary to continue analysis and assess how long the additional MD take to reduce their range.
Wider implications of the findings: The ET is an important element of the IVF cycle and it is important to ensure that this element is optimised. By performing regular QC of the results by MD at ET and sharing this data with the entire medical team permits members to optimise their technique and maximise the potential for each cycle. This requires an open-minded non-judgemental mindset from all members of the medical team.
Study funding/competing interest(s): Funding by hospital/clinic(s). No funding was obtained for this study and there is no conflict of interest for any of the authors.
Trial registration number: No Trial registration number.
Keywords: embryo transfer, pregnancy, quality control

P-573 Long-term safety of IVF, ICSI and IVM: a systematic review of animal studies

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Study question: What is the relationship between assisted reproductive technologies (ART) and long-term effects for the offspring as reported in animal studies?
Summary answer: Based on the small number of studies that we found, it seems that ART has effects in the long-term health of the offspring, but based on all included studies it was not possible to draw a coherent conclusion.
What is known already: Before the birth of the first *in vitro* fertilization (IVF) baby in 1978, aspects of IVF technology were developed in animal models. However, the introduction of human IVF into clinical practice was performed without proper safety assessment of this technology in animal studies. Consequently, the long-term safety of ART is of concern, and subject to debate.
Study design, size, duration: A systematic review based on a search in three different databases from inception until December 2014.
Participants/materials, setting, methods: We systematically searched PubMed, Embase and CAB Abstracts for relevant studies published until December 2014. Studies were eligible if they used an animal model (mammals only) that compared *in vitro* conception to *in vivo* conception, and reported at least one outcome measure focused on the ex-vivo offspring.
Main results and the role of chance: The search identified 2,300 articles, of which 46 primary studies met the inclusion criteria. There were 37 studies that reported on birth weight, and results were assessed as species-specific: studies with a mouse model were heterogeneous, while studies with a bovine model reported similar or higher birth weight after ART. The 3 studies that reported blood pressure had heterogeneous results. Out of 7 studies that reported on glucometabolism, 3 showed impaired glucometabolism after ART. Moreover, two out of five studies showed some difference in animal behaviour after *in vitro* conception. Only one study measured lifespan and this was significantly shorter in mice conceived by IVF and fed a high-fat diet.
Limitations, reason for caution: Our inclusion criteria excluded studies that were interested in the effects of particular steps of *in vitro* production. Instead of looking for any ex-vivo outcome in the offspring, we have used specific outcomes in our search to limit the amount of articles in our database search.
Wider implications of the findings: As long-term follow-up studies in human remain limited, animal studies provide an important avenue to assess the impact of ART on long-term health. Our review shows that a systematic approach to this opportunity has been lacking, as studies are species-specific, use different ART procedures and have not been established specifically to address this question. There is an urgent need for standardized animal studies specifically designed to assess the long-term safety of ART.
Study funding/competing interest(s): Funding by University(ies) – University of Adelaide & Erasmus University Rotterdam.
Trial registration number: NA.
Keywords: ART, long-term safety, offspring, animal studies

P-574 Pregnant after assisted reproduction: a risk pregnancy is born! 18-years results from a population-based registry in Flanders, Belgium

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Study question: In this population-based cohort study we examined the perinatal outcome of pregnancies after assisted reproduction (ART) including IVF/ICSI and ovarian stimulation (OS) with or without intrauterine insemination. The outcome results were compared with the results of a reference population of natural conception (NC) pregnancies.
Summary answer: The increased risk for perinatal morbidity and mortality of babies born after ART is largely attributed to a higher rate of multiple gestations but ART singletons are also at increased risk for perinatal problems when compared to NC pregnancies. Therefore all ART-pregnancies should be considered as risk pregnancies.
What is known already: Although the increased risk for perinatal morbidity and mortality of babies born after ART is largely attributed to a higher rate of multiple gestations, a significantly worse perinatal outcome for singleton pregnancies following ART compared to pregnancies after natural conception has been reported. Most studies only include IVF/ICSI pregnancies, studies describing the perinatal outcome of pregnancies after non-IVF assisted reproduction and comparative studies including IVF/ICSI, OS and NC pregnancies are scarce.
Study design, size, duration: By using the data of a population-based registry we studied the perinatal outcome of 1 079 814 births during a 18 years period (1993–2010). We examined and compared the perinatal outcome results of a large cohort of ART, OS and NC pregnancies.
Participants/materials, setting, methods: The Flanders Study Centre for Perinatal Epidemiology collects data on the perinatal outcome of all deliveries of >21 weeks and/or 500 g at birth. The following perinatal outcome parameters were studied: prematurity, low birth weight, perinatal mortality and morbidity including neonatal intracranial bleeding and need for intubation.
Main results and the role of chance: This study describes the perinatal results of a very large cohort of IVF/ICSI and OS births. Our data show that IVF/ICSI singletons had a significantly worse outcome when compared to OS and NC for almost all investigated perinatal parameters. Non-IVF OS singletons were also significantly disadvantaged for birthweight and prematurity when compared to NC singletons. The outcome of twin pregnancies was similar for the three groups unless only unlike-sex twins were studied separately. Among this subgroup, IVF/ICSI carried a higher risk for low birth weight when compared

to NC. OS unlike-sex twins were at increased risk for low birth weight and perinatal mortality when compared to NC unlike-sex twins.
Limitations, reason for caution: Although our logistic regression analysis included co-variables with a potential impact on perinatal outcome such as mode of conception, female age, fetal sex, parity and year of delivery, we couldn't correct for other prominent confounders such as smoking, obesity, insulin resistance, socio-economic status, occupation exposures, pre-existing disease, etc.
Wider implications of the findings: Our results show that all ART-pregnancies have to be considered as risk pregnancies, irrespective of the number of fetuses. ART-singletons are also at increased risk when compared to NC babies. Although IVF/ICSI singletons have the worse prognosis, OS singletons also carry a higher perinatal risk. For unlike-sex twins, results showed that both IVF/ICSI and OS pregnancies carry a higher perinatal risk when compared to NC unlike-sex twins.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Genk Institute for Fertility Technology.
Trial registration number: NA.
Keywords: assisted reproduction, perinatal outcome, pregnancy

P-575 Placental morphology in singleton births following assisted reproductive technology

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Study question: Is assisted reproductive technology (ART) treatments associated with abnormal placental morphology in humans?
Summary answer: Our study suggests that term placental weight, placental weight to fetal weight ratio, and placental volume is higher in ART conceptions when compared to natural conception.
What is known already: Some studies have found a significant increase in placenta thickness, weight, and the placental to fetal weight ratio in ART pregnancies compared to natural pregnancies. However, few studies, none with a large sample size, included detailed morphological investigations of the placenta.
Study design, size, duration: Retrospective control versus treatment study using data collected from 2003 to 2015 on 725 singleton pregnancies: 176 *in vitro* fertilization (IVF), 272 intra-cytoplasmic sperm injection (ICSI), and 277 natural conceptions (NC). Growth restricted, gestational diabetes, abnormal karyotype, pre-eclampsia and placenta previa complicated pregnancies were not included.
Participants/materials, setting, methods: We measured placental trimmed weight, diameter, and depth from IVF, ICSI, and NC pregnancies recruited from greater Vancouver. Pregnancy and neonatal details were recorded by hospital staff. We assessed the association between the type of conception and placental morphological variables such as weight, volume, density and placental/fetal weight ratio.
Main results and the role of chance: Placental weights (IVF-NC $P = 0.004$; ICSI-NC $P = 0.4$), the placental to fetal weight ratio (IVF-NC $P = 3.67E-8$; ICSI-NC $P = 0.0001$), and placental volume (IVF-NC $P = 0.06$; ICSI-NC $P = 0.017$) were larger for IVF and ICSI births compared to NC births. However, maximum placental depth and placental density were not significantly different between the three groups.
Limitations, reason for caution: The numbers in the IVF, ICSI and NC groups are not the same. Furthermore, incomplete data on smoking status, BMI, maternal age, gestational age, and ethnicity of patients as well as lack of data on quality of embryo transferred increase the possibility of a bias in our groups.
Wider implications of the findings: Our results support previous findings that placental weight and placental to fetal weight ratios are larger on average in ART births when compared to NC births, which may be indicative of intrauterine stress and predispose ART concepti to long term-health complications. The increase in mean placental volume but not mean density in the ART group compared to NC group has not been observed before and potentially suggests similar placental structural characteristics between the two groups.
Study funding/competing interest(s): Funding by national/international organization(s) – Canadian Institutes of Health Research (CIHR), grant to SM.
Trial registration number: NA.
Keywords: ART, IVF, ICSI, placenta

P-576 GnRH antagonist administered twice the day before hCG trigger, may prevent OHSS in IVF/ICSI antagonist cycles at risk for OHSS without affecting the reproductive outcomes

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Study question: In GnRH antagonist IVF/ICSI cycles at risk for ovarian hyperstimulation syndrome (OHSS), does a double antagonist dose (0,25 mg/12 h) administered the day before hCG trigger in conjunction to tapering the daily administered follicular-stimulating hormone (rFSH), is effective to prevent OHSS without affecting the reproductive outcomes?
Summary answer: GnRH antagonist dose administered twice (0,25 mg/12 h) for a single day that is the day before hCG trigger in conjunction to tapering the rFSH dose in GnRH antagonist IVF/ICSI cycles at risk for OHSS, may effectively prevent early OHSS without affecting the reproductive outcomes.
What is known already: GnRH antagonist protocol lower but do not eliminate the risk of OHSS in hyper-responding patients in IVF/ICSI cycles. GnRH agonist triggering instead of hCG for final oocyte maturation and freeze all strategy for embryos seems to be for the moment the only safe approaches to eliminate the risk of OHSS. Nevertheless, conflicting data exist regarding whether luteal support is adequate to facilitate a fresh transfer without affecting the reproductive outcomes.
Study design, size, duration: Double blinded RCT from 11/2009 to 2/2013 in a single centre. 194 patients at risk for OHSS undergoing IVF/ICSI antagonist cycle chose to proceed with embryo transfer and avoid cancellation or embryo cryopreservation were allocated into two groups by a sequence generated from a computerized random number table.
Participants/materials, setting, methods: Inclusion criteria: E2 ≥ 3500 pg/ml combined with ≥18 follicles >11 mm in diameter. Intervention group (97 patients) received a double dose of GnRH antagonist (0,25 mg/12 h) the day before hCG while another 97 patients (control group) did not. In both groups rFSH was tapered to 100 IU/24 h the day of the allocation.
Main results and the role of chance: Primary outcomes: incidence of early-onset moderate/severe OHSS was statistically lower in the intervention group compared to control group, (0% vs 12,37%, $P < 0.001$). Neither group developed late-onset moderate/severe OHSS after embryo transfer. Clinical pregnancy rate per cycle (50.52% vs 42.27%, $P = 0.249$), as well as per transfer (50.52% vs 48.24%, $P = 0.759$), were not significantly different between the two groups. Early pregnancy loss between group A and B was not statistically different (16.33% vs 17.07%, $P = 0.925$).
Limitations, reason for caution: Power calculation was based on preliminary data from an earlier pilot study on donation cycles which can introduce bias. Further RCT studies are needed to confirm our results.
Wider implications of the findings: Administration of a double GnRH antagonist dose (0,25 mg/12 h) the day before hCG trigger in conjunction to tapering the rFSH dose in patients at risk for OHSS undergoing an IVF-ICSI antagonist cycle, may represent a safe and effective alternative preventive strategy for early OHSS without compromising the reproductive outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IAKENTRO Fertility Centre.
Trial registration number: ISRCTN02750360.
Keywords: GnRH antagonists, OHSS, IVF-ICSI

P-577 The incidence of monozygotic twinning in ICSI patients following single cleavage stage or single blastocyst stage transfers

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Study question: To determine the incidence of monozygotic twinning (MZT) after single blastocyst stage transfer (SBT) compared with single cleavage stage embryo transfer (SET) in fresh and frozen ICSI cycles.
Summary answer: The incidence of MZT after single blastocyst stage transfer (SBT) is comparable with single cleavage stage embryo transfer (SET) in fresh and cryopreserved ICSI cycles.
What is known already: The primary risk factor for a dizygotic multiple birth outcome is the transfer of more than one embryo. The risk factors for monozygotic twinning are less clear. The division of the embryo at an early stage of development is estimated to occur in 0.6% of all births. Several factors have been suggested to be implicated in the splitting of the embryo with ART, including the use of ICSI, cryopreservation and extended embryo culture.
Study design, size, duration: Retrospective study between July 1st 2010 and October 1st 2014 of 2707 fresh transfers irrespective of patient characteristics (908 SBT and 1799 SET) and 1791 cryopreserved transfers (1581 SBT (vitrification) and 210 SET (freezing)). The incidence of MZT in ICSI patients following fresh or cryopreserved SET or SBT was analyzed.
Participants/materials, setting, methods: Pregnancy was defined as a viable intrauterine pregnancy (fetal heart observed) on transvaginal ultrasound between gestational weeks 6 and 7. Monozygotic twinning was identified when the number of fetal heart beats was more than one. Outcome parameters were compared using c^2 with a 5% significance level.
Main results and the role of chance: A total of 831 fresh transfer cycles (492 SBT and 339 SET) resulting in a pregnancy were identified during the study period. Ongoing pregnancy rate per transfer was significantly lower for SET cycles as compared to SBT cycles ((18.5% (333/1799) versus 23.0% (209/908)) ($p = 0.006$). MZT rate was similar after fresh SBT (0.6% (2/339)) as compared to fresh SET (1.8% (9/492)). A total of 556 frozen transfer cycles (503 SBT and 53 SET) resulting in a pregnancy were identified during the study period. Ongoing pregnancy rate per transfer was significantly lower for frozen SET cycles as compared to vitrified SBT cycles ((13.8% (29/210) versus 19.6% (310/1581)) ($p = 0.0486$). MZT rate was similar after vitrified SBT (0.6% (8/503)) as compared to frozen SET (0% (0/53)).
Limitations, reason for caution: This study is limited by its retrospective design. No correction for confounding factors such as maternal age and embryo quality has been made.
Wider implications of the findings: Our results do not support the published data in which blastocyst transfers are correlated with an increase in the incidence of twinning. Blastocysts transfers continue to offer the advantages of enhanced pregnancy rates without compromising birth outcomes in terms of monozygotic twinning.
Study funding/competing interest(s): Funding by University(ies) – Ghent University Hospital.
Trial registration number: NA.
Keywords: monozygotic, twinning, blastocyst transfer

P-578 Should a single frozen embryo transfer policy be universally applied? A retrospective analysis on 605 frozen blastocyst transfer cycles

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Study question: The aim of this study was to compare the implantation, miscarriage, clinical pregnancy, live birth and multiple pregnancy rates on single frozen embryo transfer (SFET) and double frozen embryo transfer (DFET) cycles after thawing and transferring day 5 embryos.
Summary answer: Although the positive pregnancy rates are statistically significant in favour of the DFET group, the live birth rates are comparable. The patients in the group of DFET are also exposed to significantly higher miscarriage and multiple pregnancy rates. The group of SFET has higher implantation rates.
What is known already: Well-established concerns have been expressed regarding the complications of multiple pregnancy in ART. Consequently a single embryo transfer policy has been applied in UK for patients less than 37 years old. The data on frozen embryo replacement cycles are limited and only few studies have compared SFET with DFET. As a result there is no clear consensus on the number of embryos that should be transferred on frozen cycles.
Study design, size, duration: A retrospective analysis of al frozen embryo replacement cycles between January 2011 and April 2014 was performed. Only cycles with frozen vitrified embryos on day 5 were included. Double and single embryo transfer cycles were compared for patient characteristics and treatment outcomes.
Participants/materials, setting, methods: 511 patients underwent 605 frozen embryo transfer cycles after thawing and transferring vitrified blastocyst(s) at day 5. All blastocysts were 2BB or better at the time of vitrification. All patients with vitrified blastocyst have been included regardless of age, number of previous cycles or type of infertility.
Main results and the role of chance: No difference was observed in age (35.5 ± 4.1 vs 35.8 ± 4.3, $p = 0.437$), number of cycles (2.42 ± 0.9 vs 2.57 ± 1.2, p-value 0.11) or cause of infertility. Pregnancy rates were significantly higher in the DFET group (49.3% vs 60.9% OR: 1.6, 95% CI 1.14–2.24, p-value = 0.006). However, no statistically significant difference was observed in clinical pregnancy (34.6% vs 40.9%, OR: 1.3, 95% CI 0.92–1.85, $p = 0.13$) and live birth rates (30.8% vs 34%, OR: 1.16, 95% CI 0.81–1.66, $p = 0.42$). The implantation rate was significantly higher in the SFET group (35.5% vs 27.1%, OR: 0.67, 95% CI 0.49–0.93, $p = 0.017$). The miscarriage (17.5% vs 26.9%, OR: 1.73, 95% CI 1.14–2.63, $p = 0.01$) and multiple pregnancy rate (1.5% vs 28.4%, OR: 24.68, 95% CI 3.3–184.3, $p < 0.001$) were significantly higher in the DFET group.
Limitations, reason for caution: This is retrospective analysis of embryos frozen at the blastocyst stage. Embryos frozen at cleavage stage have not been included. The selection of the embryos transferred was based on the number of available frozen embryos, preference of the couple, survival of embryos after thawing and number of previous cycles.
Wider implications of the findings: Our study has not identified a difference in live birth rates between SFET and DFET. However, patients with DFET had higher miscarriage and multiple pregnancy rates and lower implantation rates. The single embryo transfer policy has been applied in the UK for fresh IVF cycles. Our results suggest that this policy may justifiably be applied to frozen embryo transfer cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Hammersmith.
Trial registration number: NA.
Keywords: frozen embryo transfer cycle, single embryo transfer, blastocyst, vitrification

P-579 Identifying predictive factors for twin pregnancy after double frozen-thawed blastocysts transfer: analysis of 4000 frozen IVF cycles

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Study question: What are the predictive factors for twin pregnancy after receiving double frozen-thawed blastocysts transfer (DBT)?
Summary answer: The number of previous fresh cycles undergone and number of embryos frozen in the fresh cycle were independent factors affecting the risk of multiple pregnancy after DBT.
What is known already: Maternal age, the transferred embryos' developmental stage and the number of embryos transferred have been identified as key predictors of twin pregnancy in women receiving fresh embryo IVF. However, no such predictive factors have been identified in frozen IVF cycles.
Study design, size, duration: Prospectively, data on all fresh and frozen IVF cycles carried out between January 2006 and June 2014 was included. Data involving 2550 frozen-thawed blastocyst transfers were analysed, of which 1060 cycles involved the transfer of two frozen-thawed blastocysts.
Participants/materials, setting, methods: Data of women who had undergone at least one frozen embryo transfer cycle, since 2006, at a London-based centre were examined. DBT cycles with singleton or twin pregnancy were analysed using a multivariate logistic regression model, to elucidate any predictive factors of twin pregnancies.
Main results and the role of chance: An overall clinical pregnancy rate of 43% and twin pregnancy rate of 26% was observed in the 1060 DBT cycles. The number of previous fresh cycles undergone ($p = 0.03$) and number of embryos frozen during the fresh cycle ($p = 0.03$) were independent factors affecting the risk of multiple pregnancy. For every increase in the order of fresh cycle by 1, there is a reduction in the risk of multiple pregnancy by 36% (adjusted risk ratio = 0.64, 95% CI 0.42–0.97). For every increase in the number of blastocysts frozen at the time of the fresh cycle by 1, the risk of multiple pregnancy increases by 11% (adjusted risk ratio = 1.11, 95% CI 1.01–1.22). Patient age and post-thaw blastocyst re-expansion were not predictive factors of multiple pregnancy.

Limitations, reason for caution: This is a single centre study examining the risk of multiple pregnancy after frozen-thawed double blastocyst transfer. A slow-freezing technique was used for blastocyst cryopreservation.
Wider implications of the findings: This study has identified independent predictors of multiple pregnancies, following DBT in frozen IVF cycles. Future practices could potentially use these predictive factors as tools to recognise women with increased risk of multiple pregnancy, where single embryo transfer may be more suited.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Not applicable.
Trial registration number: NA.
Keywords: IVF, frozen blastocysts, DBT, predictors, twin pregnancy

P-580 Does ovarian stimulation affect embryo implantation and perinatal outcomes more in fresh IVF transfer than in frozen embryo transfer cycles?

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Study question: Does ovarian stimulation in *in vitro* fertilisation (IVF) treatment affect embryo implantation and perinatal outcomes such as preterm birth (PTB) and low birth weight (LBW) following fresh embryo transfer cycles.
Summary answer: There was no significant difference in the implantation rate (IR) and adverse perinatal outcomes of PTB, early PTB, LBW, very LBW with fresh transfer versus frozen embryo transfer cycles following stimulated IVF treatment.
What is known already: Ovarian stimulation is associated with supra physiological steroid levels which subject the endometrium to an altered endocrinological environment. Whether ovarian stimulation is detrimental to embryo implantation and subsequent perinatal outcomes through the effects on the endometrium is debatable with suggestions for avoiding embryo transfer in a fresh IVF cycle in favour of cycle segmentation with freezing of embryos and subsequent replacement either in a natural cycle or following artificial endometrial preparation in order to improve IVF outcomes.
Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of assisted reproduction treatment (ART) in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Data from 1991 to 2012 involving 78,761 singleton live births following stimulated IVF cycles with fresh embryo transfer and 349 singleton live births following frozen embryo transfer which did not have a fresh transfer in the stimulation cycle were analysed.
Participants/materials, setting, methods: Data on all women undergoing either a stimulated fresh IVF transfer cycle or a stimulated cycle with subsequent frozen embryo replacement during the period from 1991 to 2012 were analysed to compare implantation rates (IRs) and adverse perinatal outcomes of PTB, early PTB, LBW and very LBW. IR is defined as the proportion of transferred embryos that resulted in clinical pregnancies. Occurrence of a live birth at <37 weeks gestation is defined as a PTB and <32 weeks gestation as early PTB. Birth weight <2500 g is defined as LBW and <1500 g as very LBW. Perinatal outcomes were analysed for singleton live births only, defined as a singleton live birth event in which the baby is born alive. Logistic regression analysis was performed adjusting for female age, year of treatment, previous IVF cycles, previous live birth, number of oocytes and day of embryo transfer (cleavage or blastocyst stage).
Main results and the role of chance: There was no significant difference in the IR between fresh versus frozen embryo transfer cycles: adjusted odds ratio (a OR) 1.18; 95% confidence interval (CI) 0.97–1.46. There was no significant difference in the risk of PTB: a OR 0.75; 95% CI 0.44, 1.26, early PTB: a OR 0.56; 95% CI 0.27, 1.19, LBW: a OR 0.81; 95% CI 0.47, 1.38 and very LBW: a OR 0.49; 95% CI 0.19–1.23 following fresh versus frozen transfer cycles.
Limitations, reason for caution: In addition to the limitation of being an observational study, other limitations included the relatively smaller number of singleton live births in the frozen embryo transfer cycles and the inability to determine the cryopreservation method (slow freezing versus vitrification).
Wider implications of the findings: Analysis of this extensive dataset showed no detriment to embryo implantation and no increase in adverse perinatal outcomes following fresh versus frozen transfer in stimulated IVF cycles. These findings question the rationale for deferring fresh transfer and advocating frozen embryo transfer for all stimulated IVF cycles which needs addressing in well-designed randomised controlled trials. Furthermore, it would be also be of interest to query whether ovarian stimulation itself through its effects on the oocyte is associated with adverse perinatal outcomes by comparing stimulated versus natural IVF cycles.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding was sought/granted.
Trial registration number: NA.
Keywords: IVF, fresh embryo transfer, frozen embryo transfer, perinatal outcome, implantation

P-581 The effects of two different embryo culture media on the birthweight of singletons: findings after fresh and frozen-thawed embryo transfer

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Study question: Does the type of media for embryo culture result in different birthweight of singleton newborns?
Summary answer: No difference was found between the newborn birthweight of the embryos cultured in SAGE and Vitrolife media after fresh or frozen-thawed cleavage embryo transfer.
What is known already: Many studies have shown that embryo culture media used in IVF treatment may affect fetal growth and thus the birthweight of newborns. However, it is still a controversial topic. To further explore this issue, we compared the birthweight of singleton neonates born after the transfer of fresh and frozen-thawed cleavage embryos respectively cultured in the two most widely used culture media: SAGE and Vitrolife.
Study design, size, duration: A retrospective analysis was performed for 2370 singleton neonates born after IVF/ICSI cycles between August 2009 and December 2012. A comparison on the difference of birthweight was made between two different culture media, SAGE and Vitrolife.
Participants/materials, setting, methods: Patients who received blastocyst transfer, preimplantation genetic diagnosis (PGD) and donor oocytes were excluded. Only data from singletons born alive after the 28th week of gestation were included in the data analysis.
Main results and the role of chance: The newborns were divided into two groups, including 1755 cases from fresh cleavage embryo transfer and 615 from frozen-thawed cleavage embryo transfer. No differences were observed in both the absolute birthweight and the birthweight adjusted for gestational age and gender (*z*-score) of the newborns between the two culture media in each group. The sex ratio, rate of small for gestational age (SGA) and large for gestational age (LGA), as well as the rate of low birth weight (LBW) and macrosomia were all comparable between the two media in both fresh and frozen-thawed groups. Multiple linear regression analysis demonstrated that maternal weight, gestational age, frozen-thawed embryo transfer and infant gender were significantly related to neonatal birthweight.
Limitations, reason for caution: The current study is a retrospective study which is not well controlled. Moreover, in addition to gestational age and birthweight of the neonates, more research needs to be performed to evaluate the long-term effects of embryo culture medium on the health of children conceived through ART.
Wider implications of the findings: Our retrospective study demonstrated that embryo culture medium had no influence on neonatal birthweight. Further study will be required to understand its potential epigenetic changes, as well as its variation in fresh and frozen-thawed embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s). The study was funded by the Ministry of Health public welfare scientific research special fund (201402004). The authors have no conflicts of interest to declare.
Trial registration number: NA.
Keywords: neonatal birthweight, culture media, IVF/ICSI

P-582 Patients' attitudes and expectations towards frozen embryo transfer (FET) and a "freeze all"-strategy in assisted reproductive technology (ART)

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Study question: What are the attitudes towards different aspects of frozen embryo transfer (FET) and a "freeze all"-strategy in assisted reproductive technology (ART) amongst Danish fertility patients before their first cycle of *in vitro* fertilization (IVF) therapy?
Summary answer: Though the patients were concerned about the treatment delay associated with elective FET (eFET) compared with fresh embryo transfer, nearly half of the participants were in favour of eFET assuming that the clinical pregnancy rate was equivalent. The majority also indicated health of mother and child as of primary importance.
What is known already: Vitrification and blastocyst transfer has considerably improved success rates after FET and a meta-analysis has shown similar results after fresh transfer and eFET. Furthermore, the risk of ovarian hyperstimulation syndrome is eliminated in FET cycles, and FET may be beneficial to the foetus. However, the "freeze-all"-strategy is not yet implemented as standard care. One reason is the presumption of negative patient attitudes towards "freeze-all." No published data regarding patients' attitudes on a "freeze-all"-strategy exists.
Study design, size, duration: The study was designed as a cross-sectional survey including 60 fertility patients referred for their first IVF treatment during the period December 2014 to May 2015.
Participants/materials, setting, methods: All newly referred patients participating in the introductory meeting before initiating IVF-treatment at the Fertility clinic, Hvidovre Hospital, Copenhagen, Denmark were requested to fill in an online web-based questionnaire separately for men and women covering attitudes towards FET and a "freeze all" strategy, socio-demographic data, and reproductive history.
Main results and the role of chance: The overall response rate was 62% (n = 60). 69% of the females and 52% of the males responded. Of the respondents, 59% of the women and 30% of the men would choose eFET over fresh embryo transfer assuming that the probability of clinical pregnancy was the same. However, 42% of the women and 23% of the men completely agreed that they would find it difficult to postpone the embryo transfer to the subsequent natural cycle. The primary concern for both male and female participants was minimizing the risks to mother and child, as both men and women strongly agreed that whatever treatment posed the least threat to the health of the mother and the child was preferable.
Limitations, reason for caution: Selection bias cannot be excluded, as the non-response rate was 38%. The hypothetical nature of the items may limit the validity of the results. The participants were from the Capital Region of Denmark and may therefore not be representative for fertility patients in the entire country.
Wider implications of the findings: In a clinical setting with similar pregnancy rates for FET and fresh embryo transfer, these results indicate that patients, when given access to objective information on advantages and disadvantages of both fresh embryo transfer and FET, may be less prone to opt for fresh embryo transfer. This may be ground breaking for a patient-centred paradigm shift in routine IVF with a wider implementation of FET and a "freeze all"-strategy.
Study funding/competing interest(s): Funding by hospital/clinic(s). The study was supported by Hvidovre Hospital. No competing interest exists.
Trial registration number: NA.
Keywords: ART, FET, freeze-all, attitudes

P-583 Increased preeclampsia in pregnancies from double donation of gametes compared to oocyte donation only

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Study question: Is there an increased risk of preeclampsia (PE) and/or gestational hypertension (GH) in pregnancies with double donation (both semen and oocytes, DD), compared to those achieved with donation of oocyte only (OD)?
Summary answer: There was a significant increase in incidence of PE when both gametes were donated, in comparison to pregnancies where only the oocyte was donated. The patient cohorts were matched by age, parity and number of fetus in the current pregnancy.
What is known already: PE, characterized by reduced placental perfusion secondary to defective trophoblastic invasion, complicates 2–7% of pregnancies. The trophoblastic invasion is related to uterine natural killer (uNK) activity, in turn regulated by the plug of trophoblastic (fetal) HLA-C and uNK ligands. The HLA alleles expressed by the conceptus are allogenic in DD pregnancies (the mother has never been in contact with sperm or oocyte) or partially allogenic in OD, perhaps leading to higher risk of placenta maldevelopment.
Study design, size, duration: This is a retrospective analysis of 57 pregnancies reaching at least week 23 of gestation, and achieved by either DD (n = 19) or OD (n = 38). Pregnancy outcomes, PE, and GH were recorded by a survey sent to patients between September 2014 and January 2015.
Participants/materials, setting, methods: DD and OD pregnancies were matched (1:2) by age, number of fetus in the current pregnancy, and previous pregnancies in the patients. The incidence of PE and GH was compared in both cohorts. Univariate analysis was employed; differences were compared by Fisher's exact test.
Main results and the role of chance: DD and OD cohorts were similar in demographic variables and cycle characteristics. Mean patient age was 44.6 ± 3.4 and, BMI was 23.2 ± 4.4. Thirty-one percent of patients had previous pregnancies. The average day of embryo transfer was 2.6 ± 0.8 and the number of embryos transferred 2.0 ± 0.3. The majority of embryos transfers (84.2%) were fresh (81.6% of the DD and 89.5% of the OD). The incidence of PE was significantly higher in DD than in OD (21% vs 2.6%, p = 0.038), while no difference was found in GH rate (21.0% vs 13.1%, p = 0.48). Significantly more pregnancies progressed from GH to PE in DD than OD only (100% vs 20%, p = 0.048).
Limitations, reason for caution: The main limitation of this study is the small number of cases reported. Although careful pairing for GH/PE risk factors was performed, some confounding effect may still persist.
Wider implications of the findings: Pregnancies achieved by DD might be at a higher risk of PE than OD pregnancies only. This implicates an additive effect of semen without previous immunological contact with the woman in setting the risk for PE. Physicians should be aware of this possible risk in order to both counsel patients and monitor pregnancies accordingly.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA.
Keywords: preeclampsia, hypertension, oocyte donation, double gamete donation

P-584 Blastocyst grade does not affect birthweight in ART patients

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Study question: Is blastocyst morphology grade associated with birthweight of children born after ART?
Summary answer: There was no difference in the average birthweight and the rate of low birthweight across Inner Cell Mass (ICM) and Trophectoderm (TE) classification grades. However, for TE of grade C, the birthweight compared to TE grade A and B was lower and the rate of low birthweight tended to increase.
What is known already: Previously we have reported that in frozen-thawed embryo transfer cycles, the grade of TE is statistically significantly related to the rate of ongoing pregnancy and miscarriage after adjusting for confounders (Honnma et al., 2012). However, there is still scant information and no consensus on whether blastocyst grade affects fetal growth.
Study design, size, duration: A retrospective analysis of children born after frozen-thawed single blastocyst transfers (n = 800) conceived after treatment in one clinic between January 2010 and January 2013.
Participants/materials, setting, methods: The blastocysts were graded according to the Gardner grading system. ICM and TE grades were compared

with the average birthweight of children as well as the rates of low birthweight (<2500 g) for blastocysts of grades A, B, and C.
Main results and the role of chance: The average birthweight and the rates of low birthweight (%) for ICMs of grades A, B, and C were 3060.0 ± 494.5 g, 3064.7 ± 508.3 g, 2915.0 ± 31.0 g and 8.9%, 12.1%, 0% respectively. The average birthweight and the rates of low birthweight (%) for TE of grades A, B, and C were 3060.8 ± 506.9 g, 3065.2 ± 485.9 g, 2942.9 ± 549.7 g and 9.3%, 10.1%, 14.3% respectively. Overall, there was no significant difference in the average birthweight and the rates of low birthweight across the range of ICM and TE grades. However, for TE grade C, the birthweight was decreased compared to TE grades A and B and the rates of low birthweight tended to increase.
Limitations, reason for caution: Retrospective analysis with a limited sample size.
Wider implications of the findings: These results suggest that, even low grade blastocysts, can result in healthy births of similar birth weights to high grade blastocysts.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: blastocyst, birthweight, ICM, TE

P-585 Neonatal outcomes after IMSI and ICSI at the CPMA of the University of Liège

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Study question: Data of IMSI (Intracytoplasmic Morphologically Selected sperm Injection) and ICSI (IntraCytoplasmic Sperm Injection) cycles were retrospectively collected in order to compare neonatal outcome, especially the rate of malformation.
Summary answer: A non-statistically significant lower malformation rate has been observed in the IMSI group in comparison to ICSI group, whereas other outcomes were similar (mean weight, height and duration of pregnancy).
What is known already: Since 2001, living spermatozoa can be observed at a high magnification (up to 10,000 times), allowing us to identify a particular sperm head defect called vacuole. They vary in size, position and depth, and large vacuoles (more than 4% of sperm head surface) have been intensely studied by several teams. While these large vacuoles seem to be associated to DNA defects, their effects on male fertility are still discussed.
Study design, size, duration: Data of the CPMA (Centre de Procréation Médicalement Assistée) of the University of Liège were retrospectively collected. Main analyzed data were pregnancy rate, duration of pregnancy, babies height and weight, presence of malformation or not. IMSI and ICSI cycles performed with fresh semen between 2009 and 2013 were included.
Participants/materials, setting, methods: IMSI group includes 266 cycles performed in 162 patients, and ICSI group 2003 cycles in 1211 patients. Data were collected from the CPMA database. In case of data lacking, patients were contacted by post and eventually gynecologist responsible for pregnancy follow up was called by phone.
Main results and the role of chance: Mean ages were similar in IMSI and ICSI groups (34.9 vs 34.5; NS: Not Significant), whereas attempt rank was higher in IMSI cycles (3.31 vs 1.96; $P < 0.0001$). Mean number of transferred embryos was higher in IMSI than in ICSI cycles (1.82 vs 1.54 embryos per transfer; $P < 0.0001$). Pregnancy rates (positive bhCG/embryo transfer) were similar (respectively 30.8% vs 28.9%; NS) as well as neonatal data, excepted major malformation rate which was slightly lower in IMSI babies (1.75% vs 3.38%; NS). In IMSI only one malformation was described (vesico-ureteral reflux). In ICSI we reported 12 malformations (2 cardiac abnormalities, one anencephaly, one anal stenosis …), three of which required medical termination of pregnancy, and one ended in spontaneous in utero death (baby with multiple malformations including club-foot).
Limitations, reason for caution: It is clear that groups are two small to draw conclusions and that prospective randomized studies are needed to confirm these data which are consistent with previously published results (Cassuto et al., 2014). A wider multicentric retrospective study is already ongoing.
Wider implications of the findings: These data encourage us to screen IVF patients for the presence of vacuoles and to propose IMSI to couples presenting high rates of vacuolated spermatozoa. Our aim is to lower the malformation rate observed in ICSI cycles. However, more studies are needed to verify this hypothesis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Centre de Procréation Médicalement Assistée, University of Liège.
Trial registration number: IMSI babies: retrospective data.
Keywords: IMSI, malformation

P-586 Transuterine oocytes retrieval for IVF: a case-control study

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Study question: What is the incidence, risk factors, complications and cycle outcomes of vaginal oocytes retrieval requiring a transuterine approach.
Summary answer: A transuterine approach was required in 2.3% of oocyte retrievals (OCR) in our study. Higher body mass index (BMI) was the most significant risk factor for transuterine puncture. In our cohort, transuterine puncture during OCR did not increase complication rates nor affect cycle outcomes.
What is known already: The reported incidence of transuterine puncture during OCR is 1.7%–4.2%. However, there is a paucity of evidence regarding the relative safety and efficacy of this practice. Potential adverse effects of transuterine puncture during transvaginal oocytes retrieval for IVF are; needle blockage, infection, hematomas, and uterine contractions. Two previous studies have reported no effect on pregnancy rates and a non-significant trend towards higher miscarriage rates among OCRs involving transuterine puncture.
Study design, size, duration: We performed a retrospective, case-control study using an electronic database of all oocytes retrievals performed between December 2008 and October 2014. Cases were identified by documentation of transuterine puncture during OCR. The controls were matched by age and IVF treatment protocol.
Participants/materials, setting, methods: Medical records were analyzed for covariates including demographic characteristics, cause of infertility, treatment cycle and OCR details. Charts were reviewed for complications occurring up to 2 months post OCR. Hyperstimulation-related complications were excluded. Cycle outcomes included number of oocytes retrieved, pregnancy rate, implantation rate and clinical pregnancy rate.
Main results and the role of chance: Of 8023 oocyte retrievals performed, 186 cases involved transuterine puncture (2.3%). Compared to 186 matched controls, we found no significant difference in maternal age, baseline antral follicle count, previous OCR number, nor rates of endometriosis nor tubal factor. BMI was higher in the study group (26.9 ± 6.6 vs 24.7 ± 5.9, $p = 0.01$). The overall complication rate was 4.3% in both groups. Complications included urinary retention in the study group and infection in control group. Mild vaginal bleeding and abdominal pain were reported in both groups. No statistically significant differences were found in pregnancy rate (25.8% vs 28.5%), implantation rate (20.2% vs 27.6%) and clinical pregnancy rate (19.9% vs 22%) for study and control group, respectively. Prophylactic antibiotics during OCR did not reduce complications nor improve pregnancy outcomes.
Limitations, reason for caution: Reporting issues could result in underestimation of incidence and complications related to transuterine puncture. However, in our institution, proper documentation is routine.
Wider implications of the findings: There are no current published guidelines for the management of OCR by transuterine puncture. We found no difference in pregnancy rates and no increase in complication rates associated with transuterine puncture.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: transuterine, oocytes retrieval

P-587 Pregnancy outcomes in women with polycystic ovarian syndrome (PCOS) undergoing *in vitro* fertilization (IVF)

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Study question: Is PCOS an independent risk factor for increased pregnancy complications in singleton pregnancies following IVF+/- ICSI?
Summary answer: IVF pregnancies in the PCOS population are at higher risk for the development of specific pregnancy complications than those in women without PCOS, after adjusting for differences in maternal age, parity, body mass index (BMI) and time to conception.
What is known already: Meta-analyses have reported increased rates of specific pregnancy complications in the PCOS population. However, many of the included studies compared women with PCOS undergoing ART with spontaneous pregnancies in the general population. We undertook this study to assess whether PCOS contributes significantly to increased pregnancy complications when groups are matched for type of ART.
Study design, size, duration: Retrospective cohort study identifying all fresh IVF ± ICSI transfers with positive bhCG at a single tertiary reproductive endocrinology unit between December 2006 and 2012. Of the 1084 pregnancies identified, 394 resulted in singleton gestations. The other pregnancies identified had resulted in biochemical or early clinical losses, ectopic, or multiples.
Participants/materials, setting, methods: We compared complication rates among 394 singleton pregnancies (71 women with PCOS and 323 controls without PCOS), adjusting for differences in body mass index (BMI), age, and parity.
Main results and the role of chance: Women with PCOS demonstrated a significantly higher risk of developing the following pregnancy complications, after adjusting for differences in maternal age, parity, and BMI: gestational diabetes (OR 3.15, 95% CI 1.35–7.33), hypertensive disorders of pregnancy (OR 4.25, 95% CI 1.94–9.32), preterm birth <37 weeks (OR 2.30, 95% CI 1.07–4.97), and large for gestational age >90‰ (OR 2.77, 95% CI 1.21–6.35). Time to conception did not differ significantly between groups. There was no significant difference in rates of caesarian section or perinatal mortality. A significantly higher rate of ovarian hyperstimulation syndrome and biochemical pregnancies was seen among PCOS IVF pregnancies than controls.
Limitations, reason for caution: This was a retrospective review limited to a single tertiary centre, which may introduce bias. PCOS is a heterogeneous population. Although our sample size was insufficient to compare pregnancy outcomes across different phenotypes or BMI, we did include a representative variety of patients.
Wider implications of the findings: This study builds upon prior publications which have suggested increased rates of specific pregnancy complications in the PCOS population. Our results suggest that PCOS may be an independent risk factor for pregnancy complications, and this finding warrants further prospective evaluation. Women with PCOS merit close antenatal surveillance, and a better understanding of complication rates in these women is important for risk stratification and patient counseling.
Study funding/competing interest(s): Funding by University(ies) – University of Toronto, Department of Obstetrics and Gynecology.
Trial registration number: NA.
Keywords: PCOS, IVF, pregnancy complications, gestational diabetes, hypertensive disorders of pregnancy

P-588 Elective single embryo transfer (eSET) in United States IVF clinics

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Study question: What is the effect of elective single embryo transfer (eSET) rates on clinic-specific outcomes of *in vitro* fertilization (IVF) including the delivery rate and multiple birth rate?
Summary answer: Increasing rates of eSET to ≥40% of cycles was not associated with a significant decrease in clinic-specific delivery rate in women 37 years and younger and was associated with a linear reduction in the multiple birth rate after IVF for all ages.
What is known already: eSET is an effective means of reducing multiple birth rates after IVF. However, in prospective trials, eSET of cleavage stage embryos results in a reduction in the fresh cycle pregnancy rate. Relative to other countries, United States clinics perform eSET less frequently and utilize extended culture to the blastocyst stage more often. The effect of eSET rates on clinic-specific outcomes in centers performing higher rates of blastocyst transfer is not known.
Study design, size, duration: Retrospective analysis using multivariate linear regression to estimate adjusted means for clinic-level delivery and multiple birth rates according to eSET rates. Separate models estimated for all cycles and fresh cycles only, stratified by patient age and adjusted for clinic size, blastocyst transfer rate, and percent cryopreserved embryo transfer cycles.
Participants/materials, setting, methods: US Clinics performing ≥100 IVF cycles in 2012 using data reported to the Centers for Disease and Control (CDC) National ART Surveillance System. 337 clinics (74% of all US clinics) were included and were classified into 6 categories by eSET rates – <10%, 10–19%, 20–29%, 30–39%, 40–49% and ≥50%.
Main results and the role of chance: eSET rates varied considerably among clinics from 0 to greater than 60% with a mean rate of 10%. Delivery rates in fresh or all cycles did not vary substantially with increasing eSET averaging 38% delivery rate per transfer for all cycles. Women less than 35 and 35–37 years of age had equivalent delivery rates in clinics regardless of the eSET rate. In women 38 years and older, delivery rates decreased in clinics performing 40% eSET or greater. Factors directly associated with higher delivery rates included the rate of blastocyst embryo transfer and the rate of cryopreserved embryo transfer cycles. Multiple birth rates dropped in a linear fashion from 30% to 10% with increasing eSET rates from 0–9% to ≥40%.
Limitations, reason for caution: This is a retrospective cohort study comparing outcomes from individual clinics. The populations of patients treated may vary and could affect the decision to perform eSET and the outcomes of interest.
Wider implications of the findings: We found that clinics are able to perform higher rates of eSET than US national averages with no detrimental effect on delivery rates in fresh cycles or all cycles, especially for women ages 37 years and younger. Increasing utilization of eSET is associated with dramatic and linear reductions in multiple births after IVF. These results should reassure and encourage clinics to utilize eSET more often.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). University of Iowa Carver College of Medicine. United States Centers for Disease and Control (CDC).
Trial registration number: NA.
Keywords: *in vitro* fertilization, multiple gestation, pregnancy rate, single embryo transfer

P-589 Comparison of monozygotic twinning incidence following day 5 blastocyst vs. cleavage-stage single embryo transfer (SET) during IVF/ICSI cycles

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Study question: To compare the incidence of monozygotic twinning (MZT) in successful pregnancies following single day 5 blastocyst transfers vs. day 2–3 cleavage stage transfers.
Summary answer: There was no statistically significant difference in the incidence of MZT with blastocyst transfers in single embryo transfer (SET) cycles.
What is known already: The prevalence of MZT in the general population is 0.42%. Numerous studies indicate that MZT rates following ART procedures occur between 2 and 12 times higher than the natural incidence. The biological events responsible for MZT are not well understood, with various factors shown to be associated with the phenomena. These include techniques that include micromanipulation such as intra-cytoplasmic sperm injection (ICSI), as well as frozen embryo transfers (FET) and blastocyst transfers. Extended embryo culture to blastocysts, which has become increasingly popular, has been associated with an increased rate of monozygotic twins compared with cleavage-stage.
Study design, size, duration: This was a retrospective audit over a 5-year period, 2009–2013.
Participants/materials, setting, methods: All IVF/ICSI pregnancies following day 5 blastocyst or day 2/3 transfers, conceived in the study period, at Westmead Fertility Centre, a University IVF centre located in Western Sydney were included. Chi-Square or Fisher Exact tests as appropriate were used to identify associations between categorical variables.

Main results and the role of chance: By the 7 week ultrasound, comparing the incidence of MZT after blastocyst and cleavage stage transfers, there was no significant statistical difference shown. Out of 1504 SET pregnancies resulting from embryos transferred in the blastocyst stage, the total rate of monozygotic twins was found to be 1.33% (20/1504). 668 pregnancies following cleavage stage SET were included in this study; the rate of MZT in these embryos was lower, occurring at a rate of 0.75% (5/668). The incidence of MZT for the combined total (blastocyst and cleavage stage transfers) of pregnancies included in this study was found to be 1.15%. The majority of these pregnancies were derived from fresh embryo cycles (63.8%), the rest from frozen cycles (36.2%). The rate of MZT showed no significant difference between the fresh and frozen single embryo transfers, occurring at 1.08% and 1.27% respectively.
Limitations, reason for caution: as we see there is a small increase in the number of monozygotic twins in the blastocyst group, randomized trials are needed to answer this question. Due to retrospective nature of the study confounding factors like family history of twins could not be taken into account.
Wider implications of the findings: As our study did not show any statistically significant difference in the incidence of monozygotic twinning with blastocyst transfers, it is also noted that blastocyst transfers are understood to increase pregnancy rates; and this has to be taken into account when considering the most appropriate management for IVF patients and counselling women.
Study funding/competing interest(s): Funding by hospital/clinic(s) – westmead fertility centre(no funding required).
Trial registration number: Not required.
Keywords: monozygotic twinning, IVF, single embryo transfer, blastocyst, cleavage stage

P-590 Maternal and perinatal outcomes in oocyte donation pregnancies in Sweden 2003–2012

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Study question: What are the maternal and perinatal outcomes in singleton pregnancies conceived after oocyte donation (OD), as compared to *in vitro* fertilization (IVF) pregnancies with own oocytes and to pregnancies after spontaneous conception (SC)?
Summary answer: OD is associated with an increased risk for low birth weight (LBW) and preterm birth (PTB) and there is an increased risk for preeclampsia and postpartum haemorrhage (PPH) in singleton pregnancies conceived after OD, as compared to IVF with own oocytes and after SC.
What is known already: IVF as compared to spontaneous conception is associated with increased risk for pregnancy complications and adverse perinatal outcomes also in singleton pregnancies. Singleton pregnancies after OD have an increased risk for preeclampsia, PTB and LBW as compared to both pregnancies conceived after IVF with own oocytes and singletons conceived after SC. However, many studies are small and lack an appropriate control group.
Study design, size, duration: Population based cohort study performed in Sweden from 2003 to 2012 including data from all Swedish IVF-clinics. Comparison between all OD pregnancies and all IVF pregnancies and pregnancies in the general population. Only singleton pregnancies included. OD was not allowed in Sweden until 2003.
Participants/materials, setting, methods: 388 singletons conceived after OD, 27150 singletons conceived after IVF and 1090050 SC singletons. Main outcomes: PTB, LBW, small and large for gestational age (SGA, LGA), perinatal mortality, preeclampsia, placental abruption, placenta praevia, and post partum haemorrhage (PPH). Crude and adjusted odds ratios (AOR) with 95% confidence interval were calculated.
Main results and the role of chance: Singletons conceived after OD had significantly higher risks for PTB (<37, <32 weeks) as compared to IVF (AOR 1.8, 2.8, respectively) and SC (AOR 1.6, 2.1, respectively) and LBW (<2500 g, <1500 g) as compared to IVF (AOR 1.7, 2.9, respectively) and SC (AOR 1.5, 2.2 respectively). Singleton pregnancies after OD had a significantly increased risk for preeclampsia as compared to pregnancies after IVF (AOR 3.1) and SC pregnancies (AOR 2.9). Singleton pregnancies after OD had a significantly increased risk for PPH as compared to pregnancies after IVF (AOR 2.6) and SC pregnancies (AOR 2.9). The rate of LGA was significantly increased in singletons born after OD with frozen cycles as compared with fresh cycles, otherwise outcomes were similar in frozen and fresh OD cycles.
Limitations, reason for caution: In this observational study, it was only possible to adjust for maternal age, parity, year of birth, smoking, body mass index (BMI), years of involuntary childlessness, thus there may be residual confounders. Indications for OD were not available. For rare outcomes larger studies are needed.
Wider implications of the findings: The results were in agreement with the literature. The results are important for the increasing group of women with pregnancies after OD and especially for women of advanced maternal age. In Sweden only public clinics are allowed to perform OD and the maternal age limit is set to a maximum of 40–41 years. Caregivers should be aware of the increased risk of preeclampsia and adverse perinatal outcomes.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s). The study was supported by grants from the University of Gothenburg/Sahlgrenska University Hospital (LUA/ALF 70940).
Trial registration number: Not relevant.
Keywords: oocyte donation, maternal outcome, perinatal outcome

P-591 Volatile organic compounds (VOCs) that are persistently present inside and outside of the *In Vitro* Fertilization (IVF) laboratories. A 5 years evolution study

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Study question: Which are the main Volatile Organic Compounds (VOCs) that can be persistently found inside and outside the IVF laboratories and how could they have evolved in a period of 5 years?
Summary answer: Ambient air assessment in the IVF laboratory clean areas has been performed yearly to follow up the VOC concentrations surrounding the embryo environment, and at least 25 substances values have been registered per year, 6 of which exceeded 1% of the Occupational Limit Values (OLV) inside the IVF laboratories.
What is known already: Despite the efforts to clear the IVF laboratory's air through good quality management systems, including contamination preventive strategies and ambient air compounds presence controls, some of these substances may remain in harmful concentrations inside IVF laboratories, surrounding the embryos "*In vitro*" environment and affecting their development and health. The OLV aren't not sufficient because they were only designed to cover daily exposed workers without adverse effects, but not for cultured and largely unprotected cells.
Study design, size, duration: A descriptive retrospective analysis has been performed after the assessment of 54 VOCs inside the IVF laboratories over the past 5 years, since 2010–2014, by a specialized company who evaluated the VOCs concentrations.
Participants/materials, setting, methods: Environmental assessment was performed to determine the VOCs inside the IVF laboratories. Air was evaluated after a cleaning and disinfection protocol. Substances were obtained absorbing 6 L of air with an absorption pump (APEX lite). A total of 54 VOCs were simultaneously identified using the SPME (solid phase microextraction) technique.
Main results and the role of chance: Yearly ambient air assessment inside and outside the IVF laboratory registered 29 of the 54 VOCs evaluated. The most common VOCs found were Acetone, Ethyl Benzene, Limonene, Pinene, m, *p*-Xylene and Styrene. A total of 26 VOCs (89,6%) were inside the IVF in at least 2 years and also showed higher concentrations compared to the outside in at least 1 of the 5 years measured. Years 2012 and 2013 registered the higher amount of VOCs present in higher concentrations in IVF (19–21 respectively). 6 different substances exceeded the OLV 1% limit established; 4 of these (13,79%) were inside the IVF in 2010 (Hexachloro-1,3-butadiene, Tetrachloroethane) and 2012 (Acetic Acid, Tetrachloroethane, Vinyl Acetate). Nevertheless no substances were exceeded in 2011, 2013 or 2014.
Limitations, reason for caution: Specific quality standards and specific threshold levels at which contaminants cause harm to embryos have not been determined yet. For this reason it has been proposed as a quality control parameter the 1% VOCs limit for these IVF laboratories based on the literature as well as the laboratory experience**.**
Wider implications of the findings: Based on VOC measurement reports a new research has been designed in order to determine the accurate limits for some of the most common VOCs inside the IVF laboratory's air, relying upon the results obtained after exposing embryos to specific concentrations. With the

new thresholds, health regulatory bodies could be contacted looking forward to establish official OLV for IVF laboratories that will allow an acceptable embryo development.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Valenciano de Infertilidad (IVI Valencia).
Trial registration number: NA.
Keywords: IVF, laboratory, VOCs, quality

P-592 A randomized controlled trial of natural versus artificial cycle for frozen thawed embryo transfer

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Study question: Is modified natural cycle frozen thawed embryo transfer (FET) non inferior to artificial cycle FET with regard to clinical and ongoing pregnancy?
Summary answer: We found no difference in clinical and ongoing pregnancy rate between modified natural cycle FET and artificial cycle FET. Ongoing analyses considering live birth rates, cancellation rates and cost-efficiency will be presented.
What is known already: FET is of growing importance in assisted reproductive techniques. However the optimal preparation regimen remains unclear. One small RCT has indicated comparable clinical pregnancy rates between natural and artificial cycle FET. Previous systematic reviews have highlighted the need for further well powered randomised controlled trails to elucidate the optimal preparation protocol.
Study design, size, duration: Between February 2009 and April 2014 1032 patients were included in this multicentre, non-inferiority, randomised controlled trial. Patients were randomized between modified natural cycle FET or artificial cycle FET based on a 1:1 allocation using blocks of variable sizes. Stratification for initial treatment (IVF versus ICSI) was performed.
Participants/materials, setting, methods: Ovulatory patients, ages 18–40, undergoing FET after one of the first three IVF or IVF-ICSI treatment cycles were eligible for inclusion in this multicentre study. Patients undergoing FET with embryos derived from donor gametes were excluded.
Main results and the role of chance: Baseline characteristics showed no significant differences between the study groups except a longer duration of cryopreservation in patients undergoing artificial cycle FET. Clinical pregnancy developed in 94 out of the 500 patients undergoing natural cycle FET (18.8%) versus 75 out of the 469 patients undergoing artificial cycle FET (16.0%). Ongoing pregnancy was achieved in respectively 11.6% versus 9.2% of the cycles. Neither clinical pregnancy rate (OR 0.8, 95% CI 0.6–1.2, $p = 0.3$) nor ongoing pregnancy rate (OR 0.8, 95% CI 0.5–1.2, $p = 0.2$) differ significantly between natural cycle FET and artificial cycle FET. Embryo quality was the only significant confounding factor influencing both clinical and ongoing pregnancy rates.
Limitations, reason for caution: Prior to the start of the trial it was estimated that 1150 patients were needed to achieve adequate statistical power. This number was not achieved. Based on the current results a study of least double the size of the current one will be needed to rule out a type 1 error.
Wider implications of the findings: Since clinical and ongoing pregnancy rates don't differ between modified natural cycle FET and artificial cycle FET, other factors, such as cost-efficiency, clinical convenience and patients preference, should determine the choice of protocol. Data on these aspects will be presented.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Schering-Plough.
Trial registration number: NTR1586.
Keywords: frozen thawed embryo transfer, natural cycle FET, artificial cycle FET

P-593 Analysis of day 3 versus day 5 transfer data from the HFEA Database and a survey of eSET and blastocyst transfer policies in the UK

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Study question: What are the comparative outcomes of cleavage-stage transfer (CT) and blastocyst transfer (BT) according to the Human Fertilisation and Embryology Authority's (HFEA) database (2008–2012) and what are the current elective single embryo transfer (eSET) and BT policies of UK clinics?
Summary answer: BT showed a higher pregnancy and live birth rate, more male births and monozygotic twins. The predominant eSET policy of UK clinics was: patient's first cycle, age <37, ≥1 good blastocysts and surplus freezable embryos. For BT policies, the main criteria was ≥3 embryos available on day 3.
What is known already: Advances in assisted reproductive technology have led to a shift in practice from CT to BT with the aim of improving embryo selection and pregnancy rates. Multiple births have been decreasing due to the implementation of eSET policies.
Study design, size, duration: This is a retrospective cohort study using HFEA data of IVF and ICSI cycles performed between January 2008 and June 2012. The study also comprised a survey addressed to 74 IVF UK clinics.
Participants/materials, setting, methods: HFEA data from January 2008 to June 2012 from 78 clinics were analysed. Line, scatter, pie and bar charts and Wilcoxon tests (at 5% significance) were used to compare the outcomes of CT against BT. A survey addressed to UK IVF clinics ($n = 74$) assessed eSET and BT policies.
Main results and the role of chance: The use of BT rose from 12% in 2008 to 37% in 2012, with CT use decreasing from 88% in 2008 to 63% in 2012. Both overall and age-specific pregnancy and live birth rates were significantly higher for BT (p-value: <0.001). Multiple birth rates decreased for both CT and BT; in 2008, BT multiple birth rates were 28% whereas CT were 24%. By 2012 BT were 13% and CT were 17%. BT significantly favours males over females births (52:48). There was no significant difference between CT and BT regarding gestational age (singletons: 38 weeks; multiples: 35 weeks) or birth weight. The response rate of the survey was 53% (39/74). All responding clinics perform CT and BT, freeze embryos and have specific eSET and BT policies.
Limitations, reason for caution: The data requested from the HFEA were not fully provided for anonymity reasons and therefore some variables such as age-specific pregnancy and live birth rates could not be analysed fully.
Wider implications of the findings: BT is now available in all UK IVF clinics and its use has increased since 2008. All clinics have specific eSET and BT policies with the aim to reduce multiple birth rates.
Study funding/competing interest(s): Funding by University(ies) – University College London.
Trial registration number: NA.
Keywords: cleavage-stage transfer, blastocyst transfer, elective single embryo transfer, HFEA

P-594 Assisted reproduction causes placental maldevelopment and dysfunction linked to reduced fetal weight in mice

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Study question: Is reduced fetal weight associated with assisted reproduction derived from placental maldevelopment and dysfunction?
Summary answer: Yes, assisted reproduction resulted in placental overgrowth at embryonic day 18.5 (E18.5), which is associated with defects in placental layer segregation and perturbation of genomic imprinting in mice.
What is known already: *In vitro* fertilization and preimplantation embryo culture affected fetal and placental development and placental transport of amino acid and glucose during late gestation in mice.
Study design, size, duration: Blastocysts generated by assisted reproduction and *in vivo* were transferred to the uteri of pseudopregnant females. Conceptuses were collected at E14.5 or E18.5. Fetal weight, placental weight, placental structure, the expression of nutrient transporters and imprinted genes, the methylation of *H19* ICR, *KvDMR1* and *SNRPN* ICR were compared.
Participants/materials, setting, methods: Virgin 6- to 8-week-old CD1 female mice, adult CD1 males were used. Histological analyses were analyzed by standard histological and Periodic acid-Schiff staining. Gene expression was analyzed by real-time PCR. The Sequenom MassARRAY platform was used to perform the quantitative methylation analysis.
Main results and the role of chance: Assisted reproduction resulted in reduced fetal weight and placental overgrowth at E18.5, which is associated with aberrant placental morphology, most notably in the spongiotrophoblast

and labyrinth layers. Further, Assisted reproduction resulted in downregulation of most placental nutrient transporters and reduction in placental efficiency. Moreover, IVF and IVC increased the DNA methylation level of the imprinting control regions of *H19*, *KvDMR1* and *SNRPN* and disrupted the expression of imprinted genes in the placenta during mid-to-late gestation.
Limitations, reason for caution: The causal relationship between the aberrant placental maldevelopment and disrupted genomic imprinting is difficult to prove.
Wider implications of the findings: Our results from the mouse model show the first piece of evidence that ART treatment could affect fetal growth by disrupting *placentation* and placental function, suggests that perturbation of genomic imprinting resulted from embryo manipulation may contribute to these problems.
Study funding/competing interest(s): Funding by national/international organization(s). This work was supported by grants from the National Natural Science Foundation of China (81300531). The authors have no conflicts of interest to declare.
Trial registration number: It is a basic research, so have no trial registration number.
Keywords: ART, intrauterine growth restriction, placentation, metabolic syndrome, genomic imprinting

REPRODUCTIVE (EPI)GENETICS

P-595 Homozygous mutation in NTS12 associated with infertility in two brothers with "short tails" syndrome

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Study question: Can we identify a genetic defect in infertile patients with 'short tails' syndrome; a severe form of flagella pathology which causes sperm immobility and shows familial incidence?
Summary answer: We found a missense mutation (*p*.Arg1109Trp) in two infertile brothers issued from a consanguineous marriage in *NTS12* gene. The gene encodes a microtubule associated protein which allowed us to think that this mutation may disrupt the flagella building and may be responsible for infertility in the family studied.
What is known already: 'Short tails' syndrome refers to a condition of absence or severely reduced sperm motility associated to major alterations in the fibrous sheath and dysplastic development of the tail during spermatogenesis. To date the molecular default in this sperm defect is undefined.
Study design, size, duration: This is a case control study carried out on a Tunisian consanguineous family including two brothers described as having 'short tails' sperm defect associated with high percentage of head abnormalities and 13 other infertile patients presenting a similar phenotype. We included as a control group 100 fertile men.
Participants/materials, setting, methods: We used a combination of two methods; homozygosity mapping carried out on 10 patients including two consanguineous brothers and whole exome sequencing carried out on these two brothers. We screened the mutation found and other exonic mutations of the gene in other patients and fertile controls by Sanger sequencing.
Main results and the role of chance: Semen analysis shows akinetozoospermia associated with a total teratozoospermia with a predominance of short tails, microcephalic heads and abnormal acrosomes. Two brothers carried the same homozygous mutation (c.3245C > T, *p*.Arg1109Trp) in *NTS12* which encodes a protein that associates with the microtubule cytoskeleton and localizes at the centrosomes, mitotic spindle and intercellular bridge during cell division. The protein encoded is a potent microtubule-stabilizing protein whose depletion increases microtubule dynamics. Online available tools SIFT and PolyPhen predict that this variation severely affect the protein function. The residue in question (Arginine 1109) is located in a large coiled-coil region. Moreover, this mutation was absent in control group of fertile males and no variation was detected in other patients carried the 'short tails' syndrome.
Limitations, reason for caution: A limitation of this study is the low number of patients presenting with this rare form of male infertility.
Wider implications of the findings: In this study we have identified a first homozygous mutation associated to the 'short tails' syndrome. So far, we could not confirm the pathogenicity of the mutation but we suppose that it could be deleterious and may be responsible for infertility in this family.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: akinetozoospermia, short tails, missense mutation, NTS12

P-596 Digital chromatid counting in polar bodies achieves robust ploidy data of zygotes and improves pregnancy rates in IVF cycles of poor prognosis patients

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Study question: Does selection of euploid oocytes and exclusion of aneuploid oocytes improve pregnancy rates in patients of higher age and previous unsuccessful cycles?
Summary answer: Implantation rate in 56 cycles, average maternal age 37.5 years and 2.75 previous cycles was 38% thus demonstrating that polar body diagnosis with direct chromatid counting improves clinical pregnancy rates significantly. Data of ongoing pregnancies will be available.
What is known already: Aneuploidy increases with age.
Study design, size, duration: Method and study design: We have established a simple PCR method to count chromatids directly with DNA at limiting dilution and digital readout. After limiting dilution of polar body DNA into 8 PCR reaction wells (i.e., 0.25 and 0.125 genomes per aliquot in PB1 and PB2 respectively) 16 markers per chromosome for all chromosomes are amplified in a large PCR multiplex. Single marker analysis is then performed in the high throughput format 96.96. Array from Fluidigm, which allows to run 96 markers with 96 DNAs. Marker layout is such that the first round 96 markers are covering around 6 regions (2 markers per region) in the 8 most frequently aneuploid chromosomes 13, 15, 17, 18, 19, 21, 22, X. A significant number of zygotes (at least 50%) can be diagnosed as aneuploid at that stage and are excluded from further analysis. All zygotes, euploid for these 8 chromosomes, undergo a second round of single marker PCR to analyse the 8 next most aneuploid chromosomes. The few remaining zygotes are analysed with the remaining 7 chromosomes and chromosome Y as a control.
Participants/materials, setting, methods: Polar bodies.
Main results and the role of chance: Main results: From 79 cycles (average maternal age 37.5 years and 2.75 previous unsuccessful cycles), 566 zygotes were analysed and results were obtained for 553 (98%): 165 (29%) zygotes were euploid and 388 (69%) were aneuploid. In 15 cycles all zygotes were aneuploid and in 8 cycles blastocysts were cryopreserved. From the remaining 56 cycles with 1–2 transferred embryos implantation rate was 38% (21 pregnancies). Information about ongoing pregnancies will be obtained in the coming month.
Limitations, reason for caution: Copy numbers in single cells can be counted in any cell type such as oocytes, blastomeres, fibroblast and lymphoblast.
Wider implications of the findings: Polar body analysis with digital PCR from DNA at limiting dilution proofs a direct and robust method with beneficial biological outcome in couples with poor prognosis. The method allows a strategy which is fast and reduces costs to the minimum necessary to identify euploid oocytes and exclude aneuploid oocytes.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – SH-Gen Research.
Trial registration number: No register number.
Keywords: polar bodies, aneuploidy screening, digital PCR

P-597 Karyomapping: a novel alternative approach to PGD

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Study question: Could Karyomapping be an alternative approach to conventional pre implantation genetic diagnosis (PGD)?
Summary answer: Karyomapping is a novel approach which could be offered to patients with an identified genetic disorder which is less time consuming and cost effective compared to conventional PGD. This is the largest case series of patients in literature undergoing karyomapping to detect monogenic disorders in complement to other chromosomal abnormalities.
What is known already: Conventional PGD is time consuming requiring the development of a specific test for a particular disorder or couple. Karyomapping is a novel technique, whereby chromosomal abnormalities and monogenic disorders can be diagnosed concurrently. Recently, the accuracy of karyomapping has been reported as high as 97.7% when compared to target haplotyping in PGD.
Study design, size, duration: A retrospective case series comprising of 55 patients from February 2014 to date. Patients with an identified genetic disorder were referred to the Centre of Reproductive and Genetic Health (CRGH) clinic by their genetic counsellor. A complete genetic work up was conducted for the couple and the affected family members.
Participants/materials, setting, methods: Patients underwent conventional IVF/ICSI treatment and cleavage/blastocyst biopsies were performed. Biopsied cells were lysed in order to release their genetic material. The DNA was then fragmented and hybridised to a HumanKaryomap-12v2.1 beadarray. After hybridisation and staining the beadarray was scanned and results interpreted using BlueFuse Multi software (Illumina).
Main results and the role of chance: 19 Patients with an autosomal dominant (including retinoblastoma, facioscapulohumeral and muscular dystrophy, huntingtons disease, paragangliomas, Marfans syndrome), 26 patients with an autosomal recessive (e.g: beta thalassemia, sickle cell anemia, cystic fibrosis, spinal muscular atrophy), 4 patients with X linked dominant (e.g: Hypophosphatemic rickets, Incontinentia pigmenti), 6 patients with X linked recessive conditions (Retinoschisis, Norrie disease, Adrenoleukodystrophy, Ectodermal dysplasia) attended the clinic for karyomapping. The average time taken for couples to commence IVF/ICSI treatment following genetic work up was approximately 6–8 weeks. The median age and range for the patients was 32.4 (24.8–40) years. 17 patients completed ovarian stimulation, oocyte retrieval and had their embryos biopsied. Nine patients underwent a transfer of an unaffected embryo. Four patients have an ongoing clinical pregnancy. The remaining patients are awaiting a frozen embryo transfer.
Limitations, reason for caution: Absence of DNA from a family member with the known genetic condition can limit the application of karyomapping. Karyomapping does not detect new mutations and also does not detect post-zygotic chromosome duplication nor copy number variation. Hence larger studies reporting the clinical outcomes following karyomapping are essential.
Wider implications of the findings: Couples referred for PGD treatment have to wait long periods of time for development of an a-priori test before they can proceed for treatment. This wait could impede their fertility potential and also increase anxiety for couples. Conventional PGD only detects a single gene mutation in the embryo without analysing the entire genome component. Couples with multiple coexisting chromosomal abnormalities could be offered Karyomapping with whole genome sequencing rather than conventional PGD.
Study funding/competing interest(s): Funding by hospital/clinic(s) – no external funding.
Trial registration number: NA.
Keywords: karyomapping, PGD, IVF

P-598 The frequency of aneuploidy status of day 5 and day 6 blastocysts assessed by next-generation sequencing technology (NGS) application

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Study question: To assess correlation between day 5 (D5) and day 6 (D6) blastocyst morphology and ploidy status.
Summary answer: Slower developing blastocysts cryopreserved on day 6 but at the same stage of development as those developing to the blastocyst stage on day 5 do not have similar chromosomal status and therefore provide a lower chance of achieving pregnancy.
What is known already: Embryos with the highest morphological scores showed a higher euploidy rate when compared with lower quality embryos. During IVF cycles, the embryonic cohort is asynchronous in development and trophectoderm biopsy can equally be performed on D5 or D6 post-fertilization and on blastocysts of different morphological quality. It is still unknown whether blastocyst morphology and developmental rate relate to the embryo chromosomal constitution.
Study design, size, duration: Retrospective study including the data analysis of 61 blastocysts stage PGS cycles (median age 38 years, range 34–40) performed between August 2013 and November 2014 at INVICTA Fertility Centre, Poland. A total of 168 blastocysts were evaluated with the NGS protocol.
Participants/materials, setting, methods: All embryos were cultured to blastocyst stage. Trophectoderm biopsy was performed on D5 (n = 104) of development or, for slower growing embryos, on D6 (n = 64). The method involved whole genome amplification before 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: 49 (47.1%) D5 embryos were euploid, 55 (52.9%) aneuploid. In D6 group, 18 (28.1%) were euploid, 46 (71.9%) aneuploid. Difference in aneuploidy rates between D5 and D6 was statistically significant (p = 0.01). We observed no differences according to blastocyst morphology. There were 69 (80.2%) good quality embryos (>3AA score) on D5, 46 (71.9%) on D6. The euploidy/aneuploidy rates did not vary significantly between good and poor morphology embryos. For D5, the euploidy/aneuploidy rates were 49.3%/50.7% for good and 47.1%/52.9% for poor quality embryos, while for D6 the rates were 28.3%/71.7% (good) and 27.8%/72.2% (poor). Single frozen embryo transfers of 50 euploid blastocysts (D5–39, D6–11) were performed. 18 (46.2%) D5 and 3 (27.3%) D6 clinical pregnancies were confirmed. Implantation rate was 33.3% for D5, 9.1% for D6.
Limitations, reason for caution: The study is limited by sample size. A higher sample size or a prospective randomized design could be used in future studies to corroborate the current findings.
Wider implications of the findings: This study provides insight into determining ploidy status depending on the day of blastocyst biopsy. Slower developing blastocysts cryopreserved on day 6 but at the same stage of development as those developing to the blastocyst stage on day 5 do not have similar chromosomal status. Euploid embryos tend to show faster progression to the most advanced expansion stages when compared with aneuploid embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s) – INVICTA Fertility and Reproductive Center.
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, trophectoderm biopsy, next-generation sequencing, NGS

P-599 Age and ovarian reserve affect the non-coding transcriptome of human oocytes

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Study question: What is the non-coding RNA (ncRNA) transcriptome of MII oocytes matured *in vivo* from healthy women, and how is it affected by variations in age and ovarian reserve?
Summary answer: We identify a substantial expression of ncRNA in human MII oocytes; both woman age and ovarian reserve accounts for significant variations in the ncRNA transcriptome.
What is known already: Female age is crucial in determining oocyte developmental competence, as is ovarian reserve. Oocytes store large quantities of mRNA involved in early developmental events. Studies in several animal models indicate that non-coding RNAs (ncRNA), which regulate cellular processes through transcription and translation modulation, have an essential role in maternal to zygote transition, as well as germline specification and maintenance. Despite their central role in development, no characterization of ncRNA in human oocytes is available.
Study design, size, duration: This study included 36 mature (MII) oocytes from 30 oocyte donors; 9 oocytes in each of the following groups: (a) »22 year-old

and >20 AFC; (b) »33 year-old and >20 AFC; (c) »22 year-old and <10 AFC; (d) »33 year-old and <10 AFC. All stimulations were triggered with GnRH agonist.
Participants/materials, setting, methods: Total RNA was purified from individual MII and WTA2 cDNA libraries prepared; 10 mg cDNA was fragmented and biotinylated; 36 Affymetrix GeneChip HTA 2.0 arrays were hybridized, washed, stained, and scanned. Partek Genomics Suite was used to identify differentially expressed transcripts (fold change >1.5 or <-1.5; $p < 0.1$).
Main results and the role of chance: There were 43 differentially expressed NCT (non-coding transcripts) and 3 EST (expressed sequence tag) when comparing age independently of ovarian reserve, and 37 NCT and 7 EST when comparing ovarian reserve independently of age. There were 84 differentially expressed NCT including all classes of ncRNAs: 4 miRNA, 25 piRNA, 46 lncRNA, as well as 22 EST when comparing groups (a) and (d), 27.2% transcripts differences were maintained comparing groups (a) and (b); 27.7% of the differences observed where also maintained when comparing (c) and (d), 10.4% when comparing (a) and (c), and 61.8% when comparing (b) and (d). These results indicate that age is an important determinant of ncRNA abundance and that, in aged oocytes, ovarian reserve is a co-determinant of ncRNA variability.
Limitations, reason for caution: Since the MII included in this study were from young women, and ncRNA populations are understudied in human, no information can be assumed about older women. All donors received the same stimulation protocol, which could impact the array of ncRNA present in MII oocytes.
Wider implications of the findings: This study describes for the first time the ncRNA landscape of MII oocytes in the human species, providing insights into translational regulation of early embryogenesis and how this is affected by age and ovarian reserve. Moreover, we established a platform for the study of ncRNA as putative biomarkers of oocyte quality. As the oocytes analyzed were from fertile women, we can exclude an effect of genetic factor associated with infertility on the ncRNA landscape.
Study funding/competing interest(s): Funding by hospital/clinic(s).
Trial registration number: NA
Keywords: non coding RNA, transcriptome, oocyte, ovarian reserve, woman age

P-600 Medium-based noninvasive preimplantation genetic diagnosis for human a-thalassemias-SEA

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Study question: In this study, we tried to establish a novel and noninvasive medium-based testing for screening healthy embryo of patients with a-thalassemias^{-SEA} carriers who undergo *in vitro* fertilization (IVF) and preimplantation genetic diagnosis (PGD).
Summary answer: Medium-based a-thalassemias^{-SEA} detection could be a novel, quick and noninvasive method for patients with carriers who undergo PGD, while there is no significant difference in terms of diagnosis efficiency and allele drop-out (ADO) ratio between single cell based and medium-based a-thalassemias^{-SEA} detection.
What is known already: PGD is introduced for prenatal diagnosis service and blastomere biopsy at cleavage stage is mostly adopted IVF units. However, recently studies on animals suggested that blastomere biopsy may cause aberrant epigenetic modification, neurodegenerative disorders and ovary dysfunction in the offspring so that noninvasive testing method for PGD would be a favorable and urgently needed option.
Study design, size, duration: It is a diagnostic test between March and June 2014. 38 PGD cycles were performed, in which single cells biopsied from blastomeres and according spent culture media and blastocysts were collected. Blastocysts were used to verify. Another 148 media from 6 ICSI cycles were gathered.
Participants/materials, setting, methods: Among 413 Single cells that were subjected to fluorescent gap PCR analysis, 128 embryos that were diagnosed a-thalassemia SEA deletion were abandoned, and their according spent media and blastocysts were subjected to Q-PCR detection. 148 media from ICSI cycles were subjected to Q-PCR detection for quantification of cell-free DNA
Main results and the role of chance: 413 Single cells biopsied from blastomeres were turned out to be 108 normal embryos, 103 heterozygous, 128 a-thalassemia SEA deletion, 74 undetectable. The ratio of this DNA diagnosis efficiency was 82.1% (339/413). Altogether 112 out of 128 medium samples (87.5%) at day 6 was detectable, among which there were 34 heterozygous and 78 homozygous mutant. Blastocysts' verification shows 38 heterozygous and 90 homozygous mutant. 4 samples occurred ADO and its ratio was 10.5%. The *P* value of diagnosis efficiency is 0.175. Detectable ratio of medium collected at day 4 (D3–D4) after fertilization is 19.67% (12/61) with the concentration of 14.24 ± 4.76 pg/ml, which dramatically increased to 90.16% (55/61) with 48.78 ± 20.45 pg/ml at day 5 and 88.46% (23/26) with 54.35 ± 22.78 pg/ml at day 6.
Limitations, reason for caution: First of all, we are not sure to what extent that sample DNA collected from culture medium is representative of the embryo. Contamination may come from cumulus cells, thus washing off cumulus cells thoroughly is required. In addition, to prevent media contamination, individual Pasteur pipettes were used for each embryo.
Wider implications of the findings: First of all, medium-based detection could avoid lost of single cell cause of improper delivery of samples by using biopsy. Secondly, medium-based detection is more flexible and suitable for monitoring different stages of embryo development since cell-free DNA in medium is stable at different stage after D4 in comparison with blastocoelic fluid detection. Thirdly, the detection is simple and efficient. Transplantation of fresh healthy embryo could be achieved after 3 h medium-based detection.
Study funding/competing interest(s): Funding by national/international organization(s). National Natural Science Foundation of China (81370765); Guangdong Provincial Key Laboratory of Reproductive Medicine (2012A06140003) and Natural Science fund of Guangdong (S 2013040014613).
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, noninvasive, spent culture media

P-601 Ultra-PGD: preimplantation genetic diagnosis by karyomapping. Introduction to clinical genetics and results of first 136 cases

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Study question: Preimplantation Genetic Diagnosis (PGD) has helped thousands of couples avoid passing genetic disorders to their children. Established methods require a lengthy test building period, but new technology promises to revolutionize preimplantation genetics with a more rapid, higher-resolution test. Does Karyomapping provide reliable results in a shorter timeframe?
Summary answer: Karyomapping represents a major advance in preimplantation genetics. PGD tests can now be prepared within 4 weeks of receiving family DNA samples, as compared to many months previously and generate a wealth of data points for tracking the inheritance of parental genes with a high rate of success.
What is known already: Established methods for Preimplantation Genetic Diagnosis (PGD) use Short Tandem Repeats (STRs) to track heritable diseases. Several months are needed to design a custom test for diagnosing embryo biopsies. Using a microarray eliminates the need for a laborious custom test build. The ~300,000 Single Nucleotide Polymorphisms (SNPs) targeted by Illumina's Infinium HumanKaryomap-12 BeadChip can provide a richer dataset in less time than is achievable using the current STR method of PGD.
Study design, size, duration: Since it's introduction into our laboratory 136 couples seeking PGD had 938 embryos tested for 61 different genetic disorders with our "Ultra-PGD" Karyomapping protocol. Family DNAs were analyzed to ensure sufficient informative SNPs surrounded the genes of interest, followed by testing of the biopsies from day-5 frozen embryos.
Participants/materials, setting, methods: Family DNAs and amplified trophectoderm biopsies were analyzed using the Infinium HumanKaryomap-12 DNA Analysis Kit from Illumina. Beadchips were processed and scanned with an iScan array scanner. The data was processed with BlueFuse Multi software to determine inheritance.
Main results and the role of chance: We were able to determine the genetic status of 89% of the embryos tested using our "Ultra-PGD" Karyomapping protocol. This is lower than the 98% success rate for STR PGD tests on trophectoderm biopsies. Genetic status could not be determined for samples which did not amplify or failed quality-control metrics (7.7%). DNA contamination, typically maternal, accounted for an additional 1.7% of samples which could not be genotyped. DNA recombination at the disease locus also prevented genotyping of 1.9% of embryos. In all, 477 embryos (51%) were found to be

genetically unaffected. The likelihood of individual families having unaffected embryos is dependent on the number of embryos biopsied and the inheritance pattern of the disease.
Limitations, reason for caution: Karyomapping is a promising advance in PGD, but is not without limitations. The technology lacks the exceptional sensitivity of STR assays and is consequently more dependent on the quality of embryo biopsies. The poorer SNP calling found with single blastomere samples precluded the possibility of day-3 biopsies.
Wider implications of the findings: All families seeking PGD stand to benefit from a faster, lower cost and more informative test. Particularly, families who have been unable to use PGD due to limited informative STR markers have a greater chance of success with a Karyomapping PGD test. The shorter timeframe especially benefits families who are in need a faster test, such as for HLA matching for transplants.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Genesis Genetics.
Trial registration number: NA.
Keywords: PGD, karyomapping, genetics

P-602 High success rate of preimplantation genetic diagnosis in achieving healthy live-birth for very rare monogenic disorders

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Study question: What are the clinical outcomes of cycles performed for preimplantation genetic diagnosis (PGD) for very rare monogenic disorders and has the method been successful in terms of both diagnostic and clinical efficiency?
Summary answer: In this study we present our experience on clinical and technical aspects of PGD for 9 different very rare monogenetic disorders (prevalence ranging from only 7 cases reported in the literature to 1/10000) demonstrating that this technique is indeed a successful and effective prevention method for couples at risk of transmitting very rarely seen genetic disorders.
What is known already: PGD for monogenic disorders has become an effective alternative to prenatal diagnosis since its first application. Since then, it is estimated that PGD has been performed for over 350 monogenic conditions resulting in births of thousands of healthy children.
Study design, size, duration: This study consists of retrospective analysis of PGD cycles performed for very rare monogenic disorders between years 2003 and 2013. Seventeen cycles were performed for 9 couples referred for PGD with 9 rare monogenic disorders. All patients were counselled by an IVF specialist and a medical geneticist about the process of PGD and the possibilities of misdiagnosis and cancellation of embryo transfer.
Participants/materials, setting, methods: PGD set up procedure was performed initially on the parents' peripheral blood DNA samples in order to establish informative STR markers. PGD was performed on cleavage or blastocyst stage. Biopsied samples were analyzed with single-cell multiplex PCR techniques.
Main results and the role of chance: Seventeen IVF cycles were performed for PGD of 9 rare genetic diseases, which are Ehler-Danlos Syndrome Type VIIC, Co-enzyme Q deficiency, Lafora Disease, Leber's congenital amaurosis, Bartter Syndrome, Glazmann Thrombasthenia, Leukocyte Adhesion Deficiency Syndrome III, Fraser Syndrome, and Renal dysfunction-Cholestasis syndrome. Among them, Leber Congenital Amaurosis has the highest prevalence with 1/10000 and Co-enzyme Q deficiency (COQ2 gene) has the lowest prevalence with only 7 cases reported in the literature up to now. 142 embryos were biopsied and 89% of them were successfully diagnosed by PGD. In 16 cycles (94.7%) 1.6 embryos on average were transferred to the patients. Clinical pregnancy rate per embryo transfer (CPR) was 62.5% with an implantation rate (IR) of 48.1%. Live-birth rate per cycle, transfer and per patient were 41.2%, 43.8%, and 77.7%, respectively. Eleven babies were born from 7 full term pregnancies, while pregnancy is ongoing for one couple.
Limitations, reason for caution: High rate of consanguineous marriages in Turkey creates technical limitations during molecular setup of the PGD cases. It is harder to establish informative markers in consanguineous couples since most of the alleles are shared among the partners. To overcome this problem, high numbers of potential STR markers were screened during the setup procedures that at least 6 informative markers are obtained for each case.
Wider implications of the findings: Although some case reports are published on PGD of rare monogenic disorders, to the best of our knowledge this is the first study evaluating healthy live-birth outcome after PGD for very rare monogenic disorders from one center. The high efficiency of the diagnostic procedures and the high live-birth rate reported in this study indicates that PGD is highly successful when performed for couples with extremely rare monogenic disorders.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Istanbul Memorial Hospital.
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, assisted reproductive technology, rare monogenic disorders, mutation analysis

P-603 Systematic assessment the clinical outcome of preimplantation genetic diagnosis and screening based next generation sequencing

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Study question: Although SNParray based preimplantation genetic testing (SNP-PGT) and next generation sequencing based preimplantation genetic testing (NGS-PGT) have began widely applied in clinical setting, but few data are available regarding the clinical outcome evaluation.
Summary answer: No significant difference of clinical outcome (including implantation rate, clinical pregnancy rate and miscarriage rate) can be found between SNP-PGT group and NGS-PGT group. However, we found NGS provided higher accuracy in embryonic imbalanced chromosomal segmental rearrangements and even can cover 98.7% ± 3.1% of the mitochondrial DNA (mtDNA).
What is known already: NGS shows great potential of providing more powerful and cost-effective tools for PGD/PGS. Previous studies have proven that aneuploidy and imbalanced rearrangements can be detected accurately by NGS. Here we present the systematic clinical evaluation of NGS based PGD/PGS.
Study design, size, duration: This was a retrospective study of 395 couples with indications to *in vitro* fertilization and preimplantation embryos testing treatment between October 2011 and December 2013, including chromosomal translocations, inversions, advanced maternal age and recurrent pregnancy loss. Couples are randomly divided into NGS and SNParray group.
Participants/materials, setting, methods: The study was performed at the Reproductive and Genetic Hospital of CITIC-Xiangya and BGI-Health, China. In total, 1,512 blastocysts were biopsied, with 1,058 blastocysts for SNParray test and 454 blastocysts for NGS test. The clinical outcomes were calculated and statistically analyzed respectively.
Main results and the role of chance: In NGS cycles, the implantation, clinical pregnancy per ET, ongoing pregnancy rate and miscarriage rates were 52.6% (60/114), 61.3% (49/80), 52.5% (42/80) and 14.3% (7/49). In SNP array cycles, the implantation, clinical pregnancy per ET, ongoing pregnancy rate and miscarriage rates were 47.6% (139/292), 56.7% (115/203), 48.3% (98/203) and 14.8% (17/115), respectively. There were no statistical difference between SNP-PGT and NGS-PGT ($P = 0.362$, $P = 0.480$, $P = 0.522$, $P = 0.934$).
Limitations, reason for caution: This study just technically prove NGS can accurately cover the mtDNA, preliminary we found the copy number of mtDNA have direct correlation between euploidy embryos and aneuploidy embryos but further data validation are required.
Wider implications of the findings: NGS based preimplantation genetic testing can achieve the same clinical outcome as SNParray-PGT, moreover, NGS can not only accurately detect the embryos genome but also be able to cover nearly 95% of the mtDNA. Mitochondria are very crucial for the energy production needed by cell biosynthetic, metabolic and physiologic process. In the near future mtDNA may act as an important factor to assist in selecting the more viable embryos to transfer.

Study funding/competing interest(s): Funding by national/international organization(s). This study was supported by grant from the Major State Basic Research Program of China (No. 2012CB944901), Shenzhen Birth Defect Screening Project Lab (JZF No. [2011] 861).
Trial registration number: NA.
Keywords: PGD/PGS, NGS, clinical outcome

P-604 Embryo genome profiling by single cell sequencing for preimplantation genetic diagnosis in a beta-thalassemia family

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Study question: Is it feasible that embryo genome and haplotype to be analyzed by single-cell next generation sequencing (NGS) with comprehensive pre-implantation genetic diagnosis (PGD) of single-gene disorder, human leukocyte antigen (HLA) matching, sex and aneuploidy?
Summary answer: Full embryonic genome including genotypes and haplotypes can be obtained by single-cell next generation sequencing. Pre-implantation genetic diagnosis of Mendelian disorders, HLA-matching, sex and aneuploidy can be achieved comprehensively and accurately.
What is known already: Embryonic genome, including genotypes and haplotypes, contains important information for PGD. Beta-thalassemia is the most common group of monogenic disorders. PGD of single-gene disorders combining HLA-matching has emerged for couples of beta-thalassemia carriers. At delivery, the umbilical cord blood stem cell can be used to treat the affected sibling. During IVF-PGD, aneuploidy testing for embryos is necessary due to high incidence of embryonic chromosomally abnormalities. Sex information of embryos can be an indication as well.
Study design, size, duration: This is a retrospective study of PGD by NGS in a beta-thalassemia family. A couple with a beta-thalassemia daughter underwent IVF-PGD. Seven blastomeres were biopsied. Amniocentesis was performed for prenatal diagnosis. A normal female baby was born in July 2012. Each participant was provided informed consent with the institutional approval.
Participants/materials, setting, methods: A straightforward strategy to obtain full embryonic genome was developed for a beta-thalassemia carrier couple with a proband daughter. We carried out NGS for single blastomere cells, the family trio and amniotic fluid, and developed the pipeline for embryonic genome and haplotype phasing with comprehensive PGD.
Main results and the role of chance: We obtained embryo genome and haplotype by NGS. The accuracy for homozygous and heterozygous SNPs reached 99.62% and 98.39% according to amniotic fluid genome. Based on embryonic genomes, we performed comprehensive PGD. The proband sister contained a pathogenic mutation at rs7480526 in HBB gene. Genotype at rs7480526 of each embryo agreed with the haplotype. HLA-matching was analyzed according to haplotype. Haplotype of HLA genes for the transferred embryo agreed with the proband sister. Embryonic beta-thalassemia diagnosis and HLA-matching results were validated by Sanger sequencing. Embryonic aneuploidies were detected using the depth ratio of alleles in heterozygous genotypes. The transferred embryo was euploidy. Embryonic sexes were determined from heterozygosity of ChrX. The transferred embryo was female. Embryonic aneuploidies and sexes were validated by SNP-array.
Limitations, reason for caution: Embryo indels can be detected with accuracy about 89%. De novo mutations cannot be confirmed due to false positives in WGA. The cost of NGS for embryo genome would be relatively high currently. Embryo vitrification is needed recently. Ethical questions for PGD toward personal genome need to be discussed.
Wider implications of the findings: This is a retrospective study in a beta-thalassemia family for genetic diagnosis of pre-implantation embryo through whole genome, high-resolution variation detection at single cell level, and clinical models establishing for next generation sequencing application in PGD. It paves the way for PGD towards personal medicine.
Study funding/competing interest(s): Funding by national/international organization(s). This study was funded by the Laboratory of Shenzhen Birth Defect Screening Project (JZF No. [2011] 861 and JZF No. [2011] 862) and was approved by Shenzhen Municipal Commission for Development and Reform and Key Laboratory Project in Shenzhen (CXB201108250096A) and Key Laboratory of Cooperation Project in Guangdong Province (2011A060906007).
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, single cell sequencing, embryo genome profiling

P-605 Association between blastocyst morphology and euploidy rates in different age groups analyzed by aCGH and SNP PGD

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Study question: Is it possible to predict the euploidy rate of a blastocyst cohort based on patient age and morphology of the embryos *in vitro*?
Summary answer: There is a strong association between blastocyst morphology and euploidy rates in all age groups. Euploidy rate varies significantly within the same age group depending on morphology of the blastocysts.
What is known already: The vast majority of aneuploidies in preimplantation embryos are of maternal origin (either meiotic or mitotic). The proportion of aneuploid embryos progressively increases with advanced maternal age. Previous studies proved that aCGH- and SNP-based preimplantation genetic testing are the most reliable techniques to determine the genetic status of embryos *in vitro*.
Study design, size, duration: A retrospective comparative study was performed between January 2013 and January 2015. 414 cycles of IVF treatment with PGD were included in the study: in 270 cases blastocysts were analyzed by SNP, and in 144 by aCGH PGD. 2146 embryos were analyzed for euploidy rates and blastocyst morphology.
Participants/materials, setting, methods: Morphology of blastocysts was evaluated using Gardner classification before trophectoderm biopsy. Embryos were divided into three groups based on blastocyst quality: good (AA/AB/BA), fair (BB), and poor (-C or C-) and into five groups based on maternal age (SART age groups).
Main results and the role of chance: Our data demonstrated a statistically significant difference ($p < 0.05$, $\chi^2 = 9.58$) in euploidy rates between good, fair, and poor quality embryos in all age groups. The biggest difference in euploidy rates between embryos with different morphology was identified among young (<38 year-old) patients: euploidy rate for good quality embryos was 71.3% (325/456), for fair quality embryos – 54.2% (352/650), and for poor quality embryos – 30.4% (7/23). The difference in euploidy rates between embryos with different morphology has a tendency to decrease with an increase in patient age. For the patients over 40 years old, the euploidy rate for good quality embryos was 37.2% (48/129), for fair quality embryos – 24.9% (59/237) and for poor quality embryos – 3.6% (1/28), $p < 0.05$, $\chi^2 = 6.12$. The association between euploidy rates and morphology of the embryos was assessed by identifying coefficients of linear regression: for good quality embryos $y = -5.2554x + 89.235$, for fair quality $y = -4.3348 + 69.094$, and for poor quality embryos $y = -2.4784 + 31.021$.
Limitations, reason for caution: Retrospective study and heterogeneity of patients included.
Wider implications of the findings: Reliable data about correlation between morphology and euploidy of the embryos will not only help healthcare providers create the best treatment strategy for the infertile couple but will also help patients make an informed decision about the necessity of PGD based on maternal age, quality and quantity of the embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s) – none.
Trial registration number: NA.
Keywords: PGD, euploidy rate, embryo morphology

P-606 The physiological role of RNA methylation in the oocyte and embryo

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Study question: What transcripts are methylated in the oocyte? How does RNA methylation affect oocyte and early embryo development? What is the fate of methylated transcripts in the oocyte?

Summary answer: N6-Methyl-adenosine (M6A) was detected in RNA from mice and knocking down (Kd) METTL3 (RNA methylase) and Alkbh5 (RNA demethylase) with morpholinos in the sea star oocyte did not alter oocyte maturation, fertilization, nor early cleavages. However, blastulation, gastrulation, and embryo motility are delayed compared to controls.
What is known already: Methylation of the N6 position of the adenosine is one of the most prevalent internal posttranscriptional modifications of polyadenylated mRNAs and long non-coding RNAs. Methylation is performed by METTL3, METTL14 and WTAP, while demethylation is accomplished by Alkbh5 and FTO. It has been shown that perturbation of IME4, homolog to METTL3 in *Drosophila*, leads to abnormal oogenesis and Alkbh5 deficiency is implicated in spermatogenic defects in mice.
Study design, size, duration: Experimental laboratory study which is currently ongoing.
Participants/materials, setting, methods: *Patiria miniata* (sea star) oocytes, CL57B6 oocytes, and deidentified discarded human oocytes from ICSI cycles at Women and Infants Hospital of Rhode Island. Morpholinos (MOs) to Alkbh5 and METTL3 were designed based on human sequences.
Main results and the role of chance: Ovaries from mice showed expression of m6A on an RNA dot blot. Sea star oocytes injected with MOs had normal maturation and fertilization. Blastulation, gastrulation, and embryo motility were significantly impaired in the METTL3 Kd as well as with Alkbh5 Kds compared to controls. *In vitro* transcripts made with 100% N6 methylated ATP for GFP and firefly luciferase are being tested following injection in the oocyte to establish the dynamics compared to non-methylated transcripts. In an effort to minimize the possibility of chance these experiments are being performed in triplicate and will be repeated in mouse and human oocytes.
Limitations, reason for caution: One of the limitations of this project is that human oocytes cannot be fertilized due to federal restrictions involving research with human embryos. Additionally, human oocyte donation for the express purpose of research is not yet allowable in our state so the human oocytes were discarded GV or MI.
Wider implications of the findings: RNA methylation may be an important posttranscriptional modification that can report the quality of oocytes and determine embryo progression. Our future work will examine differences in methylation between young and aged oocytes as well as patterns of methylation in the ovary over the reproductive lifespan.
Study funding/competing interest(s): Funding by national/international organization(s). Reproductive Scientist Development Program (RSDP) 5K12HD000849-27 and NIH 2R01HD028152.
Trial registration number: Women and Infants Hospital Institutional Review Board 12-0045 IRB Number and Brown University IACUC: 1407000080.
Keywords: RNA methylation, oocyte competence

P-607 Influence of GDF9 polymorphisms in follicular dynamics in patients undergoing *in vitro* fertilization

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Study question: The objective of this study was to assess the influence of growth differentiation factor-9 (GDF9) polymorphisms in patients undergoing *in vitro* fertilization.
Summary answer: Polymorphisms in the GDF9 gene significantly influence oocyte development. The presence of mutant alleles 447C > T and 398C > G decrease the total number of mature follicles and the total number of oocytes collected from patients undergoing ovulation induction for *in vitro* fertilization (IVF).
What is known already: The role of oocyte-derived growth factors in either up- or down-regulating fertility is an exciting paradigm in reproduction biology. Factors such as GDF9 is known to influence the growth and depletion rates of follicles. Several genetic variants of GDF9 have recently been identified, and their correlations with premature ovarian failure suggest that these variants may contribute to aberrant follicular development and oocyte loss.
Study design, size, duration: A cross sectional prospective study was performed. The setting for this study was a fertility center with Sixty-seven women undergoing IVF treatments using r-FSH and recombinant GnRH antagonist protocol.
Participants/materials, setting, methods: We performed DNA extraction of peripheral blood, followed by polymerase chain reaction (PCR) to amplify the region of interest in the GDF9 gene. We sequenced four polymorphisms of GDF9 and analyzed their influences on patients undergoing IVF.
Main results and the role of chance: Women with the mutant allele 447C > T gene of GDF9 had a smaller number of follicles between 12 and 14 mm on the day of r-hCG administration (1.62 vs. 2.46, $P = 0.007$). In addition, women with the mutant allele 398C > G gene of GDF9 had a smaller number of follicles at least 17 mm on the day of r-hCG administration (4.33 vs. 6.49, $P = 0.001$), a smaller number of follicles between 12 and 14 mm on the day of r-hCG administration (1.42 vs. 2.25, $P = 0.017$), a lower number of follicles on the day of r-hCG administration (7.33 vs. 10.11, $P = 0.007$), and a lower number of total MII oocytes retrieved (5.38 vs. 8.84, $P = 0.017$).
Limitations, reason for caution: The limitation of our results is that we don't realized functional studies to validate the precise genetic mechanism involved in oocyte development.
Wider implications of the findings: This finding shows that in addition to playing a role in the early stages of folliculogenesis, this member of the transforming growth factor-β (TGFB) family also has an important influence on the final stage of oocyte development.
Study funding/competing interest(s): Funding by hospital/clinic(s). We are grateful for the financial support provided by Fundo de Incentivo à Pesquisa (FIPE), Hospital de Clínicas de Porto Alegre, Brazil and Clínica Pronatus – Medicina Reprodutiva, Belém Brazil.
Trial registration number: The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre *n*: 14/0070.
Keywords: GDF9, polymorphism, oocyte, follicle retrieval, FIV

P-608 The poly(A)-binding protein genes, EPAB, PABPC1, and PABPC3 are differentially expressed in infertile men with non-obstructive azoospermia

Abstract withdrawn by the author

P-609 First clinical applications of preimplantation genetic diagnosis for b-thalassemia combined with preimplantation genetic screening on blastocysts from fresh and vitrified oocytes, after next generation sequencing

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Study question: The present study reports the first clinical applications of preimplantation genetic diagnosis (PGD) for b-thalassemia combined with the diagnosis of aneuploidies (PGS) on blastocysts produced from fresh and vitrified oocytes and using next generation sequencing (NGS).
Summary answer: Applying NGS technology, it is possible to diagnose simultaneously aneuploidies and hemoglobinopathies. The use of fresh oocytes together with vitrified oocytes increases the number of available embryos to transfer.
What is known already: Until now the clinical application of PGD for single cell disease and the PGS on embryos have been applied separately. On the other side, the NGS was applied and previously validated for aneuploidies diagnosis on embryo diagnosis.
Study design, size, duration: The diagnosis of b-globinopathies and aneuploidies were performed from trophectoderm cells of blastocysts produced from fresh and vitrified/warmed oocytes in 7 infertile b-globin mutation carriers. The study was performed on a period of 4 months.
Participants/materials, setting, methods: Few trophectoderm cells were removed on day 5–6 blastocysts produced from fresh and vitrified/warmed oocytes post-ICSI. Whole genome amplification was performed on 39 samples. Sample libraries were prepared with respectively in-home Ion AmpliSeq HBB panel and Ion plus fragment library kit. Enriched barcoded-samples libraries were sequenced on Ion PGM (Life Technology).
Main results and the role of chance: 25 blastocysts from fresh and 14 blastocysts from vitrified/warmed oocytes were produced. 100% of the samples

were amplified after whole genome amplification. B-globin gene analysis and chromosomal status were completed for 37 samples. It was possible to confirm that cells belonged to embryos because of SNP and STR analysis. Respectively 2, 1 o no b-globin mutation were diagnosed in 9, 16 and 12 embryos. Fifteen blastocysts were euploid. After overall analysis, 10 embryos were available for embryo-transfer: 6 from fresh oocytes and 4 from vitrified/warmed oocytes. Seven embryos were transferred after thawing. Four clinical pregnancies were obtained (implantation rate: 57%). PGD and PGS results were confirmed after prenatal diagnosis.

Limitations, reason for caution: The first clinical results after PGD and PGS based on whole genome amplification and NGS and performed on blastocyst produced from fresh and vitrified/warmed oocytes are encouraging. Nevertheless further randomized controlled trial studies are requested specially for PGS efficacy to confirm clinical efficacy.

Wider implications of the findings: The present study shows that the single technology Next Generation Sequencing can be applied to diagnose together chromosomal abnormalities and single cell diseases. In case of limited ovarian response it is possible to combine the diagnosis of embryos from fresh and vitrified embryos. This option allows the couple to limit the cost of genetic analysis and increases the number of available embryos to transfer.

Study funding/competing interest(s): Funding by hospital/clinic(s) – unità di medicina della riproduzione – istituto HERA.

Trial registration number: No rct.

Keywords: preimplantation genetic diagnosis, preimplantation genetic screening, next generation sequencing, oocyte vitrification, clinical outcomes

P-610 Day 3 biopsy and day of transfer?

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Study question: Is their a difference in ongoing pregnancy rate between a day three transfer and a day five transfer after a day three biopsy and FISH analysis?

Summary answer: So far the ongoing pregnancy rate is 62% after a day three transfer compared to 45% after a day 5 transfer.

What is known already: Clinicians are shying away from day three biopsies with FISH analysis and moving towards trophectoderm biopsy with microarray. Day 3 transfers after day 3 biopsy has never been performed.

Study design, size, duration: Randomized controlled prospective trial. 268 patients. September 1, 2014 and the study is still ongoing. Computer randomized. Blinded to the clinician. All patients with at least four embryos on day of biopsy were included.

Participants/materials, setting, methods: All patients undergoing PGS at Fakih IVF Dubai and Abu Dhabi branches.

Main results and the role of chance: Group sample sizes of 134 in group one and 134 in group two to detect a difference between the group proportions of 0.1700. The proportion in group one (Day three transfer) is assumed to be 0.4500 under the null hypothesis and 0.6200 under the alternative hypothesis. The proportion in group two (Day 5 transfer) is 0.4500. The test statistic used is the two-sided *Z* test with pooled variance. The significance level of the test was targeted at 0.0500. Primary endpoint is ongoing pregnancy rate so far we have 87 patients: Group (day 3 transfer) 43 patients: pregnancy rate per embryo transfer 24/39 or 61.5 percent. (1 patient biochemical pregnancy counted as negative and 4 patients no embryo transfer). Group (day 5 transfer) 45 patients: pregnancy rate per embryo transfer 20/44 or 45.5 percent. (2 patients biochemical pregnancy counted as negative and 1 patient no embryo transfer).

Limitations, reason for caution: The study is still ongoing and we have to wait for the final results.

Wider implications of the findings: Reintroduction of blastomere biopsy with FISH analysis. Apparent higher pregnancy rate than previously reported in the literature with day 3 biopsy and day 5 transfers. It seems that removing one cell from a blastomere does not damage the embryo and decrease pregnancy rate as previously postulated. This finding suggests that it is the prolonged interaction of a biopsied embryo with the culture media that damages the embryo.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Fakih IVF.

Trial registration number: Pending.

Keywords: PGS, PGD, ICSI, blastomere biopsy, blastomere transfer

P-611 The –29G > A FSHR polymorphism is not associated with ovarian response in IVF/ICSI cycles

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Study question: Is there any effect of the *FSHR* polymorphism -29 G > A on ovarian response in females undergoing IVF/ICSI?

Summary answer: No significant association was detected between the *FSHR* polymorphism -29 G > A and ovarian response in females undergoing IVF/ICSI.

What is known already: *FSHR* has a major role in controlling FSH action. Altered *FSHR* expression has been proposed to impact on ovarian response. A single nucleotide polymorphism -29 G > A in the 5' untranslated region of *FSHR* is associated with altered transcriptional activity. Four previous studies have determined a significant correlation between *FSHR* (AA) genotype and poor response to IVF.

Study design, size, duration: In an observational study, we genotyped *FSHR* -29 G > A in 690 women (578 women from a tertiary referral centre for reproductive medicine in Manchester and 112 women from Mansoura fertility centre in Egypt), undergoing their first cycle of controlled ovarian hyper-stimulation for IVF/ICSI between March 2009 and December 2013.

Participants/materials, setting, methods: Blood tests were taken on day 3 of the cycle for assessment of baseline hormones and DNA extraction. Genotypes were determined using Taqman allelic discrimination assay. Correlation analysis was performed to assess effect of *FSHR* genotypes on primary outcome of response (number of oocytes retrieved and gonadotropin dose).

Main results and the role of chance: There is no evidence of any statistically significant difference (p value <0.05) in the number of oocytes retrieved and gonadotropin dose used between individuals with different genotypes.

Limitations, reason for caution: A larger sample size would be required in order to determine if the *FSHR* -29 G > A genotype has a small effect on ovarian response.

Wider implications of the findings: As a result of our finding, the *FSHR* -29 G > A polymorphism should not be used in the individualization of the treatment protocol for women undergoing IVF/ICSI.

Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Ministry of Higher Education in Egypt (Culture Affairs and Mission sector). Mansoura University. Manchester University.

Trial registration number: 08/81003/212.

Keywords: follicle-stimulating hormone, FSH receptor, ovarian response, IVF, FSH receptor polymorphism

P-612 The role of transcript expression levels of nuclear encode (TFAM) and mitochondrial encoded (MT-CO1) genes in single human oocytes maturation from women with pcos

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Study question: Does nuclear encoded (TFAM) and mitochondrial encoded (MT-CO1) genes in single human oocyte maturation difference from patients with polycystic ovarian (PCOS) compared with normal women?
Summary answer: quantification of the relative transcript abundance indicated that Different expression levels of TFAM, MT-CO1 influence human oocyte maturation in patient women with PCOS.
What is known already: Polycystic ovary syndrome (PCOS) is the most common female endocrinopathy which cause of infertility due to anovulation. Prevalence of PCOS is approximately of 15–20% of reproductive-aged women. Patients with polycystic ovarian syndrome (PCOS) have higher miscarriage rates. It is suggested that this is caused by a lower rate of mature oocytes, and a lower quality of embryos**.** Mitochondria play a critical roles in oocyte maturation. mitochondria have their own genome, which is increased during oocyte maturation. The mitochondrial transcription factor A (TFAM) controls the transcription of mtDNA and regulates the mtDNA-copy number. MT-CO1 is a respiratory chain protein encoded by heavy strand mtDNA. therefore, its transcript level may be an important role for maintaining ATP production.
Study design, size, duration: A cross-section observational study was performed of 64 Oocytes at the metaphase II (MII) stage obtained from women with PCOS as compared to 11 infertile women with male factor who undergoing IVF/ICSI protocol between January 2012 and November 2013.
Participants/materials, setting, methods: Mitochondrial related transcript expression levels of TFAM, MT-CO1 genes were performed by single-cell Taqman real time PCR-based assay.
Main results and the role of chance: For the first time, Taqman RT-qPCR analyses using single oocytes from clinical patients showed that the transcript expression levels of TFAM and MT-CO1 were significantly lower in patient women with PCOS compared with normal women ($p = 0.037$ and $p = 0.001$ respectively).
Limitations, reason for caution: The limitation of this study was small sample size which make it difficult to determine protein by western blotting and proteomics. Therefore, further studies are needed in a large sample size to determine protein levels of these genes in oocytes from women with PCOS.
Wider implications of the findings: Lower transcript expression levels of (TFAM, MT-CO1) genes in metaphase II (MII) stage of oocytes from women with PCOS probably leads to reduction of oocyte quality. These results probably enlighten a new therapeutic approaches for optimizing oocyte quality in treatment of women with PCOS.
Study funding/competing interest(s): Funding by University(ies) – Shahid Beheshti University of medical sciences.
Trial registration number: 1391-1-91-9947.
Keywords: human single oocyte, PCOS, RT-qPCR

P-613 Comparison of the live birth rate between preimplantation genetic diagnosis and natural conception in patients with recurrent pregnancy loss associated with translocation

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Study question: Can preimplantation genetic diagnosis (PGD) improve the live birth rate, as compared to natural conception, in patients with recurrent pregnancy loss (RPL) associated with translocation?
Summary answer: While PGD significantly prevented further miscarriages, there was no difference in the live birth rate.
What is known already: The reported live birth rate in patients undergoing PGD is in the range of 27–54%, and that in patients undergoing natural conception is in the range of 37–66% at first and 68–86% cumulatively. However, no prospective study has been carried out to compare the live birthrates in patients matched for age and number of previous miscarriages undergoing PGD and choosing to have natural conception.
Study design, size, duration: This cohort consisted of 126 Japanese patients with RPL associated with translocation. The 52 patients who desired natural conception and 74 patients who wished to undergo PGD after genetic counseling, between August 2003 and November 2013. The subsequent pregnancies of all the patients were followed up until July 2014.
Participants/materials, setting, methods: The live birth rate, cumulative live birth rate, miscarriage rate were compared between 37 patients undergoing PGD who were matched for age with the natural conception group, and 52 patients who did not undergo PGD and selected natural conception. PGD was performed by fluorescence in situ hybridization (FISH) analysis.
Main results and the role of chance: The live birth rates at the first trial of PGD and the first natural pregnancy after ascertainment of the carrier status were 37.8% and 53.8%, respectively (odds ratio 0.52, 95% confidence interval 0.22–1.23). The cumulative live birth rates were 67.6% and 65.4%, respectively, in the groups undergoing and not undergoing PGD. The time to pregnancy was similar in both groups. The mean number of further miscarriages till a live birth in the PGD (0.22 ± 0.42) group was significantly lower than that in the natural conception group (0.58 ± 0.78, $p = 0.012$). PGD could reduce miscarriage rate significantly. The prevalence of twin pregnancy was significantly higher in the PGD group. The cost of PGD was 6857 € per patient.
Limitations, reason for caution: Comparison was performed between 37 patients aged ≤34 years undergoing PGD and the 52 patients conceiving naturally, because the mean age of the patients undergoing PGD was significantly higher. It remained unclear whether PGD had any effect in elderly patients.
Wider implications of the findings: Information that there was no difference in the live birth rate when PGD and natural conception was compared. Couples should be fully informed of the similar live birth rates, similar time to pregnancy, the advantages of PGD such as the reduction in the miscarriage rate, the disadvantages of PGD such as the higher cost, and the advantages of natural pregnancy, such as avoidance of IVF failure.
Study funding/competing interest(s): Funding by hospital/clinic(s). This study was supported by a Grant-in-aid for Scientific Research from the Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology of Japan. All the authors declare that they have no conflict of interests.
Trial registration number: NA.
Keywords: translocation, PGD, recurrent miscarriage, recurrent pregnancy loss, live birth rate

P-614 A 24-chromosome aneuploidy detection of 100 embryos in PGS by means of NGS

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Study question: Can the Next-generation sequencing (NGS) platform Ion-Torrent™ along with low-pass whole genome sequencing, be used reliably to evaluate, in terms of Preimplantation Genetic Screening (PGS) for aneuploidy detection, many biopsied trophectoderm cells in order to confirm which euploid embryos were ready-to-transfer in an IVF cycle?
Summary answer: This validation study had provided a 100% consistency for aneuploidy screening in clinical PGS cycles by NGS in contrast to well-established microarray-based comparative genomic hybridization (aCGH), and the subsequent good prognosis for pregnancy from IVF.
What is known already: PGS involves the genetic study of the embryo's genome to determine its chromosomal arrangement, prior to euploid embryos selection and subsequent implantation in IVF. Early fluorescent in-situ hybridization (FISH) showed limitations in genome coverage that were solved by aCGH, a PGS method with demonstrated accuracy. In this way, all the chromosomes can be completely screened in a genome-wide DNA sequencing in terms of NGS data providing an alternative approach for detecting copy number variations (CNV).

Study design, size, duration: This prospective, double-blinded trial involved genetic testing with both NGS and array-CGH techniques of 100 blastocysts from 29 patients (median age 40 ± 5.57 years) recruited in a total of 30 clinical IVF cycles at the fertility clinic IVF-Spain (Alicante) from May 2013 to December 2014.
Participants/materials, setting, methods: After oocyte insemination, 100-blastocyst trophectodermal biopsy was performed on day 5. Whole genome amplified DNA (PicoPlex® technique) followed both NGS and aCGH. CNV analysis was performed with BlueFuse Multi software (aCGH) and Ion Reporter™ Software v4.2. (NGS), which determined the ploidy status with 0.01× read coverage.
Main results and the role of chance: An amount of 42 of 100 embryos were euploid. Thirteen (46.4%) of 28 embryos transferred achieved a successful pregnancy. Four (30.8%) patients have already given birth and 7 (53.8%) pregnancies are still in progress; 2 (15.4%) of them are *dizygotic twin pregnancies.* In respect to aneuploid embryos, 15 of 58 (25.9%) presented trisomies, 20 (34.5%) monosomies, 9 (15.5%) full gain and losses, 3 (5.2%) were chaotic and 6 (10.3%) had partial imbalances. Along the whole process, no technical failures, false positives and negatives were detected. There was a significant concordance (98%; 98/100; 95% confidence interval [95% CI]: 94.75–100) between aCGH and NGS results, including balanced translocations. The two discordant results showed a monosomy on one chromosome by aCGH, while the NGS screening was highly accurate.
Limitations, reason for caution: Although reliability of Next-generation sequencing (NGS) platform Ion-Torrent™ along with low-pass whole genome sequencing for aneuploidy detection and the subsequent higher pregnancy outcomes, further data in a randomized controlled trial are required in order to achieve a broad-based clinical application.
Wider implications of the findings: This supposes an opportunity to model the possible NGS throughput capacity of a single instrument and one chip in both PGS and even PGD (Preimplantational Genetic Diagnosis). In just one biopsy, a combined 24-chromosome aneuploidy and single-gene disorders detection by means of NGS is assumed. Couples could have access to a more affordable PGS/PGD service. Definitively, a higher probability of implantation is achieved and thus drastically improve the IVF success rates.
Study funding/competing interest(s): Funding by national/international organization(s) – Funding by commercial/corporate company(ies). Funding by national/international organization(s): The Torres Quevedo Program from the Spanish Ministry of Economy and Competitiveness, with specific projects PTQ-12-05687 for Penacho, V., PTQ-13-06028 for González-Reig, S., and PTQ-11-04960 for Alcaraz, L. A. Funding by commercial/corporate company(ies): Bioarray S. L. (Elche, Alicante, Spain) and IVFSPAIN (Alicante, Spain). No competing interests are declared.
Trial registration number: This is not a clinical trial so it not linked to a trial registration number.
Keywords: NGS, aCGH, PGS, aneuploidy screening, balanced translocation

P-615 Warming-biopsy-PGS-revitrification-rewarm-ET: a viable strategy for patients who want to minimize the number of frozen cycles before becoming pregnant

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Study question: Do the benefits outweigh the risks for the strategy of warming, biopsy and re-vitrification to allow for Pre-Genetic Screening (PGS) of embryos currently in storage?
Summary answer: The case studies exemplified how this strategy benefited patients with numerous cryopreserved embryos. Knowing the genetics of cryopreserved embryos may remove the need for patients to undergo multiple unsuccessful cycles, avoiding unnecessary financial and emotional burden. To reduce the risks associated with this strategy, an excellent cryopreservation program is required.
What is known already: PGS of embryos has been demonstrated to be a useful tool for embryo selection. Improvements in cryopreservation survival and implantation rates have demonstrated a reduction in the risk bestowed on an embryo due to vitrification compared to previous cryopreservation techniques. Re-cryopreserving embryos has been demonstrated to not compromise embryo viability by a number of publications (Farhat et al., 2001; Smith et al., 2005, Sheehan et al., 2006, Hashimoto et al., 2007, Kumasako et al., 2009).
Study design, size, duration: Four case reports involving PGS of surplus embryos. Patients A, B, C and D (aged 35, 34, 37 and 29 respectively) had 6, 9, 8 and 10 surplus blastocysts cryopreserved (COOK or Kitazato vitrification protocols). Patients had 1, 3, 13 and 2 previous embryo transfers (respectively) with or without implantation.
Participants/materials, setting, methods: Embryos were warmed using the same warming protocol they were vitrified with and the trophectoderm biopsied after warming. Biopsies were tubed and analysed with array-CGH. Blastocysts were re-vitrified with Kitazato-Cryotop Vitrification system. Normal embryos were transferred in a subsequent Frozen Embryo Replacement Cycle (FERC) where embryos were re-warmed using Kitazato.
Main results and the role of chance: Patient A: All six embryos were euploid. Patient returned for a FERC where a single embryo was warmed and transferred leading to a single foetal heart. Patient B: None of the embryos were diagnosed as euploid. Patient returned for a fresh cycle with PGS analysis instead of having a FERC. Six embryos were euploid and the patient is currently being prepared for a FERC for a single euploid embryo transfer. Patient C: All 8 embryos were diagnosed as aneuploid. Patient was advised to proceed with egg donation. Patient D: After two miscarriages following eSET, surplus embryos were analysed with PGS and 3 embryos were euploid. The patient is currently being prepared for a FERC cycle for a single euploid embryo transfer.
Limitations, reason for caution: These case studies demonstrate how re-vitrification can be used as a strategy for patients who have large numbers of embryos already frozen and did not have the opportunity to perform PGS before freezing to benefit from PGS. Refreezing does carry risks and preference should be to perform biopsy before cryopreservation.
Wider implications of the findings: Case study A demonstrated that embryos re-vitrified in our cryopreservation programme are viable. Case Studies B, C and D demonstrated that this strategy saved the patient having to undergo numerous FERCs with negligible chance of success, protecting the patient from considerable financial and emotional affliction.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Boston Place Clinic.
Trial registration number: NA.
Keywords: biopsy, PGS, cryopreservation, re-freeze, frozen embryo replacement

P-616 Carrier testing for twelve common severe recessive pediatric diseases by comprehensive targeted sequencing assay in Chinese population

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Study question: To develop an efficient method for carrier status testing for common severe recessive pediatric diseases.
Summary answer: Carrier screening by next generation sequencing together with other conventional assay made available to the general population could reduce the incidence of severe recessive pediatric diseases even causing by complicated mutations.
What is known already: Carrier screening for a specific disease, for several diseases with high frequency in some region or for several hundreds diseases by next generation sequencing had been reported previously. Identifying carrier persons and couples can provide them with a variety of reproductive options. A comprehensive assay for common severe recessive pediatric disease genes can be useful to assess carrier burden for these diseases in China.
Study design, size, duration: YH genome DNA sample and 100 samples with mutation identified were used for evaluate the power of mutation detection. And then carrier screening was performed for a total of 523 asymptomatic adults including two groups with associated family history or not with.
Participants/materials, setting, methods: All exons and selected intergenic and intronic regions of 15 target genes were enriched by hybrid capture, sequenced by next-generation sequencing (NGS), analyzed with bioinformatics software, and reviewed evidence with stringent rules. Complementary testing also was designed to cover some hot mutations in technical limitation of NGS.
Main results and the role of chance: In the 101 validation samples, the average depth for the targeted regions was more than 200 fold, with 99% coverage.

The mutation detection had ~98% sensitivity and ~100% specificity for substitution, small insertion/deletion, and gross deletion. In 438 individuals with non family history, the average genomic carrier burden for deleterious mutations was 0.19, and about 18% carried at least one deleterious mutations. For the high reproductive risk in this group, we found one carried a deleterious mutation on X-linked recessive genes in 282 females, but none was match in autosomal recessive genes in 100 couples. And in the 85 individuals with family history about the 12 diseases, the average genomic carrier burden was up to 0.66, and about 56% carried at least one deleterious mutations.

Limitations, reason for caution: This screening methods is not able to distinguish two *SMN1* copies on one chromosome (in *cis* configuration) or on two chromosome (in *trans* configuration), and some complicated rearrangement mutations of CYP21A2 can not be detected. For the two diseases, the mutations included in the carrier testing is limited.

Wider implications of the findings: This targeted sequencing assay can used to evaluate the screening program in China for control of common severe recessive pediatric diseases in the population of conception or pre-conception couples.

Study funding/competing interest(s): Funding by national/international organization(s). Tianjin Municipal Science and Technology Special Funds for Enterprise Development.

Trial registration number: NA.

Keywords: carrier testing, next-generation sequencing, severe recessive pediatric diseases

P-617 Human ART culture media influence the epigenetic pattern of Lit1 in mouse preimplantation embryos

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Study question: Do different human ART culture media have an effect on the methylation status of maternally imprinted genes in murine pre-implantation embryos?

Summary answer: The epigenetic profile of the maternally imprinted gene *Lit1* is changed during in-vitro-culture (IVC) of murine pre-implantation embryos using KSOM(aa) and the human sequential culture media HTF and Multiblast (Irvine Scientific, Bruckberg, Germany), but not ISM1 and Blast Assist (Origio, Berlin, Germany) compared to the *in vivo* control.

What is known already: In ART cycles pre-implantation embryos are cultured at a vulnerable growth phase in an artificial environment. We have shown that different human embryo culture media have an impact on the developmental potential of pre-implantation embryos in mice. During *in vitro* culture epigenetic profiles of imprinted genes change which may result in epigenetic diseases. It has been proposed that ART children carry higher risks of genomic imprinting disorders.

Study design, size, duration: For methylation analysis we used 248 mouse zygotes, cultured until 4.5 days post coitum (dpc) either in two different human sequential media (ISM1/Blast Assist or HTF/MultiBlast) or in KSOM(aa) (*in vitro* control). Blastocysts obtained from the uterus on dpc 3.5 served as *in vivo* control.

Participants/materials, setting, methods: Per group 6 to 10 superovulated female mice (B6C3F1, 6–8 weeks) were mated with C57Bl/6 males. DNA was isolated from *in vivo* and *in vitro* blastocysts and inner cell masses (ICMs), which were obtained via immunosurgery. PCR and pyrosequencing were conducted for two maternally imprinted genes (*Mest*, *Lit1*).

Main results and the role of chance: Methylation levels for *Lit1* did not differ significantly between individual blastocysts of the HTF/MultiBlast group and the *in vitro* control (HTF/MultiBlast: 43.44 ± 11.80% $N = 25$; *in vitro*: 43.01 ± 18.03% $N = 23$). For *Mest* we found lower methylation levels compared to *Lit1*, differences between the treatment groups were not significant (HTF/MultiBlast: 38.44 ± 29.49% $N = 8$; *in vitro*: 31.59 ± 16.20% $N = 44$). However, in individual ICMs the methylation level for *Lit1* in the ISM1/BlastAssist group and *in vivo* control was significantly higher compared to the HTF/MultiBlast and *in vitro* group (ISM1/BlastAssist: 61.67 ± 9.1% $N = 15$; *in vivo*: 56.64 ± 7.99% $N = 12$; HTF/MultiBlast: 42.43 ± 12.21% $N = 18$; *in vitro*: 46.66 ± 13.64% $N = 47$; $p \leq 0.0001$). Again, methylation levels for *Mest* were lower compared to *Lit1*, differences between the treatment groups were not significant (HTF/MultiBlast: 28.32 ± 11.74% $N = 17$; *in vitro*: 33.46 ± 11.84% $N = 26$; *in vivo*: 38.69 ± 16.02% $N = 13$).

Limitations, reason for caution: This was an experimental study using a mouse model. Results cannot be directly transferred to the human situation. Still, our study allows indirect assessment of whether the development of pre-implantation embryos is affected by the use of different culture media.

Wider implications of the findings: Analysis of whole individual blastocysts showed methylation levels of 30–40% for *Mest* and *Lit1*, which is in line with previous findings. However the obtained values show a high heterogeneity. Values obtained for the ICMs are more homogeneous and the epigenetic profile is significantly different between the experimental groups. Therefore analysis of individual ICMs provides a closer insight into epigenetic mechanisms, which could be linked to metabolic changes during pre-implantation development of murine embryos.

Study funding/competing interest(s): Funding by University(ies) – University of Muenster medical school IMF grant No. 11 12 12.

Trial registration number: NA.

Keywords: ART culture media, murine pre-implantation embryo, epigenetics, imprinting

P-618 Meiotic segregation analyses of reciprocal translocations in sperm and embryos: no support for predictive value regarding PGD outcome

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Study question: Is there a correlation between the number of balanced sperm and the number of chromosomally balanced embryos during PGD and could a sperm fluorescence in situ hybridisation (FISH) analysis prior to preimplantation genetic diagnosis (PGD) predict the outcome?

Summary answer: We found that the proportion of balanced sperm was much higher than the number of balanced embryos during PGD, without any linear correlation.

What is known already: Previous studies have suggested that men with more than 60% unbalanced sperm has a poor reproductive prognosis and that there is a linear correlation between the proportion of unbalanced sperm and the proportion of unbalanced embryos.

Study design, size, duration: Prospective cohort study. Ten male reciprocal translocation heterozygotes were included. We analysed 1000 sperm from each patient and between 3 and 29 embryos from the total of PGD cycles that the couples went through. Each couple went through 1–4 PGD cycles for a period of up to 4 years.

Participants/materials, setting, methods: Ten male translocation heterozygotes that went through PGD at the Stockholm PGD centre at Karolinska University Hospital. FISH analysis of sperm and embryos was performed with the same DNA probes.

Main results and the role of chance: The most common segregation mode in the whole sperm count was alternate (51.5%) followed by adjacent 1 (18%), adjacent 2 (13%), 3:1 (13%), 4:0 (0.5%) and other 4%. The total number of embryos was 160 and the segregation modes were as follows; Alternate 21%, Adjacent 1 23%, Adjacent 2 13%, 3:1 20%, 4:0 1% and other 22%. We computed the equation suggested with our data in order to predict the proportion of abnormal embryos in our cohort, and compared the results to the actual outcome. No reliable linear correlation between the levels of unbalanced sperm and unbalanced embryos was found. Four out of ten couples achieved a pregnancy and these translocation heterozygotes had a level of balanced sperm between 28 and 67%.

Limitations, reason for caution: The number of patients included is low and the difference in the number of counted cells between sperms and embryos may be a factor that affected the outcome.

Wider implications of the findings: Our results indicate that a sperm FISH analysis prior to PGD is not a reliable predictor of the PGD outcome. PGD is a valuable reproductive alternative for translocation heterozygotes with reproductive problems and should be offered to these couples.

Study funding/competing interest(s): Funding by national/international organization(s). This work was supported by grants from the Swedish Medical Research Council and the Stockholm County Council.

Trial registration number: NA.

Keywords: chromosomal translocation, preimplantation genetic diagnosis, sperm FISH, meiotic segregation, predicted prognosis

P-619 FISH is equivalent to SNP array for preimplantation genetic diagnosis on young translocation carriers undergoing blastocyst biopsy

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Study question: Is fluorescence in situ hybridization based preimplantation genetic diagnosis (FISH-PGD) more cost-effective than single nucleotide polymorphism (SNP) array based strategy for young translocation carriers undergoing day 5 or 6 trophectoderm biopsy?
Summary answer: For young translocation carriers (age ≤35), D5 FISH-PGD generated higher clinical pregnancy than D3 FISH-PGD, and is equivalent to D5 SNP-PGD.
What is known already: D3 FISH-PGD is gradually replaced by comprehensive chromosome screening (CCS) for translocation carriers. But CCS technique also has some disadvantages, such as more expensive and uncertainty of the minute chromosome segment abnormality, especially the segment abnormality is *de novo*. The incidence of aneuploidy in embryo is increased with the advanced maternal age. Trophectoderm biopsy on D5 decreases risk of embryo damage compared with D3 biopsy.
Study design, size, duration: This was a retrospective study for 1338 chromosome translocation carriers with the female age under 35 years old, who were treated by D5 FISH-PGD between November 2012 and August 2014, D3 FISH-PGD between January 2005 and October 2011, and D5 SNP array-PGD between November 2011 and October 2014.
Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. Totally 1338 couples were recruited, including 447 couples treated by D5 FISH-PGD, 427 by D3 FISH-PGD and 464 by D5 SNP array-PGD. 4115 8-cell embryos and 4143 blastocysts were biopsied with balanced embryos being transferred.
Main results and the role of chance: Reliable results obtained in D5 FISH-PGD, D3 FISH-PGD, and D5 SNP-PGD were 97.0% (2092/2156), 90.1% (3707/4115) and 95.87% (1905/1987), respectively. In D5 FISH-PGD group, the proportions of normal/balanced embryos, clinical pregnancy rate and early miscarriage rate were 59.59%, 66.67% and 15% respectively for Robertsonian translocation carriers (ROBs), and 35.41%, 52.45% and 14% respectively for reciprocal translocation carriers (RECs). In D3 FISH-PGD group, 31.86%, 41.60% and 12% respectively were for ROBs, and 16.62%, 41.87% and 15% respectively were for RECs. In D5 SNP-PGD group, were 44.79%, 73.17% and 10% respectively were for ROBs, and 31.12%, 55.0% and 19% respectively were for RECs. D5 FISH-PGD was significantly better than D3 FISH-PGD in clinical pregnancy rate; and no significant difference exists compared with D5 SNP-PGD.
Limitations, reason for caution: There is a chance of selective bias due to not a RCT analysis. The final outcomes of D5 FISH-PGD and D5 SNP-PGD have not been obtained as the late pregnancy loss may occur. We cannot exclude differences between the final data and the data in the present submitted abstract.
Wider implications of the findings: The adoption of FISH-PGD combined with D5 or D6 trophectoderm biopsy and FET is an alternative approach for young translocation carriers. Due to the high cost for SNP-PGD, the couples with relative poor economic conditions may benefit from this strategy.
Study funding/competing interest(s): Funding by national/international organization(s). This work was supported by a grant from the Major State Basic Research Development Program of China (No. 2012CB944901) and National Science Foundation of China (No. 81222007). The authors have no competing interests to declare.
Trial registration number: NA.
Keywords: fluorescence in situ hybridization, preimplantation genetic diagnosis, single nucleotide polymorphism, trophectoderm biopsy, translocation

P-620 The origin and significance of additional aneuploidy events in couples undergoing preimplantation genetic diagnosis for translocations by array comparative genomic hybridisation

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Study question: To what extent do the aneuploidies that occur in addition to the translocation imbalances in embryos from carriers of translocations undergoing PGD by aCGH persist in the embryo? Do these additional abnormalities have a meiotic or mitotic origin?
Summary answer: From a total of 127 aneuploidy events seen on follow up, 106 (83%) were either concordant with the aneuploidy seen on diagnosis or showed a complementary aneuploidy event. Of the 83 embryos, 33 (39%) had meiotic and 37 (44%) had mitotic aneuploidy and 13 (15%) had both types of events.
What is known already: Diagnostic application of aCGH in PGD for reciprocal and Robertsonian translocations has revealed 45–55% embryos with additional aneuploidies with or without translocation related imbalances. The occurrence of these extra abnormalities with the balanced form of the translocation reduces the number of embryos suitable for transfer. The origin and significance of these aneuploidies can only be determined by full follow up studies, of which few have been carried out.
Study design, size, duration: This study targeted whole chromosome and segmental aneuploidies present in addition to the balanced or unbalanced translocations detected on aCGH diagnosis. Whole untransferred embryos collected on day 5/6 of embryo development were spread for FISH or tubed for aCGH analysis. Aneuploidies were classified as meiotic or mitotic after follow up.
Participants/materials, setting, methods: In total, 85 embryos from 23 infertile or sub-fertile translocation carriers (average maternal age, 35 ± 4.2 years) undergoing 31 cycles of PGD by aCGH were included. Sixty-three were followed up by FISH and twenty-two by aCGH with high resolution Agilent oligonucleotide 8 × 60 k and/or BlueGnome 24 Sure BAC microarrays.
Main results and the role of chance: Conclusive follow up results were obtained for 83/85 embryos, 63 by FISH and 20 by aCGH. From a total of 127 aneuploidy events seen on follow up, 106 (83%) were either concordant with the aneuploidy seen on diagnosis or were complementary. Meiotic aneuploidy affected 39% of embryos and 44% had mitotic events; 15% had both types. Meiotic and mitotic events were almost equal (62 versus 65), 97 affected whole chromosomes (59 meiotic, 38 mitotic) and 30 were segmental (3 meiotic, 27 mitotic). With the exception of chromosomes 4 and Y, all were found to be aneuploid, chromosome 22 was most frequently affected by meiotic errors, followed by chromosomes 15, 16 and 19. Chromosomes 2 and 19 had the highest number of mitotic anomalies, then chromosomes 3 and 16.
Limitations, reason for caution: Two methods, aCGH and FISH were used to determine the prevalence of additional aneuploidies at later stages of embryo development. When aCGH is performed on whole embryos mosaicism below the level of 25% is difficult to detect. FISH is better for assessing the exact level of mosaicism.
Wider implications of the findings: Aneuploidies of meiotic origin and the majority of abnormalities of mitotic origin were widespread in the whole embryos followed up. All the embryos diagnosed as abnormal (translocation balanced or unbalanced) after aCGH diagnosis at cleavage stage would have remained unsuitable for transfer if tested at later stages of development and those abnormalities found after diagnosis at the blastocyst stage were confirmed in the rest of the embryo. Additional aneuploidies are thus confirmed as significant findings.
Study funding/competing interest(s): Funding by University(ies) – University College London (UCL).
Trial registration number: NA.
Keywords: aneuploidy, aCGH, translocation, meiotic error, mitotic error

P-621 Polymorphism of CAG and GGN repeats in androgen receptor gene in women with polycystic ovary syndrome

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Study question: This study was designed to investigate the polymorphism of CAG and GGN repeats in AR gene, XCI pattern and the expression of AR in Chinese women with polycystic ovary syndrome (PCOS).
Summary answer: In our study, the prolonged GGN repeat in AR gene, and the increased mRNA expression of GGN trinucleotide repeat, was found in PCOS patients, suggesting that the polymorphism of GGN repeat in AR gene was associated with pathophysiology of PCOS.
What is known already: Polycystic ovary syndrome (PCOS) is a common cause of anovulatory infertility, one of characteristics of PCOS is androgen excess, hyperandrogenism or overaction of androgen, which may be related to androgen receptor (AR). The activity of AR is physiologically modulated by its polyglutamine and polyglycine tracts, variable size, in N-terminal transactivation domain. Those polyglutamine and polyglycine tracts are encoded by a highly polymorphic CAG and GGN repeat sequence in exon 1 of *AR* gene located on X chromosome.
Study design, size, duration: A total of 156 women consisting of 80 PCOS cases (aged 21–34 years) and 76 controls (aged 21–34 years) were recruited, in our center from 2012 to 2014. Ovarian tissues from 5 adults women with normal menstrual cycles (during transsexual operation) and 7 PCOS cases (during surgical treatments) were collected.
Participants/materials, setting, methods: The frequency distributions of CAG and GGN repeat alleles, as well as their X-inactivation patterns, were compared between control group and PCOS group. Expression of AR mRNA was tested by qPCR in ovarian tissues of 7 PCOS patients and 5 normal women, while cellular location of AR protein by immunohistochemistry.
Main results and the role of chance: Based on the mean biallelic trinucleotide repeat lengths, we divided the PCOS and control groups into shorter repeat (CAG ≤ 22, GGN ≤ 16) and longer repeat groups (CAG > 22, GGN > 16) to evaluate the effect of the two repeat lengths in PCOS. PCOS cases had significantly higher frequency of longer GGN biallelic mean (29.8%) and X_weighted_biallelic_mean (33.3%) than controls (6.1% and 3.2%, respectively) ($\chi^2 = 9.219$, df = 1, $p = 0.002$; $\chi^2 = 8.717$, df = 1, $p = 0.003$). The *GGN* repeat mRNA levels of ovarian tissue could be measured in both groups, and were approximately 1.8-fold higher in the controls than in the PCOS cases ($p = 0.022$).
Limitations, reason for caution: The size of participants and sample in our study is few, and we did not obtain the clinical data of control group because of ethical issue.
Wider implications of the findings: To our knowledge, this is the first report of an association between the GGN polymorphism and PCOS. Moreover, our study provides evidence of a significant relationship between mRNA expression and trinucleotide repeat length polymorphism, which should be confirmed with further research. Replication of our findings would shed light on the role of androgen metabolism in the development of PCOS and provide useful insights into the possible role of anti-androgen medication for treating this disease.
Study funding/competing interest(s): Funding by national/international organization(s). Major State Basic Research Development Program of China (973 Program) (2012CB944703, 2012CB944902), the Health Commonweal Project of China (201402004), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).
Trial registration number: Our study is not RCT.
Keywords: androgen receptor, polycystic ovary syndrome, polymorphism, hyperandrogenism, endocrinology

P-622 An association between thrombophilias and pregnancy loss: should we test?

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Study question: Are genetic variants in thrombophilia-related genes (FV Leiden, FII Prothrombin, FXII, FXIIIAI, and FGB) significantly associated with self-reported recurrent pregnancy loss (RPL) in women seeking treatment at fertility centers as compared to a general population sampled from the 1000 Genomes Project?
Summary answer: We observe a significant association of all studied variants with all women seeking fertility treatment when compared to the control group; further, we find that two variants found in FXII (rs1801020) and FXIIIAI (rs3024477) significantly differ among women with a self-reported history of RPL and the control group.
What is known already: RPL affects 1–5% of individuals diagnosed with infertility. Over 50% of cases are due to aneuploidy, but genetic variants have also been purported to increase RPL risks, such as thrombophilias. Results from studies on thrombophilias are contentious and the role they play in RPL is unclear. Consequently, the benefits of treatment plans guided by thrombophilias, such as low-dose aspirin or low-dose heparin during an at-risk pregnancy, remain a topic of debate for best practices.
Study design, size, duration: We employed a retrospective case-control study to identify significant associations between thrombophilia-related variants and RPL. A total of 2201 females referred for carrier screening and consented to research in a de-identified manner were enrolled into the study. Patients were enrolled between January 2013 and October 2014.
Participants/materials, setting, methods: Illumina's Infinium HD Genotyping Platform identified variants: FV Leiden (rs6025); FII Prothrombin (rs1799963); FXII (rs1801020); FXIIIAI (rs5985; rs3024477); FGB (rs1800790). RPL was defined as ≥2 miscarriages before 20 weeks gestation. The 1000 Genomes Project was used as the control. Fisher's Exact Test calculated significance of genotype frequencies ($p \leq 0.05$).
Main results and the role of chance: We find that all 6 tested thrombophilia variants differ significantly between all female study participants treated at fertility centers and the control group: FV Leiden (rs6025 – $p = 3.56 \times 10^{-5}$); FII Prothrombin (rs1799963 – $p = 1.14 \times 10^{-7}$); FXII (rs1801020 – $p = 2.2 \times 10^{-16}$); FXIIIAI (rs5985 – $p = 6.56 \times 10^{-7}$; rs3024477 $p = 3.43 \times 10^{-6}$); FGB (rs1800790 $p = 4.96 \times 1013$). Only FXII (rs1801020 – $p = 1.82 \times 10^{-6}$) and FXIIIAI (rs3024477 – $p = 0.00496$) significantly differ between study participants with a self-reported history of RPL and the control group. The other variants were not found to be significantly associated with self-reported RPL: FV Leiden (rs6025 – $p = 0.103$); FII Prothrombin (rs1799963 – $p = 0.565$); FXIIIAI (rs5985 – $p = 0.646$); FGB (rs1800790 $p = 0.121$).
Limitations, reason for caution: Participants were categorized for RPL based on self-reported histories taken during genetic counseling sessions for the prescribed carrier screening. Self-reported histories may not be completely accurate and we may have incorrectly categorized participants if they did not accurately report multiple miscarriages.
Wider implications of the findings: Routine thrombophilia-related testing in women diagnosed with infertility or RPL is not performed. However, our findings suggest that women diagnosed with idiopathic infertility may benefit from screening to guide reproductive treatment and pregnancy monitoring. A subset of participants may have unknowingly experienced multiple miscarriages, as 42% report never being pregnant. Women diagnosed with RPL may benefit from FXII and FXIIIAI screening. Interestingly, FXII is a phospholipid-binding protein implicated in antiphospholipid syndrome, another RPL risk.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Recombine.
Trial registration number: NA.
Keywords: thrombophilia, infertility, genetics, genetic testing, recurrent pregnancy loss

P-623 Preimplantation genetic diagnosis (PGD) for fragile X (FX) syndrome: direct allele repeat size detection and advantages of blastocyst stage biopsy

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Study question: Is blastocyst biopsy followed by embryo freezing advantageous over cleavage stage biopsy followed by fresh embryo transfer when it comes to PGD for FX with direct allele repeat size detection?

Summary answer: Diagnostic and clinical outcome data showed significant advantages of repeat size detection in trophectoderm samples for FX PGD. Furthermore, higher implantation and pregnancy rates were observed for FX patients who had undergone PGD cycles involving blastocyst biopsy followed by embryo cryopreservation versus cleavage stage biopsy with subsequent fresh transfer.
What is known already: PGD allows the selection of embryos that are unaffected by inherited disorders following *in-vitro* fertilization (IVF). The main difficulty for FX patients is that some carriers have primary ovarian insufficiency, making it difficult to stimulate the ovaries to produce the multiple eggs necessary for PGD. On the technical side, limited amounts of embryonic material available for the diagnosis makes it difficult to detect the alleles' repeat sizes using standard polymerase chain reaction (PCR) methods.
Study design, size, duration: Data were collected from 27 cleavage stage and 22 blastocyst FX PGD cycles (average maternal age of 29.2 and 35.8, respectively) performed between January 2012 and December 2014. A total of 216 cleavage stage and 115 blastocyst embryos were assessed (on average 8 and 5.2 embryos per cycle, respectively).
Participants/materials, setting, methods: Single blastomere or trophectoderm biopsied samples were sent from 25 different IVF clinics in the USA to a single reference PGD laboratory. DNA was processed and analysed via capillary electrophoresis and Karyomapping (Illumina, USA). When requested, comprehensive chromosome screening (CCS) was also performed in parallel to FX testing for blastocysts.
Main results and the role of chance: A diagnosis was obtained for 94.8% (109/115) of blastocysts and 88% (190/216) of cleavage stage embryos ($p = 0.05$). Linkage analysis testing was carried out for all samples. Repeat size assessment and CCS were only possible for blastocyst biopsies. The proportions of embryos found to be available for transfer after linkage analysis alone was 43.7% for cleavage stage and 56.9% for blastocysts. Repeat size assessment indicated 22.9% more blastocysts (having intermediate or premutation allele repeat range results) to be considered for transfer. Despite of the additional CCS, 47.7% of blastocysts were considered for transfer showing similar proportion to the cleavage stage group. Preliminary clinical outcome data showed a significantly higher pregnancy rate for blastocysts compared to cleavage stage embryos ($p = 0.04$); 72.7% (8/11) vs. 33.3% (3/12), respectively.
Limitations, reason for caution: Although knowing repeat size increases the number of embryos available for replacement, transfer of embryos with premutations should be done only after thorough consultation with the patient since individuals with premutations are at increased risk for fragile X-associated symptoms.
Wider implications of the findings: Even though less embryos were available for transfer (52 vs. 83), the blastocyst stage testing strategy followed in this study resulted in significantly higher pregnancy rates compared to cleavage stage. Opting for an IVF/PGD strategy that enables better embryonic selection and more comprehensive diagnosis, leading to achievement of a healthy pregnancy in a shorter period of time is advantageous, especially in cases hindered by poor ovarian response.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Institutional funding. None of the authors have any competing interests.
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, cleavage stage, blastocyst, fragile X

P-624 Maternal dietary exposure to bisphenol-A (BPA) increases embryo size and alters key epigenetic regulators in the blastocyst

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Study question: What role does maternal BPA ingestion play in the morphology and epigenetic regulation of preimplantation embryos?
Summary answer: Maternal dietary BPA exposure is associated with increased blastocyst size, as well as upregulation of a pluripotent gene, possibly via DNA methylation.
What is known already: BPA exposure may contribute to increasing infertility rates. BPA negatively impacts embryo transport, development, and implantation in mice. BPA exposure has been reported to alter the murine epigenome in somatic cells and alter imprinting genes in post implantation embryos. Preimplantation embryo development is a time of extensive epigenetic reprogramming. The plasticity of the preimplantation embryo may render it vulnerable to environmental insults, such as BPA exposure.
Study design, size, duration: In this IACUC approved animal study, female Sprague Dawley rats were fed AIN control or 250 mg/kg body weight/day BPA diets during the periconceptional time period. A total of 119 control (10 confocal imaged, 50 PCR analyzed) and 96 BPA (5 confocal imaged, 50 PCR analyzed) embryos were obtained.
Participants/materials, setting, methods: Embryos were retrieved from the uterine horns on gestational day 5 then measured using AxioVision software. Blastocysts were fixed, DAPI stained, and imaged using confocal microscopy. Total RNA was isolated, followed by reverse transcription and quantitative real-time PCR for *18S, Oct4, Dnmt1, Dnmt3a, Dnmt3b, Tet1, Tet2,* and *Tet3*.
Main results and the role of chance: A significant difference in the average area (control 5768.87 $\mu m^2 \pm 1038.60$ vs BPA 6649.37 $\mu m^2 \pm 1386.06$; $p < 0.01$) was noted between groups. There was no difference in the number of cells per embryo (control 23.6 nuclei ± 7.47 vs BPA 23 nuclei ± 8.86; $p = 0.90$). Significant upregulation of pluripotency gene *Oct4* was noted in BPA exposed embryos (fold change 4.32; $p < 0.01$). Epigenetic enzymes involved in *de novo* methylation, *Dnmt3a* (fold change 1.80), *Dnmt3b* (fold change 1.52), *Tet1* (fold change 2.05), *Tet2* (fold change 1.43), and *Tet3* (fold change 52.61), were up regulated in BPA exposed embryos, while methylation maintenance enzyme *Dnmt1* (fold change 0.52) was down regulated, though these changes were not statistically significant.
Limitations, reason for caution: We noted that some epigenetic enzymes may be dysregulated by BPA. However, due to individual variation, significant differences were not detected. Increased sample size will aid in clarifying the role of BPA in modulating the epigenome of preimplantation embryos and the impact of epigenetic dysregulation in advancing embryo morphology.
Wider implications of the findings: Aberrations in *de novo* methylation and methylation maintenance in BPA exposed embryos may be related to increased pluripotency gene expression and embryo size. Given that advanced embryo morphology is often used as a good prognostic feature for embryo selection in the *in vitro* fertilization laboratory, BPA exposure could result in selection for epigenetically dysregulated embryos using this strategy. Additionally, there may be health consequences for the resulting BPA exposed offspring due to the Barker hypothesis.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s). Funding by commercial/corporate company(ies). Center for Environmental Genetics New Investigator Scholar Award NIH/NIEHS P30ES006096. Center for Environmental Genetics Innovator Award. NIH/NIEHS P30ES006096. NIEHS ES019480. NIEHS ES020988. The Patty Brisben Foundation.
Trial registration number: NA.
Keywords: epigenetics, methylation, BPA, blastocyst, morphology

P-625 A combined day 5/day 6 trophectoderm biopsy strategy followed by frozen embryo transfers can maximize the embryo utilization and clinical outcome in comprehensive chromosomal screening (CCS) cycles

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Study question: This study questions whether performing a trophectoderm biopsy on embryos that developed into good quality blastocysts on day 6 bring additional benefits in the cycle outcome as compared to day 5 biopsies in CCS cycles.
Summary answer: Day 6 trofectoderm biopsy increases the total number of analyzable as well as chromosomally normal embryos at least by 25%. Also, among the embryos biopsied, the distribution of chromosomal abnormalities as well as clinical pregnancy rates are found to be similar for both day 5- and day 6-blastocysts.
What is known already: Contemporary preimplantation genetic screening (PGS) requires a trophectoderm biopsy on developing good quality blastocysts.

However, not all fertilized embryos develop to blastocyst stage at similar rates. If fresh transfer is planned, only the embryos that are available for biopsy on day 5 are taken into account for analysis. However, some embryos in the same cohort can only reach the blastocyst stage on day 6 hence are not included in the same analysis. The data regarding the chromosomal status as well as clinical performance of day 6 biopsied embryos are scarce and conflicting.

Study design, size, duration: This retrospective comparative study has been performed in Bahceci Fulya Assisted Reproductive Technology Centre between January 2013 and December 2014. It includes 425 consecutive CCS cyles in which 1155 blastocysts were biopsied and analyzed for 24 chromosomes on day 5 and day 6.

Participants/materials, setting, methods: Patients in the study group were CGS candidates having advanced maternal age, recurrent implantation failure and recurrent abortion or combinations. According to the nature of the CCS strategy, whenever the embryos have reached the hatching blastocyst stage, they were biopsied and immediately vitrified after the biopsy. Once a chromosomally normal embryo was found, embryo transfer (ET) was planned for the next suitable time frame with hormone replacement therapy. Within the study period, 743 embryos were biopsied on day 5 and 412 on day 6 and vitrified individually.

Main results and the role of chance: Mean female age in the study group was 36.1, ± 5.1. A total of 146 ETs were performed in these cases. In 102 cycles, utilization of only day-5 biopsied blastocysts gave a 59.8% pregnancy rate while in 44 cycles, transfer of only day-6 biopsied blastocysts resulted in 59.0% pregnancy rate. Results of the CCS analysis as well as the distribution of chromosomal abnormalities for each biopsy day are shown in table.

Day of embryo biopsy	Day 5	Day 6
# of embryos biopsied	743	412
# of embryos analyzed	717 (96.5%)	390 (94.7%)
Normal	180 (25.1%)	100 (24.2%)
Abnormal	537 (74.8%)	290 (70.3%)
Complex abnormalities	115 (21.4%)	59 (20.3%)
Aneuploidies involving a single chromosome	281 (52.3%)	167 (57.8%)
Trisomy	153 (54.4%)	86 (51.5%)
Monosomy	82 (29.2%)	61 (36.5%)
deletion/duplication	46 (16.4%)	20 (12.0%)

Limitations, reason for caution: High risk of embryonic aneuploidies due to the nature of the cases as well as the limited number of the embryos available for ET can be the limitations in this study.

Wider implications of the findings: Our results show that in CCS cycles, biopsy and analysis of day 6 blastocysts should not be ignored since it can bring additional benefit in the clinical outcome by increasing the probability of finding normal embryos as well as successful pregnancies in the routine preimplantation genetic screening programme.

Study funding/competing interest(s): Funding by hospital/clinic(s). This study received no funding and the authors do not have any competing interests.

Trial registration number: NA.

Keywords: PGS, trophectoderm biopsy, comprehensive chromosomal screening, frozen embryo transfer

P-626 Comparison of clinic outcomes of preimplantation genetic screening (PGS) in elder patients, repeated miscarriage or those once with fetus of abnormal CGH

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Study question: To retrospectively compare the differences of miscarriage rate after PGS with whole genome amplification (WGA) and single-nucleotide polymorphisms (SNPs) between advanced maternal age (AMA), repeated miscarriage (RM) and former abortion with abnormal fetal CGH (AC).

Summary answer: PGS show a tendency of more advantages in decreasing miscarriage rate in AMA and AC compared to RSA group.

What is known already: It is supposed that PGS can decrease miscarriage rate by a better selection of euploid embryos. Present indications of PGS include AMA and RM, but which patients group will benefit more from PGS is still in debate.

Study design, size, duration: Patients with unexplained recurrent miscarriage (≥3 repeated spontaneous miscarriage, $n = 38$), ≤2 miscarriage but with abortus of abnormal CGH (AC, $n = 36$) or of advanced maternal age (AMA, $n = 27$) were selected for PGS by SNP. The study was carried out from September 2010 to November 2013. Only those have at least one transferrable blastocysts were selected.

Participants/materials, setting, methods: Laser assisted trophectoderm biopsy was performed on day 5 or 6 post oocytes retrieval. No more than 5 trophoblast cells were biopsized and then vitrified. Less than 2 blastocysts were transferred in the FET cycles. Clinic pregnancy rate (PR), miscarriage rate (MR) and CGH analysis of abortus was measured.

Main results and the role of chance: Of the 101 cycles, PR was relatively lower in AMA (37.04%, 10/27) than in AC (66.67%, 24/36) and RM (68.42%, 26/38) ($p < 0.05$), possibly because of the differences in maternal ages (38–45, 23–42, 30–44, respectively). But all the 10 pregnant women in the AMA group go well through the two trimester, i.e., no one result in miscarriage, while miscarriage rate of AC (12.50%, 3/24) also show a tendency to be lower than that of RM group (26.92%, 7/26) ($P > 0.05$). Of the 10 miscarriage cases, 7 performed CGH and no abnormalities were found with the abortus.

Limitations, reason for caution: The study is limited for its retrospective design. A statistic difference may be reached only after we increase the sample size.

Wider implications of the findings: Our study shows that PGS decrease the miscarriage rate in AMA, mainly by rule out aneuploidy embryos. The miscarriage rate in RM remains high, possibly due to unresolved pathologies causing spontaneous miscarriage. Therefore PGS is a useful tool for AMAs.

Study funding/competing interest(s): Funding by University(ies) – No applicable.

Trial registration number: NA.

Keywords: PGS, miscarriage rate, RM, AMA

P-627 Mosaicism and DNA methylation at imprinted genes varies with severity of oligozoospermia in infertile men

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Study question: Does DNA methylation at imprinted genes display increased abnormalities with increasing severity of oligozoospermia in infertile men?

Summary answer: Our data suggests that DNA methylation at imprinted genes does display errors that increases with the severity of oligozoospermia in infertile men, however, the men with the most severe oligozoospermia did not display the greatest number of errors. We also observed a similar pattern for mosaicism in sperm methylation.

What is known already: Previous studies have shown that there may be an upper threshold of sperm concentration, below which DNA methylation errors at imprinted genes become more prevalent in the sperm of infertile men.

Study design, size, duration: Our case control study includes a total of 50 patients, which includes nine fertile control men and 41 oligozoospermic men, subdivided into three categories based on sperm concentration: eight with 5–15 million/ml, 23 with 1–5 million/ml, and 10 with <1 million/ml.

Participants/materials, setting, methods: We examined the methylation status of three imprinted genes (*H19*, *IG-GTL2*, and *MEST*) in the ejaculate sperm of reproductive age men using bisulfite cloning and sequencing. We categorized clones into four groups based on the percentage of abnormal methylation present. DNA fragmentation and *MTHFR* genotype were examined.

Main results and the role of chance: We analyzed DNA methylation at three imprinted regions in 851 clones, in which DNA fragmentation and *MTHFR* genotype were not found to be significant confounding factors. Furthermore, these clones underwent exclusion criteria to reduce amplification bias. Clones with altered DNA methylation in the *H19* gene were found more frequently in men with oligozoospermia, specifically those with a sperm concentration of 1–5 million/ml compared to fertile control men. These men showed significantly fewer clones with normal methylation (51% vs. 75% in controls; $P = 0.006$). Furthermore, these men also showed significantly more clones that were completely abnormally methylated (10% vs. 0% in controls; $P = 0.034$). Inter- and intra-individual mosaicism in sperm methylation was observed consistently throughout the study.

Limitations, reason for caution: The number of patients in each category of oligozoospermia are not the same. There may be potentially significant findings in the other categories if the sample sizes were similar. Additionally, only a few sperm were cloned per patient.
Wider implications of the findings: Our results support other studies showing methylation defects at the *H19* gene in the sperm of oligozoospermic men. Additionally, we show that DNA methylation may not be a contributing factor to male infertility in the most severe cases of oligozoospermia. The aetiology of these cases may not have a significant epigenetic component. We speculate that mosaicism in sperm methylation may also be characteristic of infertility.
Study funding/competing interest(s): Funding by national/international organization(s) – Canadian Institutes of Health Research.
Trial registration number: NA.
Keywords: DNA methylation, mosaicism, oligozoospermia, DNA fragmentation, MTHFR genotype

P-628 Carrier screening of 58,000 patients in the IVF setting utilizing next generation DNA sequencing detects common, rare and otherwise undetectable mutations in prevalent, society-recommended diseases

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Study question: The objective of this study was to evaluate the clinical effectiveness of NGS in detecting common and rare mutations across a large number of patients in a clinical setting, among 14 diseases recommended for carrier screening.
Summary answer: Due to the more extensive set of pathogenic mutations detectable for the genes assessed, NGS enables more comprehensive examination of carrier status, and is, therefore, able to yield higher detection rates resulting in fewer missed carriers than if traditional carrier tests were used.
What is known already: Carrier screening for specific genetic disorders is recommended by ACOG, ACMG, and societies representing the Ashkenazi Jewish population. Due to cost considerations and limitations of the technologies employed, traditional carrier screening assays are designed to look for only the most common mutations within a gene. This older approach can yield high detection rates in specific populations (e.g., the Ashkenazi Jewish); however, it's suboptimal for other ethnicities or for patients of mixed or unknown ethnic background.
Study design, size, duration: Using NGS, we evaluated carrier status for up to 14 disorders (as ordered by physicians in IVF centers) for 58,338 patients.
Participants/materials, setting, methods: A high-throughput and proprietary methodology (comprised of multiplex gene capture, NGS and computational analysis) has been applied to over 58,000 patient DNA samples across ~400,000 individual tests, as of this writing. Clinical reports were issued on the presence or absence of disease-causing mutations in genes associated with society-recommended, recessive genetic disorders.
Main results and the role of chance: Among the first 58,338 clinical samples evaluated, our NGS-based tests routinely detected common mutations among 14 disorders, as well as numerous rare mutations that would not be detected by traditional screening assays. 2768 (4.7%) of patients were found to be carriers of 403 distinct pathogenic mutations among the 14 diseases. Of the 2768 carriers detected, 13.2%–20.9% would have been missed by traditional carrier tests, putting these reproductive couples at increased risk of conceiving a child with a debilitating or fatal genetic disorder.
Limitations, reason for caution: Not applicable.
Wider implications of the findings: Next generation DNA sequencing (NGS) is able to detect five- to ten-fold the number of pathogenic mutations with potentially higher detection rates in all ethnicities compared to traditional carrier screening tests. Consequently, NGS is expected to provide a more comprehensive determination of carrier status.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Good Start Genetics, Inc., Cambridge, MA. All authors are employees of Good Start Genetics.
Trial registration number: NA.
Keywords: NGS, next generation DNA sequencing, carrier screening, pathogenic, IVF

P-629 Significant skews in methylation fractions in previously described genotypes and sub-genotypes of the FMR1 gene

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Study question: Since genotypes and sub-genotypes of the *FMR1* gene have been associated with different ovarian aging patterns, we here investigated whether patterns of methylation skew differ among them, and whether AMH levels, reflective of functional ovarian reserve (FOR, i.e., the small growing follicle pool) are affected by *FMR1* methylation patterns.
Summary answer: Women with normal (*norm*) *FMR1* genotypes ($CGG_{n=26–34}$) differed significantly in mean skew of methylation from women with heterozygous (*het*) genotypes (one allele either $CGG_{n<26}$ or $CGG_{n>34}$). With *norm FMR1* increasing skew was associated with increasing AMH but with *het FMR1* with decreasing AMH.
What is known already: As previously reported [PLoS ONE 2010; 5 (12): e15303], patients can in their *FMR1* gene be classified as *norm* if both alleles are in normal CGG range (n = 26–34), as homozygous (*hom*) if both alleles are outside normal range and as *het* if only one allele is outside normal range. *Hom* and *het* patients can be further divided into sub-genotypes *low* ($CGG_{n<26}$) and *high* ($CGG_{n>34}$). Women with *norm*, *het-low* and *het-high* and *hom FMR1* demonstrate distinctively different declines in FOR, as assessed by AMH and, as we also previously reported, in methylation patterns of the *FMR1* gene.
Study design, size, duration: We here prospectively in a collaborative study between an academically affiliated private fertility center (CHR) and a laboratory corporation with a novel proprietary *FMR1* assay (Asuragen), investigated 74 patients over a 6 month period.
Participants/materials, setting, methods: 52 infertility patients and 12 egg donors were evaluated at their initial consultation for CGG repeat numbers on the *FMR1* gene, methylation and serum AMH, using a high performance *FMR1*PCR (Chen et al., Genet Med 2011; 13: 528–538). This assay allows for determination of CGGn, intermittent AGG genotypes and methylation status of both allele.
Main results and the role of chance: Average age was 34.3 ± 7.2 years (patients 36.6 ± 5.9; donors 24.5 ± 2.4). After adjustments for age and donor/patient status there was a significant difference in mean skew between patients with *norm FMR1* and *het FMR* genotypes (including *het-norm/low* and *het-norm/high* subgenotypes; *norm*, 9.6 ± 7.13; *het*, 15.8 ± 10.2, P = 0.008). Increasing skew was associated with increased baseline AMH among *norm FMR1* patients, while *het* patients demonstrated decreasing AMH with increasing skew (P for interaction = 0.039). No significant differences in methylation percentages or skew were observed between donors and patients.
Limitations, reason for caution: Because of small sizes of study groups, here presented findings should be considered preliminary.
Wider implications of the findings: Neither in reproductive medicine nor in other specialty areas affected by the *FMR1* gene, is the molecular biology of the gene yet well understood. Here presented findings suggest that increasing methylation skew affects AMH values in opposite ways in women with *norm* and *het FMR1* genotypes, thus suggesting a possible role of methylation skew in the gene's function, calling for further exploration.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for Human Reproduction; Foundation for Reproductive Medicine; Asuragen, Inc.
Trial registration number: NA.
Keywords: fragile X mental retardation (FMR1) gene, anti-Mullerian hormone (AMH), functional ovarian reserve (FOR), methylation

P-630 Preimplantation genetic screening (PGS) in IVF cycles with frozen embryo transfer (FET) – 2 year experience of one PGS center

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Study question: Does preimplantation genetic screening (PGS) improve pregnancy rates (PRs) in IVF-cycles with embryo transfers (ET) of frozen embryos? Do PGS results and clinical outcome differ in relation to advanced maternal age (AMA)?
Summary answer: Our results demonstrate an overall increase in PRs after PGS. Although the proportion of aneuploid embryos strongly increases with AMA, PRs did not significantly differ from younger patients when PGS was applied. PGS can overcome adverse effect of AMA.
What is known already: The benefit of comprehensive chromosomal screening is still controversially discussed. Recently, a significant increase in PRs in young good-prognosis-patients was reported when performing PGS on trophectoderm (TE) samples by aCGH followed by fresh ET. Further, a benefit of PGS was stated in patients with AMA. Various factors, e.g., effect of post-zygotic mosaic, biopsy procedure, embryo classification prior biopsy, contributing sperm factor, trial bias, number of embryos transferred to mention just a few, influence reported results.
Study design, size, duration: PGS data from a 2 year period (2013–2014) after FETs were retrospectively analyzed in correlation to maternal age. Patients were subdivided into 3 age groups. Clinical outcome (pregnancy rates) was compared with age-matched outcome of FETs without PGS (noPGS). PGS analysis was performed on TE samples by aCGH (BlueGnome 24 sure).
Participants/materials, setting, methods: Group I: ≤32 year: (A) PGS/FET: 27 egg donors; 192 TE-samples; 24 FETs; (B) noPGS/FET: 372 FETs. Group II: ≤38 year: (A) PGS/FET: 30 patients; 162 TE-samples; 19 FETs; (B) noPGS/FET: 200 FETs. Group III: >38 year: (A) PGS/FET: 61 patients; 261 TE-samples; 28 FETs; (B) noPGS/FET: 95 FETs. For statistical analysis Chi-Square test was used.
Main results and the role of chance: Although the proportion of aneuploid embryos was clearly increasing with AMA (group IA: 86/192; 45%; group IIA: 94/162; 58%; group IIIA: 195/261; 75%, $P < 0.00001$), PRs did not significantly differ among the PGS groups (IA: 16/24; 67%; IIA: 13/19; 68%; IIIA: 16/28; 57%, P 0.674). Overall PR after PGS was significantly improved in comparison with FETs without PGS (45/71; 63% vs. 329/666; 49%, P 0.025). Comparing PRs in individual age groups there was improvement seen in all 3 groups. However, only in the PGS group ≤38 year significant difference could be observed in comparison to the non-PGS group (group IA: 16/24; 67% vs. group IB: 219/372; 59%, P 0.451; group IIA 13/19; 68% vs. group IIB: 74/200; 37%, P 0.008; group IIIA: 16/28; 57% vs. group IIIB: 36/95; 38%, P 0.077).
Limitations, reason for caution: It is known that number and probability of euploid embryos decreases with age. Further, a number of embryos are not eligible for TE-biopsy, and PGS-results are sometimes not interpretable. Several stimulated cycles might be necessary to perform one ET, therefore PGS might not be the best therapy for all AMA-patients.
Wider implications of the findings: PGS is a useful method for choosing viable embryos for FET, particularly in patients with AMA since the proportion of aneuploid embryos significantly increases in the older patients groups. According to our results PGS followed by FET improves PR in comparison with FET without PGS. However, further studies of large cohort of patients are needed to confirm these findings.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Zentren Prof. Zech.
Trial registration number: No trial registration number.
Keywords: PGS, AMA, FET, trophectoderm, chromosome

P-631 Endogenous retrotransposon expression and de novo retrotransposition events after incorporation of exogenous retroelements in human spermatozoa

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Study question: To investigate the expression of L1, HERV-K10 and SVA retrotransposons in human spermatozoa as well as the potential incorporation of exogenous human and mouse retroelements in human sperm genome favoring new retrotransposition events.
Summary answer: Our results show that (a) L1, HERV-K10 and SVA retrotransposons are transcriptionally active in human spermatozoa and (b) the spontaneous interaction of human spermatozoa with exogenous human and mouse retroelements and their subsequent incorporation into their genome result in *de novo* retrotransposition events.
What is known already: Retrotransposons have played a crucial role in the human genome structure and evolution. Retrotransposition events in human oocytes have been well documented, but their presence in human spermatozoa is still unclear. Mammalian spermatozoa can spontaneously bind exogenous DNA molecules and internalize them into their nucleus. The presence of a functional endogenous reverse transcriptase in murine mature spermatozoa, which has the ability to convert the internalized molecules into cDNA copies, explains the occurrence of retrotransposition events.
Study design, size, duration: Retrotransposon RNA expression was studied in fifty semen samples, 25 from normozoospermic and 25 from oligozoospermic patients. The potential incorporation of exogenous retroelements in human spermatozoa was studied in 108 men, incubating spermatozoa with human LINE-1, HERVK-10 and mouse VL30 retrotransposons, in a period of 2 years.
Participants/materials, setting, methods: RT-PCR analysis was performed in order to confirm the retrotransposon expression in ejaculated human spermatozoa. Exogenous retroelements were tagged with an enhanced green fluorescence (EGFP) gene-based retrotransposition cassette and the *de novo* retrotransposition events were tested using PCR, FACS analysis and confocal microscopy.
Main results and the role of chance: The RT-PCR products detected in human semen samples were specific transcripts of L1, HERV-K10 and SVA elements, providing evidence for retrotransposon expression in spermatozoa. Human sperm cells are capable of internalizing exogenous EGFP-tagged retroelements into their genome, favoring retrotransposition events. The 16.67% of the samples analyzed by FACS and PCR were positive for retrotransposition. The respective retrotransposition frequencies for the L1, HERV-K10 and VL30 retrotransposons were 0.34 ± 0.13%, 0.37 ± 0.17% and 0.3 ± 0.14% per sample containing 10000 spermatozoa. The retrotransposition frequencies were not affected significantly by the duration of incubation, while the increase of plasmid DNA concentration was accompanied by a slight increase of the retrotransposition rates.
Limitations, reason for caution: The possible truncation of retroelements after a retrotransposition event as well as the inadequate fluorescence detection due to very low EGFP expression levels might compromise the retrotransposition rates.
Wider implications of the findings: Controlled retrotransposition events may be beneficial for the quality of human spermatozoa and their contribution to the zygote, whereas the uncontrolled retrotransposon expression and the subsequent genome remodeling could explain the defective development, function and genetic constitution of human spermatozoa and the fairly high rates of *de novo* genomic aberrations in human embryos.
Study funding/competing interest(s): Funding by national/international organization(s). This study has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.
Trial registration number: NA.
Keywords: HERV-K10, human spermatozoa, LINE-1, retrotransposon expression, SVA

P-632 Is interchromosomal effect (ICE) related to the sex of the translocation carrier?

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Study question: To ascertain if the sex of the carrier influences the rate of aneuploidy for chromosomes not involved in the translocation.
Summary answer: Embryos from couples with a male translocation carrier have a higher chance of having aneuploidy or complex abnormal embryos with abnormalities – unrelated to the chromosomes involved in the translocation compared to embryos from couples with a female translocation carrier.
What is known already: The first studies on sperm for male translocation carriers via FISH mostly supported the theory of ICE. Further FISH studies of ICE on embryos from couples with one translocation carrier were limited because of low number of analyzable chromosomes and small sample size. Due to these limitations, some studies had contradictory results. Currently, Comprehensive Chromosome Screening techniques are able to study all chromosomes making them the most useful tools for ICE studies on embryos.
Study design, size, duration: This is a retrospective study involving 78 reciprocal translocation carrier cycles and 559 embryos. This study extended from cases tested between 6/11/2013 and 4/7/2014.
Participants/materials, setting, methods: This is a retrospective study involving 78 reciprocal translocation carrier cycles and 559 embryos. This study extended from cases tested between 6/11/2013 and 4/7/2014.
Main results and the role of chance: (a) Overall, the chances of having a normal or balanced embryo in reciprocal translocation carrier cycles is 17.9% regardless of the carrier's sex. (b) 50% of the embryos that are balanced or normal for the translocation have abnormalities involving other chromosomes. (c) Female carriers showed a higher proportion of unbalanced embryos (60.3%) than male carriers (55.0%), but with no statistical significant value. Male carriers produce a higher number of abnormal but not unbalanced embryos (20.5%) compared to female carriers (13.2%), and these differences are statistically significant ($p = 0.05$), pointing out an ICE effect on the chromosomal segregation of male translocation carriers. (d) The average maternal age for both groups is about the same: 33.62 for the female carrier group and 33.69 for the male carrier group.
Limitations, reason for caution: This study does not consider male factor effect. Age of male partner is also not considered.
Wider implications of the findings: The ICE effect may explain higher percentages of aneuploidy in embryos in male carriers with other structural abnormalities.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – N/A.
Trial registration number: NA.
Keywords: ICE, PGD, translocation, aCGH, embryo

P-633 Reverse transcriptase inhibition by lamivudine and nevirapine impairs gametogenesis in mice

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Study question: To explore the effect of reverse transcriptase inhibition on gametogenesis in male and female mice with the antiretroviral drugs Lamivudine and Nevirapine and examine testicular and ovarian defects concerning the growth of germ cells, the morphology, the DNA integrity and their capability for fertilization.
Summary answer: Our results show that the reverse transcriptase inhibition by the use of antiretroviral drugs impairs the normal process of gametogenesis. We observed degeneration on the seminiferous tubules structure, increase on sperm cell morphological abnormalities and DNA degradation. In ovaries the inhibition resulted in the formation of multiple cysts and anovulation.
What is known already: Reverse transcriptase (RT) controls RNA virus life cycle and has been the target of many antiretroviral drugs used in HIV and Hepatitis patients. RT expression in cells is related to retroelements and is usually high in gametes, embryos, non-differentiated cells and cancer tissues, thus indicating a role in the process of cell growth and differentiation. Antiretroviral drugs affect semen quality in HIV and Hepatitis patients and impair preimplantation development in mouse embryos.
Study design, size, duration: Immature male and female FVB/N mouse 21 days old, were treated with a nucleoside analog reverse transcriptase inhibitor Lamivudine in a dose of 50 mg/kg and a non-nucleoside reverse transcriptase inhibitor Nevirapine in a dose of 30 mg/kg, for 7 weeks. After treatment, sperm parameters, testicular and ovarian morphology were examined.
Participants/materials, setting, methods: Testes and ovaries were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin. Histological examination was made under light microscopy. Papanicolaou staining used for sperm morphology analysis and SCSA analysis for DNA sperm integrity. *In vivo* and *in vitro* fertilization of male and female gametes was also checked.
Main results and the role of chance: The inhibition of reverse transcriptase via the use of antiretroviral drugs markedly impairs gametogenesis in male and female reproductive organs in mice. Seminiferous tubules showed signs of apoptotic Sertoli cells and deregulation of sperm cell production and differentiation. Sperm cells DNA integration was adversely affected and sperm morphological abnormalities were increased. *In vivo* and *in vitro* capacity of fertilization was not affected, although prolonged mating period was observed. Ovaries were characterized by the presence of three times higher number of antral and very few corpus luteum. We conclude that the reverse transcriptase inhibition impairs the process of gametogenesis possibly through the disruption of normal cell growth and differentiation. We also examined the side effects of antiretroviral drugs on other molecular targets apart from reverse transcriptase.
Limitations, reason for caution: Non – nucleoside reverse transcriptase inhibitors and especially nucleoside analog reverse transcriptase inhibitors exert a toxic effect on mice testes and ovaries not only through the inhibition of reverse transcriptase but also through other pathways such as mitochondrial activity inhibition on DNA polymerase.
Wider implications of the findings: Our findings show that the use of antiretroviral drugs affects the process of spermatogenesis and oogenesis in mice and potentially the reproductive outcomes of HIV and Hepatitis patients. Men and women, undergoing such treatment should be regarded as a high risk infertility group in need for a specialized approach.
Study funding/competing interest(s): Funding by national/international organization(s). This study has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.
Trial registration number: NA.
Keywords: gametogenesis, reverse trascriptase inhibition, lamivudine, nevirapine

P-634 Comparison of egg donation cycle outcome with morphologically high quality blastocyst transfer and genetic embryo selection through PGS

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Study question: Is an egg donation cycle with one transfer of 2 euploid blastocysts after genetic embryo selection through PGS a superior method of treatment when compared to an egg donation cycle outcome with 3 double morphologically high quality blastocyst transfers.
Summary answer: Through the genetic selection of blastocysts using PGS we are giving patients the highest chance of becoming pregnant in one double blastocyst transfer procedure with a pregnancy rate of 95% per transfer. This makes PGS a method of choice in egg donation cycles in the future.

What is known already: For many women today egg donation represents the final step in infertility treatment. They delay family planning due to the cultural revolution not being aware that their best quality eggs are those when they are under 35 years of age. Combining egg donation treatments with genetic screening we may achieve the most efficient treatment option, decreasing the perinatal risks by performing only eSET as PGS showed for IVF cycles.
Study design, size, duration: In 2014 retrospectively we reviewed outcome of 80 egg donation treatments. Exclusive egg Donation (16–20 fresh donor eggs guaranteed) was offered to both groups. First group of 40 patients, six blastocysts were used in three double-embryo transfers. Second group of 40 patients euploid blastocyst selection by PGS took place.
Participants/materials, setting, methods: All 80 donors were under 30 years old, proven fertility, stimulated in the same pattern with rFSH 250 IE. All 80 recipients were enrolled after exclusion of anatomical factors. Male factor was excluded, through enrolment of normozoospermic patients.
Main results and the role of chance: Retrospectively we reviewed outcome of two groups with Exclusive egg donation treatment with genetic selection of blastocysts and without it. In the first group after 3 double blastocyst transfers of morphologically high quality, we achieved a 72.0% pregnancy rate per transfer and a cumulative pregnancy rate of 86.0% per cycle. In the second group with the addition of PGS and identification of euploid embryos, we observed 95% pregnancy rate after just one double-embryo transfer ($p = 0.006$) and mean euploidy rate of 66.3% ± 10.1. Through the genetic selection of blastocysts, patients are given the highest chance of becoming pregnant in one double blastocyst transfer procedure with a pregnancy rate over 95% per transfer. This makes PGS a method of choice in egg donation cycles in the future.
Limitations, reason for caution: Trophectoderm biopsy is an invasive procedure, where few cells are removed and sent for genetic analysis. Blastocyst is then frozen until the results and transfer day. There is a fear that through poor vitrification not all biopsied embryos survive and with fresh blastocyst transfer we could achieve better results.
Wider implications of the findings: Previously reported 32% blastocyst aneuploidy rate obtained from IVF cycles of women under 35 years old cannot be confirmed in our study. Our retrospective analysis shows blastocyst euploidy rate between 10 and 70% per cycle in young egg donors. We cannot rely on donor's age and speculate blastocyst euploidy. Each morphologically good blastocyst must be individually screened through PGS to be sure of its euploid core in order to obtain the highest implantation and pregnancy rates.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University associated Private clinic and its Foundation.
Trial registration number: NA.
Keywords: egg donation, PGS, euploidy

P-635 The impact of next-generation sequencing (NGS) preimplantation genetic diagnosis (PGD) on pregnancy rate after frozen blastocyst embryo transfer

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Study question: To assess pregnancy rate after comprehensive chromosome screening using next-generation sequencing-based preimplantation genetic diagnosis (NGS PGD) in trophectoderm after frozen blastocyst embryo transfer.
Summary answer: We confirm that NGS can be applied clinically for the purpose of detecting aneuploidy in human preimplantation embryos. Furthermore, data from this trial indicated that trophectoderm biopsy followed by frozen transfer of euploid embryos was associated with very good implantation and clinical pregnancy rates.
What is known already: Currently used PGD methods of aneuploidy screening have been reported to improve pregnancy rates. However, most of them are based on low-density array comparative genomic hybridisation (aCGH), with results based on less than 2700 probes. New technical possibilities, such as next-generation sequencing (NGS) methods, have shown promise of improved genetic diagnostics.
Study design, size, duration: Prospective case control study included analysis of results from 114 embryos obtained from 32 patients (mean age 37) performed between September 2014 and November 2014 at INVICTA Fertility Centre, Poland.
Participants/materials, setting, methods: Embryos were cultured to blastocyst stage, trophectoderm biopsy was performed. Torrent Suite Software and was used for chromosome copy number variation analysis.
Main results and the role of chance: We transferred 43 (1.3 per transfer) vitrified blastocysts during frozen embryo transfer in the investigated group and 50 (1.5 per transfer) in the control group, resulting in a total of 20 embryos implanted in the PGD group and 12 in the control group. The primary outcome measure of pregnancy rate per transfer was approximately 25% higher in the PGD group compared to the control group (59.4% vs. 35.3%, respectively; $p < 0.04$). The difference in the implantation rate (secondary outcome measure) was greater than 20% (46.5% vs. 24.0%; $p < 0.02$).
Limitations, reason for caution: Limitations of this study are small size of the investigated group and lack of randomization.
Wider implications of the findings: These findings suggest that NGS may be a useful tool for embryo selection that gives normal embryos priority for transfer. NGS technology allows personalisation of the method and therefore could be used for all cases in which PGD is indicated.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Invicta Ltd., Gdansk, Poland. There are not any commercial association of the author of any co-authors that might pose a conflict of interest.
Trial registration number: The registration number on www.irct.ir is IRCT2014062318202N1.
Keywords: next-generation sequencing, preimplantation genetic diagnosis, aneuploidy screening, semiconductor-based sequencer, frozen embryo transfer

P-636 Mitochondrial DNA copy number assessed using next generation sequencing (NGS), as a selection criteria for viable embryo for transfer

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Study question: Assessment of correlation between mitochondrial DNA (mtDNA) copy number and ploidy status of embryo.
Summary answer: Elevated mitochondrial DNA copy number is observed in abnormal embryos, thus indicating that this parameter assessment could become a viable additional tool for prioritisation of embryos for transfer.
What is known already: The number of mtDNA copies can affect all vital processes which take place in cells but mostly the specialized ones requiring most of the energy for their specialized functions. This could also influence functioning of the gametes and disturb reproductive possibilities It known that number and activity of mitochondrion correlates with embryo quality but the exact relationship has not yet been established.
Study design, size, duration: We set out to retrospectively analyse the amounts of the mtDNA among 359 embryos biopsied either on day 3 or day 5 of development. The study was performed between August 2013 and November 2014 at INVICTA Fertility Centre, Poland.
Participants/materials, setting, methods: Samples analysed during preliminary validation were derived from cytogenetically characterised human cases and their embryos were previously confirmed to be aneuploid during routine preimplantation genetic screening using next generation sequencing. MtDNA relative amount was assessed as number of reads for mtDNA/number of reads for genome DNA ratio × 1000.
Main results and the role of chance: Our results showed a significant difference in mtDNA amount depending on embryo biopsy day. Results from day 3 biopsy showed much higher mtDNA to genome DNA ratio than those from day 5 (5.54 vs. 1.2, $p < 0.001$, respectively). We found a significant difference in mtDNA amount in day 3 biopsy samples between normal and aneuploid embryos (6.3 ± 7.5 vs 7.1 ± 5.8, $p = 0.004$, respectively). In day 3 group no correlation was found between mtDNA copy number and implantation, embryo quality, embryo's sex or patient's age. In day 5 group there was no difference in mtDNA amount between euploid/aneuploid embryos (1.5 ± 1.4 vs 2.1 ± 2.9, $p = 0.72$). We observed a significant difference in mtDNA amount only when comparing transferred embryos that implanted to the ones that did not (1.9 ± 1.3 vs 1.3 ± 1.8, $p = 0.002$, respectively).

Limitations, reason for caution: The findings are contrary to some recently published studies. A future prospective randomized study could potentially support the current findings.
Wider implications of the findings: The findings contribute information on cell physiology and are of important clinical significance supporting PGD performance purposefulness.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Invicta Ltd., Gdansk, Poland. There are not any commercial association of the author of any co-authors that might pose a conflict of interest.
Trial registration number: NA.
Keywords: mitochondrial DNA copy number, next generation sequencing, preimplantation genetic diagnosis, embryo transfer prioritization, aneuploidy

P-637 Telomere length: correlation with aneuploidy risk and implantation potential of human oocytes and cleavage stage embryos

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Study question: Could the measurement of relative telomere length (RTL) in oocytes and cleavage stage embryos identify aneuploidy and help determine which embryos have the greatest reproductive potential?
Summary answer: Measurement of RTL does not reliably identify aneuploidy in oocytes or embryos, although it is correlated with specific types of chromosomal errors and patient indications. Importantly, RTL showed an association with pregnancy in both oocytes and cleavage stage embryos, suggesting its potential use as an additional biomarker of embryo viability.
What is known already: Telomeres play important roles in ensuring the success of gametogenesis and mitotic divisions in embryos. The regulation of telomere length (TL) in human oocytes and embryos is poorly understood. Previous studies have shown that TL decreases from the oocyte to the cleavage stage, before being reset in blastocysts. An association between TL and reproductive competence has been suggested and TL deficiency has been implicated in aneuploidy. However, the evidence is limited with disagreements between studies.
Study design, size, duration: Human polar bodies and blastomeres were subjected to whole genome amplification (WGA). The amount of telomere DNA was assessed in the WGA products using quantitative real-time PCR. The products were also subjected to comprehensive chromosome screening (CCS), allowing the corresponding oocytes and embryos to be classified as euploid or aneuploid.
Participants/materials, setting, methods: 216 first polar bodies (PB) and 491 blastomeres (from 36 and 71 cycles, respectively) were analyzed. Maternal ages ranged from 28 to 44 years (mean 38.11) for the PBs and 19–47 (mean 37.5) for blastomeres. Real-time PCR was used to assess telomere DNA quantity and RTL was calculated from the results.
Main results and the role of chance: There was no clear correlation between RTL and aneuploidy in oocytes or embryos; however, a trend was observed with shorter telomeres detected in aneuploid samples from patients of advanced reproductive age. Furthermore, RTLs in PBs with chromatid errors only were shorter than in PBs with gains or losses of entire chromosomes ($p = 0.021$). RTL in embryos was associated with indication and clinical outcome. However, in oocytes this relationship was only observed in samples from younger women (<38 years). Patients with previous implantation failure (PIF) or miscarriage (PM) had embryos with shorter telomeres compared to other patients ($p < 0.01$). In oocytes shorter telomeres were observed in PIF versus PM patients ($p < 0.01$). Interestingly, oocytes and embryos from patients who achieved a clinical pregnancy showed longer telomeres ($p < 0.01$).
Limitations, reason for caution: The use of WGA prior to telomere quantification does not distort the results obtained. However, it is possible that this strategy might reduce the capacity to resolve small differences in telomere length. A relative quantification of telomere length is provided, but the actual number of telomere repeats was not calculated.
Wider implications of the findings: Telomere assessment has potential to improve our understanding of preimplantation development and the mechanisms causing aneuploidy. Identification of the embryos having greatest reproductive potential is critical for the success of IVF treatment. Aneuploidy screening and morphological evaluations help to reveal viable embryos, but cannot guarantee implantation. Telomere length measurement may represent a new independent parameter for assessing the viability of chromosomally normal embryos, and may also reveal the embryos that would benefit most from CCS.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Reprogenetics – None of the authors have any competing interests.
Trial registration number: NA.
Keywords: telomere length, human oocytes, cleavage stage embryos, aneuploidy, implantation potential

P-638 Mild oxidative stress is beneficial for increase in telomere length

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Study question: Telomeres are highly conserved hexameric repeats of DNA that confer chromosome stability and maintain genomic integrity. As telomeres are Guanine rich repeats, they are highly prone to oxidative damage. In this study we wanted to know the impact of oxidative stress on telomere length.
Summary answer: Sperm DNA integrity is important for accurate transmission of genetic information to the offspring. Telomere is an essential component of Sperm DNA, if shortened can cause loss of paternal genome integrity, which is important for proper fertilization and healthy embryo. Oxidative stress causes loss of telomere integrity.
What is known already: Telomere shortening results from normal cell division, reactive oxygen species, genotoxic insults, and genetic predisposition. Reactive oxygen species (ROS) shorten telomeres by oxidizing the guanine rich telomeric DNA and triggering a DNA damage response which leads to excision of telomere repeats. In differentiated spermatozoa, telomeres play a fundamental role in the organization of the sperm nucleus. After fertilization, sperm telomeres are the first site in the sperm genome to respond to oocyte signals for pro-nucleus formation.
Study design, size, duration: This is a case control study. The study included 112 cases and 53 controls. Cases and controls were recruited from January, 2013 to July, 2014.
Participants/materials, setting, methods: The study included 112 infertile men and 53 fertile controls. ROS estimation was done by chemiluminescence method. The average telomere length from the sperm DNA was measured by quantitative Real Time PCR. 8-Hydroxy-2-deoxy-Guanosine (8-OHdG) level was assessed by Cayman's ELISA kits. DNA fragmentation Index (DFI) was assessed by Sperm Chromatin Structure Assay (SCSA).
Main results and the role of chance: The mean ROS was significantly elevated in cases (66.61 ± 28.32 RLU/s/million sperm) compared to controls (14.04 ± 10.67 RLU/s/million sperm). The 8-OHdG level in patients were 30.92 ± 3.27 pg/ml and in controls 14.29 ± 2.24 pg/ml. The mean DNA Fragmentation Index (DFI%) in patient was 36.11 ± 13.69 and in controls 24.17 ± 8.7. The mean telomere length was significantly lower in patient group (ROS > 35) as compared to patient group (ROS < 22) but it significantly increased in the patient group (ROS = 22–35) as compared to patient group (ROS < 22).
Limitations, reason for caution: The findings of this study should be validated in more samples.
Wider implications of the findings: In this study we found that in infertile patients oxidative stress leads to sperm DNA damage and telomere shortening. Elevated ROS levels lead to telomere shortening but seminal ROS to a particular level (ROS = 22–35 RLU/s/million sperm) are protective in maintaining telomere length.
Study funding/competing interest(s): Funding by national/international organization(s) – Department of Biotechnology (DBT), INDIA.
Trial registration number: NA.
Keywords: infertility, oxidative stress, telomere

P-639 Targeted copy number analysis for preimplantation genetic screening

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Study question: Can next-generation DNA sequencing (NGS) be applied in a targeted fashion to measure chromosome copy number in limiting numbers of human cells?
Summary answer: We have developed a targeted, PCR-based approach that determines chromosome copy number with analytical accuracy sufficient for use in preimplantation genetic screening (PGS).
What is known already: PGS is used to assess the chromosome copy number of embryos. Although growing evidence indicates that euploid embryo transfer increases implantation rates and decreases miscarriage rates, PGS adoption has been limited at least in part due to the high cost of traditional PGS approaches. However, increased use of trophectoderm biopsy followed by vitrification and subsequent frozen embryo transfer coupled with streamlined workflows employing next-generation DNA sequencing (NGS) are poised to enable broader PGS adoption.
Study design, size, duration: 12 pg DNA (~2 diploid cells) purified from cell lines, or lysate derived from 1 to 5 cultured fibroblast cells, served as template for the PCR reactions. The products were sequenced to generate count data for each chromosome, and these data were subsequently used to infer karyotypes.
Participants/materials, setting, methods: We have developed and implemented an automated PCR-based method that amplifies regions from each chromosome and simultaneously attaches the sequencing adapters and sample-specific barcodes necessary for multiplexed NGS. Because the PCR primers are human-specific, this method is robust to non-human DNA contamination.
Main results and the role of chance: Using purified gDNA template derived from 19 aneuploid and 16 euploid cell lines, a total of 41 true aneuploid chromosome calls, 3630 true diploid chromosome calls, 1 incorrect aneuploid (false positive) chromosome call, and 0 incorrect diploid (false negative) chromosome calls were made. The incorrect aneuploid call was in a sample that contains additional correctly called aneuploid chromosomes, thus yielding perfect sample-level specificity, and perfect sample- and chromosome-level sensitivity. Aneuploidies detected included trisomies 2, 8, 9, 13, 18, 20, 21, 22, 2 + 21, and 16 + 21, XO, XXXX, XXY, and XYY. The technique also detected trisomy 9, 13, 18, 21, XXY, and XYY when lysate from one, two, or five fibroblasts was used as template.
Limitations, reason for caution:.
Wider implications of the findings: Collectively, our results indicate that this simple, targeted approach for determining chromosome copy number can deliver the level of accuracy required to perform NGS-based PGS.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Good Start Genetics, Inc.
Trial registration number: NA.
Keywords: PGS, next-generation DNA sequencing

P-640 Does the pregnancy rate following transfer of vitrified-warmed embryo biopsied and analyzed by CGH change in different maternal age ranges?

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Study question: Does patients age affect clinical outcome in vitrified-warmed embryos biopsied with comparative-genetic array (CGHa) technique transfer?
Summary answer: In spite of an increase in embryo transfer cancelation rate due to aneuploidies detected in all embryos as patients age is higher, maternal age did not affect IVF clinical outcome in vitrified-warmed embryos transferred cycles since pregnancy rates are similar between groups with at least one euploid embryo transferred.
What is known already: Maternal age is the best predictor of clinical IVF outcomes. In women older than 35 years old, IVF success rates declines progressively and this is more remarkable after forties. Moreover, aneuploidy and abortion rates turns higher as women's age increases. Oocyte aging can cause not only cell cytoplasm alterations, but also nuclear genetic abnormalities due to altered chromosome alignment. Those abnormalities lead to aneuploidies, which can be responsible for implantation failure and abortion.
Study design, size, duration: This retrospective observational study evaluated 299 cycles with 841 embryos biopsied and evaluated with CGHa. Patients underwent standard protocols for ovulation induction and endometrial preparation at a private Assisted Reproduction Center between 2011 and 2014. Participants were split in three groups according with age: <35 year-old (n = 69); 36–39 year-old (n = 90); ≥40 year-old (n = 140).
Participants/materials, setting, methods: Embryos were cultured until D5 and blastocysts biopsied and vitrified using standard protocols. Genetic analysis were done with aCGH. Euploid embryo-transfer were performed on the next menstrual cycle with endometrium preparation. We compared demographic characteristics to evaluate similarity between groups, and primary outcome was ongoing-pregnancy rate in vitrified-warmed transferred cycles.
Main results and the role of chance: Patients age were <35 year-old: 32.8 ± 2.3; 36–39 year-old: 37.8 ± 1.1; ≥40 year-old: 41.7 ± 1.7. The number of embryos biopsied was higher in patients <35 year-old (4.0 ± 2.4) compared to 36–39 year-old (2.9 ± 1.9; $p = 0.001$) and ≥40 year-old (2.6 ± 1.8; $p < 0.001$). The percentage of normal embryos was also higher for younger patients (<35 year-old: 48.2%) compared to 36–39 year-old (24.6%; $p < 0.001$) and ≥40 year-old (15.4%; $p < 0.001$), while the cycle cancelation rate due to non-normal embryos for transfer was higher for older patients ≥40 year-old (67.1%) compared to 36–39 year-old (56.7%; $p < 0.001$) and <35 year-old (18.8%; $p < 0.001$). On the other hand, when we evaluated cycles transferred, older patients had little lower number of transferred embryos (≥40 year-old: 1.3 ± 0.5) related to <35 year-old (1.6 ± 0.5; $p = 0.015$) but no different of 36–39 year-old (1.5 ± 0.6; $p = 0.178$), and ongoing pregnancy rates (<35 year-old: 46.4%; 36–39 year-old: 46.2%; ≥40 year-old: 56.5%; $p = 0.524$) were not affected.
Limitations, reason for caution: This is a retrospective observational study in which we compared clinical outcomes of vitrified-warmed embryo transfer, biopsied with CGHa analysis in according with patients age. Severe male factor, previous recurrent abortion or implantation failures could interfere in genetic results.
Wider implications of the findings: Vitrified-warmed blastocysts biopsied and evaluated by CGHa analysis technique transfers have similar clinical outcomes independently of women age. Genetic abnormalities embryos rate and cycle cancellation progressively increases in older patients, as is expected. However, for an euploid transferred cycles, patients age does not affect clinical outcomes suggesting that negative outcomes are placed exclusively to oocyte chromosome alterations and does not outstanding to other patients characteristic as endometrium.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Huntington Reproductive Medicine.
Trial registration number: NA.
Keywords: CGH, age, IVF, embryo, vitrification

P-641 Human preimplantation embryo pluripotency and DNA integrity are affected by induced retroelement expression

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Study question: To investigate the effects of LINE-1 retrotransposition on methylation and DNA damage and also to determine the effects of LINE-1 retrotransposition on stage-specific expression of three major transcriptional factors OCT-4, SOX2, NANOG, during preimplantation stages of human embryo development.
Summary answer: Our data provide novel evidence that LINE-1 retrotransposition in human preimplantation embryos induce double strand breaks and interferes with the expression patterns of pluripotency factors OCT-4, SOX2, NANOG during human preimplantation development. Furthermore, they demonstrate potential changes in methylation reprogramming at the cleavage stage.
What is known already: The epigenetic regulatory mechanism of DNA methylation plays crucial role in cell differentiation, regulation of gene expression, genome reprogramming and silencing of repetitive elements. In mammals, epigenetic reprogramming occurs during early development. OCT-4, SOX2 and

NANOG, are crucial for the establishment and maintenance of pluripotency and self-renewal of ESCs in preimplantation embryo. LINE-1 have been implicated in many human disorders, chromosome rearrangements, gene silencing and are found to be expressed in undifferentiated human ESCs.
Study design, size, duration: *In vitro* preimplantation development of human embryos generated from oocytes and transfected through sperm with active LINE-1 were compared with control embryos. Retrotransposons were tagged with EGFP gene-based retrotransposition cassette in order to demonstrate new retrotransposition events when inserted into the embryonic genome.
Participants/materials, setting, methods: Immature oocytes at GV and MI stages and MII oocytes were donated for research to the IVF center of University Hospital of Ioannina, by infertile couples. Retrotransposition events in oocytes and in embryos were confirmed by fluorescent microscopy. Methylation status, pluripotency and DNA double strand breaks were also examined.
Main results and the role of chance: Embryos infected by retroelements introduced at ICSI in comparison to uninfected, are characterized by accelerated asymmetrical cell division, multiple cellular fragments, cleavage arrest and embryo degeneration. This is also evident from the early expression of the OCT4 and the absence of SOX2 and NANOG which are expressed at the morula stage of controls. In addition, double strand DNA breaks in each particular blastomere at the cleavage stage, coincided with methylation changes and insufficiency of pluripotency factors expression. We conclude that the very early initiation of DNA DSBs as the result of the RE activity, interferes with further preimplantation embryo development to the blastocyst stage and hinders the positive influences of OCT4, SOX2, NANOG on the formation of the morulae, the ICM and the trophectoderm.
Limitations, reason for caution: The injected quantity of LINE-1 cassette may exert a spectrum of damaging effects in the early embryo beyond the designated markers used in this study and confer to the early embryo arrest.
Wider implications of the findings: Our findings show that active retroelements produce DNA breaks as they create new integration sites in the genome, thus may interrupt human embryo development and lead to morphological and genomic abnormalities. The absence of SOX2 and NANOG, apart from the embryo destruction by the active retroelements, may be also influenced by interference of the retroelements with methylation. Uncontrolled human retroelements may cause the high percentages of abnormal embryos in clinical practice.
Study funding/competing interest(s): Funding by national/international organization(s). This study has been co-financed by the European Union (European Regional Development Fund-ERDF) and Greek national funds through the Operational Program "THESSALY-MAINLAND GREECE AND EPIRUS-2007–2013" of the National Strategic Reference Framework (NSRF 2007–2013).
Trial registration number: NA.
Keywords: LINE-1, double-strand breaks, human preimplantation embryo development, pluripotency, methylation

P-642 Chromosomal polymorphic variants increase the embryo aneuploidy rate in IVF cycles

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Study question: Could chromosomal polymorphic variations increase the embryo aneuploidy rate in blastocysts obtained after an IVF cycle?
Summary answer: Differences in embryo aneuploidy rates are detected in patients with and without chromosomal polymorphisms. We show that carriers of polymorphisms have a higher risk of embryonic aneuploidy.
What is known already: Chromosomal polymorphic variants are considered as normal but previous studies have reported that they are associated with infertility and recurrent abortions although the way is unknown. On the other hand there is a high incidence of chromosome abnormalities in human gametes and embryos that leads to failure of IVF cycles, including oocyte donation cycles. This could be due to aggressive stimulation, male factor or other issues, but there are causes that are yet to be defined.
Study design, size, duration: A retrospective study was performed. We included the array-CGH results of 524 embryos from 231 comprehensive chromosome screening (CCS) cycles performed between 2013 and 2014 at Instituto Bernabeu, Alicante, Spain. The main outcome measures were embryo aneuploidy rate and implantation rate.
Participants/materials, setting, methods: We included 524 embryos (301 embryos from oocyte donation cycles and 223 from IVF cycles with own oocytes). Whole chromosome imbalances by array-CGH in trophectoderm cells from D5 embryos were detected. Array-CGH analysis was performed using Agilent SurePrint G3 8 × 60 k CGH microarrays previous whole genome amplification (WGA) of genomic DNA.
Main results and the role of chance: Significant differences were reported in the embryo aneuploidy rate between carriers and not carriers of chromosomal polymorphisms (46.8% vs. 34.9%; OR = 1.638, 95% CI = 1.005–2.668). We show that these differences occur in oocyte donation cycles but not in cycles with own oocytes, perhaps because the oocytes donors are a homogeneous group without confusion factors like woman age. The presence of a chromosomal polymorphism in the oocyte donor (and not in the male partner) has a higher risk for embryonic aneuploidy. Moreover, we observed that the implantation rate from euploid embryos transferred were lower in polymorphisms carriers group (42.9% vs. 57.3%) although the difference in this case was not significant.
Limitations, reason for caution: The present work suggests that chromosomal polymorphisms could be responsible for a higher percentage of aneuploidies in embryos from IVF cycles, mainly in young woman. More studies are needed to elucidate how these polymorphisms affect to mechanisms of meiotic segregation.
Wider implications of the findings: This study reveals a higher aneuploidy rate in blastocysts from chromosomal polymorphisms carriers than in embryos from individuals with normal karyotype, showing a relationship between these both phenomena. Karyotyping of individuals, patients and donors, before an IVF cycle is important because the incidence of chromosomal abnormalities, including polymorphic variants, is quite high and application of array-CGH in these cases will improve the IVF results selecting euploid embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu Foundation.
Trial registration number: This study was not registered.
Keywords: chromosomal polymorphic variations, embryo aneuploidy, array-CGH

P-643 The inter-chromosomal effect, a reality: an example of a study on paternal meiosis

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Study question: What's the origin of prenatal aneuploidy in male carriers of translocations: Is there any inter-chromosomal effect???
Summary answer: This cytogenetic evaluation confirmed that the inter-chromosomal-effect (ICE) truly exists and could contribute to higher rates of abnormal prenatal aneuploidy, resulting in a small increase in the risk of miscarriage and birth of children with congenital abnormalities and a potential reduction in fertility.
What is known already: Many researchers have sort to investigate this phenomenon. Some of them did not report any evidence of ICE in several translocation carriers. Some of the analyzed patients had normal semen parameters; therefore the authors suggested that ICE could be restricted to patients with abnormal semen parameters. However, many reports detected an ICE in different translocation carriers.
Study design, size, duration: Five male carriers of translocations (3 reciprocal and two Robertsonian), were included in this study. In addition 7 fertile men with normal 46, XY karyotypes and normal sperm characteristics were recruited as a control group for the analysis of the ICE.
Participants/materials, setting, methods: In all cases, fluorescent in situ hybridization specific for chromosomes X, Y, 18, 21 and 22 was realized. The Mann-Whitney U-test was used to compare the aneuploidy rates between patients and controls.
Main results and the role of chance: In the ICE evaluation, all translocation carriers (both Robertsonian and reciprocal translocation carriers) showed

significantly increased frequencies of disomy of all investigated chromosomes, and diploid gametes compared with the control group ($p < 0.05$). However, disomy XY was not significantly different between controls and patients ($p > 0.05$). We also observed a considerable inter-individual variability in disomy and diploidy rates observed in our group of translocation carriers.
Limitations, reason for caution: The limitations of the available cytogenetic technologies have meant that the existence of an ICE remains a subject of debate. The analysis of additional chromosomes would have been useful in order to draw legitimate conclusions and more elucidate possible mechanism for generating chromosomal imbalances.
Wider implications of the findings: This study demonstrates that besides the direct effect on the chromosomes involved in the rearrangement, there may also be an impact on the segregation of other, structurally normal, chromosomes during meiosis. This might be a consequence of disrupted chromosome alignment on the spindle, or due to interference with other key aspects of the chromosome segregation process, leading to a generalized increase in the risk of producing aneuploid paternal gametes.
Study funding/competing interest(s): Funding by hospital/clinic(s). Departments of Cytogenetic and Reproductive Biology and Gynecology and Obstetrics, Farhat Hached University Teaching Hospital, Sousse, Tunisia.
Trial registration number: NA.
Keywords: prenatal aneuploidy, male carriers, inter-chromosomal effect

P-644 Increased incidence of partial Y chromosome microdeletions in male newborns conceived by assisted reproductive technologies

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Study question: To investigate the incidence of Y chromosome microdeletions in male newborns conceived by intracytoplasmic sperm injection (ICSI), *in vitro* fertilization (IVF), and natural conception (NC).
Summary answer: We found a statistical significance in the proportion of babies with partial Y chromosome microdeletions between the ICSI, IVF, and NC populations.
What is known already: Vertical transmission of Y microdeletions from father to son via ICSI have been widely shown.
Study design, size, duration: A total of 186 male newborns were recruited, including 35 babies conceived by ICSI, 37 babies conceived by IVF, and 114 babies conceived naturally. The Yq genetic status of the newborns was determined according to 18 Y-specific STS markers covering three azoospermia factor (AZF) sub-regions and internal control sequences.
Participants/materials, setting, methods: Genomic DNA was extracted from umbilical cord blood according to the protocol provided with the QIAamp DNA Blood Mini Kit. A two-tailed Fisher's exact test was used to compare the frequency of patients with and without detected Y chromosomal microdeletions in the ICSI, IVF, and NC populations.
Main results and the role of chance: We found that partial AZF microdeletions were identified in 8 (22.9%) of 35 ICSI newborns, 4 (10.8%) of 37 IVF newborns, and 1 (0.9%) of 114 NC newborns. There was a statistically significant difference in the proportion of babies with partial Y chromosome microdeletions between the ICSI, IVF, and NC populations. When analyzed individually, only the SY114 and SY152 STS markers showed a statistically significant difference in incidence between the three cohorts.
Limitations, reason for caution: We did not study the Y chromosome microdeletions in fathers, therefore cannot analyze whether the deletions were inherited or *de novo*.
Wider implications of the findings: Our studies indicate that the population of male children conceived through ARTs, particularly ICSI, are at an increased risk of genetic defect in the form of partial Y chromosome microdeletions. The growing population of ART-conceived children emphasizes the importance of studying genetic repercussions of these procedures in regards to the future fertility of males conceived *in vitro*.
Study funding/competing interest(s): Funding by national/international organization(s) – Canadian Institutes of Health Research.
Trial registration number: NA.
Keywords: ICSI, ART, Y chromosome microdeletion, infertility, IVF

P-645 The type of chromosomal alteration that is detected in aneuploid embryos is related to maternal age

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Study question: Is there any relationship between maternal age and type of chromosome aberrations seen in blastocysts generated by IVF cycles?
Summary answer: Despite embryonic trisomies increase with maternal age, the rate of monosomies in blastocysts is independent of the age. Only significant changes related with advanced maternal age were detected in chromosomes 2, 11, 13, 15, 16, 21 and 22.
What is known already: The aneuploidy is one of the major factors affecting the embryo outcome. The rate of embryonic aneuploidy increases with maternal age. Discarding affected embryos, after a Comprehensive Chromosome Screening (CCS) by array Comparative Genomic Hybridization (aCGH) give us an implantation rate that does not depend on maternal age. Thus, CCS corrects the effect of maternal age in embryo implantation.
Study design, size, duration: This is a retrospective observational study performed between January 2013 and January 2015 at Instituto Bernabeu. Alicante. Spain. The study includes the data analysis of 585 blastocysts with conclusive CCS results obtained from 197 patients.
Participants/materials, setting, methods: We included 518 blastocysts from patients aged ≤40 years old and 67 over 40 years old. Whole chromosome imbalances by array-CGH in trophectoderm cells from D5 embryos were detected. Array-CGH analysis were performed using Agilent SurePrint G3 8 × 60 k. The association between variables was analyzed using Logistic Regression and Chi-square Test.
Main results and the role of chance: 41.4% of the embryos analyzed were aneuploids of which 65.3% had monosomy and 46.2% trisomy. There was no significant difference in the number of monosomies in aneuploid blastocysts between patients ≤40 years old (67.7%) and >40 years old (61.4%) $p = 0.422$. On the other hand, trisomies increase in a statistically significant way between both age groups (42.6% vs 65.9%; $p = 0.005$). After the individual analysis of chromosome, it was observed that most do not modify their levels of alteration with increasing maternal age, only on chromosomes 2, 11, 13, 15, 16, 21 and 22 a statistically significant increase was observed. Surprisingly, on chromosome 3, the opposite effect is observed, this chromosome appears altered less frequently in aneuploid embryos with higher maternal age.
Limitations, reason for caution: The study is limited by its retrospective nature. A higher sample size or a prospective randomized design should be used in future studies to corroborate the current findings.
Wider implications of the findings: This investigation reveals that all types of embryonic aneuploidies are not equiprobables. Trisomies are less frequent than monosomies but trisomies augment with increasing maternal age mainly on chromosomes 2, 11, 13, 15, 16, 21 and 22.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Instituto Bernabeu.
Trial registration number: NA.
Keywords: maternal age, CCS, chromosome aberrations, aneuploid embryo, trisomies

P-646 Preimplantation genetic diagnosis (PGD) for inherited disorders using single nucleotide polymorphism (SNP) arrays: clinical outcomes of 100 cycles with transferable embryos

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Study question: Is linkage analysis using a universal SNP array based protocol, known as Karyomapping, a successful PGD treatment strategy for patients requesting embryo testing for inherited disorders?

Summary answer: The high clinical pregnancy rate, including patients from multiple fertility centers throughout the USA, was similar to outcomes achieved by the most successful *in-vitro* fertilization (IVF) programs. Additionally, as lengthy patient-specific test developments were unnecessary, more patients were treated with faster time to embryo diagnosis, transfer, and pregnancy.
What is known already: Karyomapping utilizes a universal protocol, applicable to virtually all patients, for linkage-based PGD. It eliminates the need to develop and optimize patient-specific protocols, drastically reducing the waiting time for initiation of IVF treatment. This technology was previously validated using the gold-standard polymerase chain reaction methodology (with 100% concordance) and has proven to be highly efficient in providing a diagnosis for even the most complex of cases, including those not feasible using conventional PGD methods.
Study design, size, duration: Among the 130 Karyomapping cases performed between December 2013 and September 2014, clinical outcome and follow-up data was collected for 100 PGD cycles that had embryos suitable for transfer. Seventy-two out of the 100 cycles had undergone embryo transfer at the time of data collection.
Participants/materials, setting, methods: Patients were referred from over 50 IVF centers in the USA and Canada. Blastocyst stage embryos were biopsied and frozen for transfer in subsequent cycle(s). Samples were whole genome amplified and analyzed using Karyomapping combined with direct mutation detection as needed and/or independent aneuploidy screening when requested (76% cases).
Main results and the role of chance: The average number of embryos analyzed and eligible for transfer per cycle was 6.15 and 2.5, respectively. The aneuploidy screening, carried out in 76% of cases, likely reduced the number of transferable embryos but improved clinical outcome. The implantation rate was 71.4% (60/84) and the clinical pregnancy rate per cycle started was 72.2% (52/72). Most transfers involved a single thawed blastocyst at a time, except for six patients who had two embryos transferred in one cycle (8%). Two twin pregnancies were identified (resulting from one single and one double embryo transfer). The average maternal age was 32.66 years (range: 25–41). Currently, there are 11 live births and 35 ongoing pregnancies. Prenatal and perinatal testing was performed in four cases confirming PGD in all instances.
Limitations, reason for caution: The collection of clinical outcome and PGD follow-up data can be challenging, particularly when dealing with a large number of referring centers and international patients. Furthermore, few patients elect to undergo prenatal or perinatal testing to confirm the diagnosis, making it more difficult to reliably assess accuracy of PGD.
Wider implications of the findings: With the number of couples undergoing carrier screening steadily increasing, Karyomapping allows IVF groups and PGD laboratories to deal with the rapidly growing demand for embryo testing for inherited disorders. Ongoing follow-up data analyses will help us better determine the clinical accuracy of PGD using this technology. The application of Karyomapping and simultaneous comprehensive chromosome screening of embryos will speed up the testing process and further improve clinical outcomes.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Institutional funding. None of the authors have any competing interests.
Trial registration number: NA.
Keywords: preimplantation genetic diagnosis, single gene disorders, linkage analysis, karyomapping, human embryos

P-647 Differences in chromosomal abnormalities observed in preimplantation embryos according to male or female meiosis disruption in isolated male factor (MF) and advanced maternal age (AMA)

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Study question: Are chromosomal abnormalities different in preimplantation embryos from MF and AMA couples?
Summary answer: Significantly higher incidence of chromosomal abnormalities in AMA patients than in MF, mainly due to complex abnormalities. Interestingly, in MF a higher incidence of aneuploidies for chromosome 2 and for sex chromosomes were observed, whereas in AMA patients the significantly higher aneuploidy rate was for chromosomes 15, 16 and 22.
What is known already: Aneuploidy in human embryos increases with advancing maternal age, due to the presence of aneuploid oocytes with a meiotic origin. Preimplantation Genetic Screening (PGS) is commonly indicated for the selection of euploid embryos to improve clinical outcome. Male meiotic impairment can also result in increased risk of aneuploidy offspring. Therefore, PGS could be also applied in couples with severe MF infertility.
Study design, size, duration: Two ongoing RCT studies (2012–2014) in two groups: AMA (75 PGS cycles in women between 38 and 41 years of age), and MF (44 cycles in severe MF, with sperm concentration <2 million sperm/mL, women age <38 years). In both studies, without a history of implantation failure or recurrent miscarriage.
Participants/materials, setting, methods: In both RCTs, patients were randomly allocated into two groups: conventional blastocyst transfer or PGS cycle. For the purpose of this study, only embryos in the PGS groups were considered. Cleavage stage biopsy and array CGH analysis of single blastomere. Statistical comparisons using Fishers 'exact test ($p < 0.05$).
Main results and the role of chance: In AMA group, 376 embryos were informative, 77.9% abnormal, 57.2% with complex abnormalities, 12.7% with chaotic pattern and 6.9% with segmental aneuploidies. In MF group, 277 embryos were informative, 66.4% abnormal, 5.8% with complex abnormalities, 15.9% with chaotic pattern and 6.1% with segmental aneuploidies. AMA group showed a significant increase in chromosomal abnormalities ($p = 0.0013$), mostly due to the significant increased for complex abnormalities ($p < 0.0001$) compared to MF group. Aneuploidy analysis for individual chromosomes in AMA group showed a significant increase in aneuploidy for chromosomes 15 ($p = 0.02$); 16 ($p = 0.03$) and 22 ($p = 0.0033$). In MF a significant increase was observed for chromosome 2 ($p = 0.045$) and sex chromosomes ($p = 0.04$). No statistical differences were observed in embryos with chaotic pattern or segmental aneuploidies and for the other individual chromosomes.
Limitations, reason for caution: This is an Interim analysis and the study needs to be completed to draw stronger evidence. This is not a blinded study. The results are valid for these specific populations.
Wider implications of the findings: These results would help to understand the origin of chromosomal abnormalities observed in preimplantation embryos and products of conception. Additionally, the cytogenetic studies on sperm could be re-oriented to the analysis of different chromosomes that are not commonly considered at high aneuploidy risk, such as chromosome 2.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – BlueGnome (Illumina), IVI, Igenomix.
Trial registration number: ClinicalTrials.gov NCT01571076.
Keywords: RCT, advanced maternal age, male factor, aneuploidy, embryo selection

P-648 CGH is mainly worth for older patients: comparison of outcomes on unselected patients receiving frozen-thawed blastocysts from elective cycles with or not day 5/6 CGH-biopsies

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Study question: Do trophectoderm biopsy performed by array-comparative genomic hybridization (aCGH) at day 5/6, associated with frozen-thawed transfers improve clinical outcomes in all ages compared to elective non-biopsied frozen-thawed transfer in an unselected population?
Summary answer: Trophectoderm biopsy performed at the ideal embryo development (day 5 or 6) associated with vitrification at blastocyst stage possibly enhances pregnancy rates mainly in older patients, where the chromosome alterations are higher. However, in younger patients, assuming lower aneuploidy rates, the CGH selection does not lead to a significant improvement.
What is known already: Failures of IVF cycles may be explained by poor embryo potential, due to aneuploidies. Trophectoderm biopsy, with aCGH, has been shown to produce a better selection. If the embryo does not reach the proper cell expansion on day 5, an aggressive procedure could damage the implantation ability, postponing to an accurate moment and driving to vitrification and transfer in a different cycle. Deferring cycles in unselected patients is also a trend to enhance pregnancy rates.
Study design, size, duration: This is a retrospective observational study including 375 cycles of vitrified-thawed blastocyst transfers from 2013 to 2014 in a private center where we evaluated clinical pregnancy outcomes of frozen-thawed biopsied-euploid embryos (n = 149) compared to unselected frozen-thawed transfers with no chromosomal analyses (n = 226).
Participants/materials, setting, methods: All patients received same endometrium preparation and progesterone supplementation. Frozen-thawed embryo transfers were stratified by age in both groups: with CGH transfers (FET-aCGH group: ≤35 year-old: n = 44; 36–39 year-old: n = 47; ≥40 year-old: n = 58) compared to deferred transfers with no aCGH (FET group: ≤35 year-old: n = 103; 36–39 year-old: n = 72; ≥40 year-old: n = 51).
Main results and the role of chance: In the FET-CGH group, number of biopsied embryos was higher in patients ≤35 year-old (4.1 ± 2.4) compared to 36–39 year-old (2.9 ± 1.9; p = 0.001) and ≥40 year-old (2.4 ± 1.7; p < 0.001). The percentage of normal embryos was also higher for younger patients (≤35 year-old: 54.27%) compared to 36–39 year-old (29.28%) and ≥40 year-old (17.19%), while the cycle cancelation rate due to aneuploidies was higher for older ≥40 year-old (65.52%) against 36–39 year-old (46.81%; p < 0.05) and ≤35 year-old (11.36%; p < 0.05). Combining CGH-FET and FET groups and applying a multiple logistic regression analysis showed a significant chance to become pregnant, per started cycle, only for those who underwent CGHa at ≥40 year-old (OR: 4.7; p = 0.013). The CGH analyses did not influence the pregnancy chance per started cycle in patients ≤35 year-old: (OR: 1.7; p = 0.248) or 36–39 year-old (OR: 1.2; p = 0.683), adjusted for the number of embryos transferred in both groups.
Limitations, reason for caution: This study evaluated unselected patients undergoing frozen-thawed embryos transfer and factors that could imply in pregnancy success, as sperm quality and factor of infertility, were not taking into account.
Wider implications of the findings: Our findings showed a high aneuploidy rate mainly on patients older than 40 year-old, which had beneficial on embryo selection to accomplish better pregnancy rates per started cycle and also a better counseling after CGH. On deferred transfers the low pregnancy rates in olders is probably correlated with chromosomal abnormalities. In younger patients (39 year-old or less), euploid embryos are more expected and other factors may influence the outcomes and questioning the CGH analysis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Huntington Reproductive Medicine.
Trial registration number: NA.
Keywords: CGH, frozen-thawed embryo, vitrification, pregnancy, IVF

P-649 Are false positive rates comparable at day-3 blastomere versus day-5 trophectoderm biopsies using arraycgh for preimplantation genetic screening?

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Study question: Are day-3 blastomere biopsy or day-5 trophectoderm biopsy representative of the whole embryo when analysed by array-Comparative Genomic Hybridization (arrayCGH)?
Summary answer: The results obtained in both types of embryo biopsies showed similar concordance rates when whole blastocysts were re-analysed using the same technique. Therefore, both biopsy strategies can be used for clinical analysis in PGS using arrayCGH.
What is known already: ArrayCGH technique is applied in clinical routine for PGS using either day-3 or blastocyst embryo biopsies. Mosaicism appears during embryo development and it has been described at both cleavage and blastocyst stage. Day-3 embryo biopsies have been discredited as not representative of the whole embryo due to mosaicism. On the contrary, trophectoderm biopsies may provide more accurate diagnosis due to the higher number of cells that can be analysed simultaneously.
Study design, size, duration: Blinded study in which 109 embryos previously diagnosed as chromosomally abnormal by arrayCGH were re-analyzed. All the embryos were re-analysed using arrayCGH on the whole blastocyst. Samples were collected from October 2012 to September 2014.
Participants/materials, setting, methods: Group A: 50 embryos previously diagnosed as aneuploid by arrayCGH on single blastomeres on day-3; Group B: 59 embryos diagnosed as aneuploid by arrayCGH on trophectoderm biopsies on day-5/day-6. Whole chromosome aneuploidies were considered except partial chromosome gains are losses. Re-analysis was performed following the same arrayCGH protocol used for PGS (BlueGnome Ltd, Cambridge, UK).
Main results and the role of chance: High concordance rates were observed in both, day-3 and trophectoderm biopsies when compared with the re-analysis in the whole blastocyst (49/50 (98%) and 57/59 (96.6%) respectively). Specifically, in 39 out of 50 (78%) embryos in group A, and 48 out of 59 (81.4%) of group B, all the aneuploidies reported remained in the re-analysis of the whole blastocysts. At least one aneuploidy observed in the biopsies was confirmed in 6/50 (12%) in group A, and in 7/59 (11.9%) in group B. Complementary aneuploidy for a given chromosome (e.g., monosomy vs. trisomy) was observed in 4/50 (8%) in group A, and 2/59 (3.4%) in group B. The discrepancy in group A showed a mosaic profile in the blastocyst and, in group B the two discrepancies were full euploid blastocysts.
Limitations, reason for caution: Sample size could be increased to confirm the findings. ArrayCGH technology does not allow the detection of mosaicism rates lower than 25–30%.
Wider implications of the findings: Both types of biopsies showed similar false positive rate. Therefore, both approaches are valid for clinical routine in PGS and more patients would be benefited of PGS by choosing a personalized approach based on their clinical background, number and quality of the available embryos.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – IGENOMIX.
Trial registration number: NA.
Keywords: arryCGH, preimplantation genetic screening, whole blastocyst reanalysis, blastomere biopsy, trophectoderm biopsy

P-650 Function of TETs in chromatin remodeling during spermiogenesis: a crucial point towards production of potent spermatozoa and in safeguarding of male fertility

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Study question: Among all cell types spermatozoa are known to have the lowest genome-wide 5-cytosine-methylation. Ten eleven translocation enzymes (TETs) catalyze in somatic cells the 5C-hydroxymethylation (5hmC) towards DNA-demethylation. Here, we asked the function of TETs in spermatogenesis and their role in production of fertile sperm and maintenance of male fertility.
Summary answer: We found that TET2 and TET3 are expressed in last steps of spermatogenesis and accompany the 5hmC-process. Notably, we could reveal that several conditions typical for male infertility (low sperm concentration, low sperm motility, oligo- and asthenozoospermia, low fertilization and pregnancy rates) are significantly associated with reduced TET expression.
What is known already: Our knowledge about the function of TETs and the impact of 5hmC in human spermatogenesis is still very sparse. Few existing studies are focused on the role of TETs in germ cell cancers (maintenance of active demethylation mechanisms in carcinoma-in-situ and seminomas in order to keep the hypomethylated cancer-genome) and do not deliver a consistent picture about their impact in normal spermatogenesis.
Study design, size, duration: We analyzed TET2- and TET3-expression (mRNA and protein), and 5-hmC, respectively, in normal human spermatogenesis and mature spermatozoa. We compared TET-mRNA-amount in spermatozoa of fertile donors and sub-fertile men, who underwent ART (assisted reproductive technology), and correlated the data to different fertility parameters (spermiogram according to WHO criteria and ART-outcome).
Participants/materials, setting, methods: We examined TET2- and TET3-expression in testis tissue samples exhibiting normal spermatogenesis by in-situ-hybridization (ISH), immunohistochemistry (IHC) and western blot (WB), and 5hmC – by immunofluorescence (IF). Ejaculates from 51 fertile and 44 sub-fertile men (fertility clinic Munich) were utilized for mRNA-studies in spermatozoa by RT-qPCR. Statistics were done using SPSS-20.
Main results and the role of chance: TET2-mRNA and – protein expression could be found in cytoplasm of pachytene-spermatocytes (stage I) up to elongated spermatids (stage II). TET3-mRNA-expression was present in cytoplasm of pachytene-spermatocytes (stage III) up to elongated spermatids (stage II), whereas TET3-protein-expression was examined in nucleus of round spermatids (stage I up to stage IV). 5-hmC was detected in elongated spermatids (stages V and VI). Analyzing mature spermatozoa (note: expressional inactive cells), we could reveal the presence of non-degraded TET2- and TET3-protein as well as a verifiable amount of non-degraded TET2- and TET3-mRNA. Comparing fertile donors with sub-fertile patients we observed that low level of TET-mRNA in spermatozoa is significantly associated with low sperm-concentration and -motility, with oligo- and asthenozoospermia, and moreover, with low fertilization and pregnancy rates.
Limitations, reason for caution: Mature spermatozoa are expressional inactive cells. To be able to quantify TET-mRNA in spermatozoa we tested in spermatozoa (healthy donors and patients) the stability of three standard house keeping genes known from somatic cells (*a-Tubuline*, *β-Actin* and *GAPDH*), and have chosen *GAPDH* as the most stable and suitable.
Wider implications of the findings: Our study shows that the final step of spermatogenesis comprising the huge genome-wide epigenetical modifications like chromatin-hypercondensation by nucleosome-to-protamine exchange and DNA-hydroxymethylation are accompanied and supported by TETs, and are crucial for production of "feature-complete" sperm cells with the ability to fertilize the oocyte. Evaluation of TET-function in spermatozoa of men could be further used for the development of clinical applicable test-systems with prognostic and diagnostic validity.
Study funding/competing interest(s): Funding by national/international organization(s) – German Research Foundation (Deutsche Forschungsgemeinschaft).
Trial registration number: DFG-KFO181_Period-2_Project-SCHA1531/1-1.
Keywords: sperm-epigenome, TET

P-651 Preventing the discard of potentially transferable embryos by next generation sequencing-based preimplantation genetic screening

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Study question: Can next generation sequencing (NGS)-protocol be applied for aneuploidy screening of human embryos to improve the detection of potentially transferrable mosaic embryos?
Summary answer: The higher dynamic range of NGS methodology allows an improved discrimination between mosaic and fully aneuploid embryos compared with array comparative genome hybridization (aCGH)-based preimplantation genetic screening (PGS), thus preventing the discard of potentially viable embryos.
What is known already: Aneuploidy screening of embryos at blastocyst stage can be jeopardized by the presence of chromosomal mosaicism, a phenomenon characterized as a mixture of diploid and aneuploid cell lines in the same embryo. Although many of such embryos do not implant, some may undergo through a natural mechanism of aneuploidies rescue during development resulting in euploid embryos. Therefore, an improved discrimination between mosaic and full aneuploid embryos provides a chance to prevent discarding of these embryos, allowing couples to consider the option of their transfer.
Study design, size, duration: This study was organized into two steps. The first involved mixing experiments with different euploid and aneuploid single cell ratios to establish a correspondence curve between aneuploidy percentage and copy number recorded by NGS. In the second step, blastocysts previously diagnosed with aCGH were analyzed by the NGS-based PGS protocol.
Participants/materials, setting, methods: Four hundred thirty-one whole genome amplification (WGA) products of embryos obtained from 196 PGS cycles performed during September 2013/May 2014 were selected for NGS analysis. WGA products included 51 diploid/aneuploid mosaic and 380 aneuploid embryos. NGS analysis was performed using MiSeq instrument. CNV analysis was accomplished by BlueFuse software.
Main results and the role of chance: Results of reconstitution experiments demonstrated that embryos constituted by aneuploid and euploid blastomeres could be consistently identified by NGS. Application of NGS-protocol to clinical samples showed concordant results in all the 51 mosaic embryos detected by aCGH and revealed that 40 out of 380 (10.5%) of aCGH-diagnosed aneuploid embryos were indeed mosaic embryos. After genetic counseling, 19 out of 51 mosaic embryos were transferred in 18 women, 8 of which had positive hCG levels. Two pregnancies resulted biochemical, 6 continued and were confirmed by at least one fetal sac and heart beat. Four pregnancies were ongoing and two went to term resulting in the birth of 2 healthy babies born.
Limitations, reason for caution: The NGS method provides great magnitude results due to the opportunity to avoid discarding of potentially viable embryos. However, this study is limited by the small amount of sample, thus larger studies are needed to confirm the significance of this procedure.
Wider implications of the findings: The possibility to improve the identification of mosaic embryos by NGS could represent a useful tool to increase the chances for infertile patients to achieve a pregnancy following an IVF treatment. This study demonstrates that NGS technique has a substantially increased sensitivity for detection of mosaicism over aCGH. Our results indicate that the transfer of mosaic embryos had the potential to result in healthy live births, thus avoiding discarding of potentially euploid embryos.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – 'GENOMA Group'.
Trial registration number: NA.
Keywords: preimplantation genetic screening, array comparative genomic hybridization, next-generation sequencing, embryo aneuploidy, mosaic embryos

P-652 Epigenetic alterations of aromatase coding gene, CYP19A1, in cumulus cells of MII oocytes from infertile endometriosis patients

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Study question: Does endometriosis alters expression of *CYP19A1* gene in cumulus cells (CCs) of endometriosis patients and is there any association between altered *CYP19A1* expression with epigenetic changes on its promoter?
Summary answer: Aromatase decreased in CCs of MII oocytes from infertile endometriosis patients compared to control group. There are harmonious patterns between mRNA expression of *CYP19A1* gene and epigenetic alterations of its promoter PII region. Decreased aromatase expression may reduce the competence of the oocyte in endometriosis patients.
What is known already: Aromatase, the key enzyme of estrogen biosynthesis, is encoded by the *CYP19A1* gene. Aromatase plays a pivotal role in ovarian functions, folliculogenesis and acquisition of oocyte competence. Among the various promoters of *CYP19A1*, the promoter PII is the most active ones in ovarian cells. Previous studies showed that changes in gene expression of aromatase are associated with pathogenesis of endometriosis but no epigenetic marks have been reported for aromatase regulation in CCs of endometriosis till date.
Study design, size, duration: Case–control study was conducted on 10 infertile endometriosis patients and 10 patients with tubal factors of infertility who underwent ovarian stimulation with GnRH agonist for Intracytoplasmic Sperm Injection. Cumulus oocyte complexes were obtained from follicles during ovarian puncture. Only the CCs from MII oocytes were selected for this study.
Participants/materials, setting, methods: Total RNA extraction and cDNA synthesis were performed using Micro-RNeasyand QuantiTect Whole-Transcriptome Kits, respectively. Relative expression of *CYP19A1* gene was examined by Quantitative real-time PCR. The DNA binding of MeCP2 and specific histone modifications in PII promoter region of *CYP19A1* gene were examined by Chromatin Immunoprecipitation (ChIP) assay.
Main results and the role of chance: Our data revealed that the mean relative expression of *CYP19A1* gene was significantly lower in CCs from infertile endometriosis patients compared with the control group ($P < 0.05$). In CCs of endometriosis patients, incorporation of MeCP2 in promoter PII of *CYP19A1* is significantly higher than that of control group ($P < 0.05$). Furthermore, a significant hypoacetylation at lysine 9 of histone 3(H3K9ac) of promoter PII was observed in patients affected endometriosis, whereas no significant difference of methylation level atlysine 9 of histone 3 (H3K9me2) was detected between patients and control groups.
Limitations, reason for caution: For getting more complete information, we need to analyze a larger number of endometriosis patients and control groups.
Wider implications of the findings: For the first time our results have shown that decreased *CYP19A1* expression in cumulus cells of endometriosis patients might be the result of epigenetic alterations in regulatory region of*CYP19A1*, either through DNA methylation or histone modifications. Changes in gene expression of aromatase may impair the development of the follicles and follicular steroidogenesis leading to poor oocyte quality and maturity in endometriosis patients. These alterations may have close relationship with endometriosis-associated infertility.
Study funding/competing interest(s): Funding by University(ies), Funding by national/international organization(s) – Iran University of Medical Sciences, Tehran, Iran, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Trial registration number: NA.
Keywords: endometriosis, aromatase, cumulus cell, epigenetic

P-653 First trimester combined screening test in ART pregnancies derived from blastocyst transfer

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Study question: Is first trimester combined screening for major fetal trisomies influenced by assisted reproduction techniques (ART) pregnancies from blastocyst transfer, with or without cryopreservation?
Summary answer: In ART pregnancies from blastocyst transfer, with or without cryopreservation, both the nuchal translucency (NT) measurement and free b-hCG concentration are higher as compared to spontaneous conceptions, whereas PAPP-A does not show any significant difference.
What is known already: Previous studies on ART pregnancies, from fresh and frozen-thawed embryos, showed controversial results concerning differences in NT values, free β-hCG and PAPP-A measurements compared to natural conceptions. Most studies showed no discrepancy of NT measurements, lower levels of PAPP-A and increased levels of free β-hCG. No published data are available analysing differences in the various components of first trimester combined screening between ART pregnancies after blastocyst transfer, with or without cryopreservation, and pregnancy conceived naturally.
Study design, size, duration: Retrospective case-control analysis involving 198 pregnancies recruited between 2012 and 2014. Forty-seven women conceived with fresh blastocysts from in vitro fertilization cycles (fresh-blasto), 51 with frozen-thawed blastocysts (frozen-blasto) and 200 were natural cycles (control group).
Participants/materials, setting, methods: Consecutive singleton pregnancies with euploid fetuses underwent ultrasound assessment at 11^{+0}–13^{+6} weeks with measurements of crown rump length (CRL), NT, free b-hCG and PAPP-A concentrations. Biochemical measurements were converted into multiples of the median (MoM). Sonographic measures were converted into delta values (A = observed-expected). Pregnancy outcomes were recorded.
Main results and the role of chance: The median NT was significantly higher both in frozen-blasto group (median: 0.27 mm; IQR: 0.02–0.44; $p < 0.001$) and in fresh-blasto group (median: 0.17 mm; IQR: 0.04–0.39; $p < 0.05$) as compared to control group (median: 0.00 mm; IQR: -0.19–0.12), whereas it was not different in the frozen-blasto compared to the fresh-blasto group. The median free β-hCG MoM was significantly higher both in frozen-blasto group (median 1.34; IQR 0.84–2.06; $p = 0.001$) and in fresh-blasto group (median 1.06; IQR 0.80–1.74; $p = 0.015$) as compared to control group (median 1.02; IQR 0.77–1.26), and it was also higher in frozen-blasto group compared to fresh-blasto group ($p < 0.001$). The three groups showed no significant differences in the median PAPP-A MoMs.
Limitations, reason for caution: This explorative study is based on a limited number of patients with blastocyst transfer and therefore needs independent confirmation.
Wider implications of the findings: Increased NT measurement and free β-hCG concentrations in pregnancies conceived after blastocyst transfer may lead to a higher false positive rate of the first trimester combined test for trisomy 21, with a consequent increased rate of unnecessary invasive tests and related fetal loss. If our results will be confirmed, first trimester screening algorithms may benefit from adjustments in the reference values of NT and free β-hCG, in pregnancies conceived through fresh and frozen blastocyst transfer.
Study funding/competing interest(s): Funding by hospital/clinic(s) – San Raffaele Hospital.
Trial registration number: NA.
Keywords: ART, frozen blastocyst, first trimester test

P-654 Preimplantation genetic screening results performed in women aged 40 years old or older

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Study question: Analyze the preimplantation genetic screening (PGS) results performed in women aged 40 years old or older.
Summary answer: Our results confirm the high incidence of aneuploidies in women older than 40 years. When the patient has euplodies embryos to be transferred, the pregnancy rate is similar to the women in the thirties.

What is known already: Preimplantation genetic diagnosis (PGD) was initially developed to conceive healthy children in couples with genetic disorders or with sex chromosome syndromes. In patients with advanced maternal age is not uncommon that more than one-half of oocytes retrieved to be aneuploid. These women produce a high percentage of aneuploid embryos, which may explain the low implantation rate and recurrent miscarriages. Currently, preimplantion genetic screening (PGS) has been offered for many patients around the world.
Study design, size, duration: Retrospective cohort study between June 2012 and November 2014.
Participants/materials, setting, methods: Forty biopsy cycles was performed in women aged 40 years old or older (mean age 41.9 years). All patients had undergone in vitro fertilization (IVF) by intracytoplasmic sperm injection (ICSI). A total of 155 embryos were biopsied and PGS with the use of array comparative genomic hybridization (aCGH) was performed.
Main results and the role of chance: A total of 155 embryos were biopsied on day 3 or day 5 of culture, in 40 cycles of biopsy. These embryos, just 16 were considered euploid (10.3%). The euploid embryos were transferred in 13 cycles, 8 fresh cycles and 5 frozen embryo transfer. One euploid embryo has not been transferred and remains frozen. The pregnancy rate per transfer was 53.8% (7/13), and 17.5% (7/40) when considered the number total of cycles. One miscarriage was observed (14.3%).
Limitations, reason for caution: The study was conducted with a small number of cycles. These patients in the study may have had implantation failure or repeated miscarriages previously, which could explain the high percentage of aneuploidies found.
Wider implications of the findings: PGS does not improve ongoing pregnancy rate but can decrease miscarriages by aneuploidy. Patients with advanced maternal age may benefit with PGS, reaching more healthy babies born.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fertilitat Centro de Medicina Reproductiva.
Trial registration number: NA.
Keywords: PGS, age, pregnancy rate, implantation rate

P-655 Assessment and validation of a next generation sequencing-based protocol for detection of chromosomal abnormalities in human preimplantation embryos

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Study question: Is the 'VeriSeq PGS' assay (Illumina, USA) as accurate in detecting chromosomal abnormalities in human blastocysts when compared to an established, clinically applied method [array-comparative genomic hybridization (aCGH)]?
Summary answer: The assay was highly accurate in detecting abnormalities affecting whole chromosomes while, it was also found to be able to detect partial gains and losses of chromosomal material arising *de novo* or as a consequence of genetic predisposition (e.g. one of the parents being a translocation carrier).
What is known already: Recent randomized trials have indicated that comprehensive chromosome screening (CCS) of preimplantation embryos enhances *in vitro* fertilization (IVF) success rates. However, although methods currently being used are highly accurate in detecting aneuploidy, mosaicism, smaller deletions and duplications, and even single nucleotide changes may have an impact on reproductive outcome. NGS potentially can offer increased precision and higher analytical depth.
Study design, size, duration: A retrospective assessment of whole genome amplification products (WGA) obtained from 65 clinical PGS cycles involving trophectoderm biopsy of blastocysts was carried out. A translocation cycle involving chromosome segments as small as 9.9 Mb [t(1;21)(p36.23;q22.2)] was also assessed. A total of 378 blastocysts (10 from the translocation cycle) were assessed.
Participants/materials, setting, methods: Aliquots of SurePlex whole genome amplification products previously assessed clinically with 24sure microarrays (Illumina) for aCGH were used to carry out the VeriSeq PGS assay following manufacturer's instructions. A MiSeq desktop sequencer was utilized for next generation sequencing (NGS). The BlueFuse Multi analysis software was used for interpretation of results.
Main results and the role of chance: A total of 9,072 chromosomes were assessed, 365 of which were determined previously by aCGH to carry abnormalities spanning across the entire chromosomal complement. 30/365 were partial aneuploidies affecting only parts of different chromosomes and having a size range of 9.9–139.5 megabases (Mb) (including the translocation cycle samples). Diagnosis reached through the NGS method was in complete concordance (100%) with diagnosis reached through aCGH for all 378 embryos (176 euploid, 202 aneuploid). Furthermore, NGS analysis agreed with aCGH analysis for 9,071/9,072 (99.99%) of chromosomes assessed. NGS analysis detected 1 abnormality (trisomy) in an otherwise abnormal embryo that was not detected by aCGH. All 30 partial aneuploidies were successfully detected by NGS. Results regarding the 10 translocation samples were in complete agreement between the two methods.
Limitations, reason for caution: Only a few samples with partial aneuploidies or inherited chromosomal rearrangements were assessed. Errors caused by the amplification method would affect both aCGH and NGS results and are not measured in this study.
Wider implications of the findings: The VeriSeq PGS assay accurately identifies aneuploidies affecting whole chromosomes and chromosome segments as small as ~10Mb. The results of this study indicate that the method can be used clinically for comprehensive chromosome screening of blastocysts. NGS-based aneuploidy screening is expected to substantially contribute to better care of IVF patients by potentially offering higher precision for diagnosis.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – NA.
Trial registration number: NA.
Keywords: PGS, NGS, veriseq, miseq, aCGH

P-656 Development of a rapid low-pass whole genome sequencing technique for the diagnosis of microdelection and microduplication in human embryos prior to implantation

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Study question: Aneuploidy is common in human embryos created using in vitro fertilization (IVF) techniques. Reliable chromosome screening methods are crucial for single cells biopsied from preimplantation embryos to ensure transfer of euploid embryos.
Summary answer: The results demonstrate that next-generation sequencing (NGS) is highly accurate for diagnosis of microdelection and microduplication in cells from human preimplantation embryos.
What is known already: NGS has been shown to be accurate for preimplantation aneuploidy screening at a low cost.
Study design, size, duration: Excess blinded DNA from cells derived from preimplantation genetic diagnosis (PGD) cycles of 25 couples at risk of aneuploidy was evaluated by NGS with the use of the Ion Torrent Personal Genome Machine (PGM) (Life Tech).
Participants/materials, setting, methods: Single Nucleotide Polymorphism (SNP) array and fluorescence in situ hybridization (FISH) were also run on blinded excess DNA from the same samples. Finally, results obtained from NGS, SNP and FISH from the same embryos were unblinded and all three independent methods evaluated for consistency.
Main results and the role of chance: NGS provided 46% (13/25) equivalent PGD diagnoses of compound point mutations and small deletions and insertions compared with SNP–based analyses. In 5 samples (25%), there was complete discrepancy between the two methods. In the other 7 samples, there was partial discrepancy between SNP–based analyses and NGS-base analyses. Further evaluation with FISH indicated that NGS diagnosis was the same as FISH.
Limitations, reason for caution: Wider implications of the findings should be confirmed in large-scale studies.

Wider implications of the findings: NGS can provide more reliable blastocyst PGD results, which would be widely used for preimplantation genetic diagnosis.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by National Science Foundation of China (No. 81222007). The authors have no competing interests to declare.
Trial registration number: NA.
Keywords: next-generation sequencing, single nucleotide polymorphism array, preimplantation genetic diagnosis, microdelection, microduplication

P-657 Human leukocyte antigen (HLA) matching of preimplantation embryos using a polymerase chain reaction (PCR) based methodology and karyomapping

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Study question: Can universally-applicable methods for preimplantation HLA-matching be established using PCR-based protocols or single nucleotide polymorphism (SNP) arrays (Karyomapping; Illumina, USA)? If so, which of these two methods is most advantageous when it comes to patient care?
Summary answer: Two universally-applicable PGD/HLA-matching methods were successfully established (one based on PCR, the other on Karyomapping) and applied clinically to *in vitro* fertilization (IVF)/PGD cycles. Karyomapping was determined to be highly advantageous as it required a shorter test preparation time and permitted more comprehensive assessment of the region of interest.
What is known already: Linkage analysis through assessment of short tandem repeats (STRs) in the human major histocompatibility complex (MHC) region is the method of choice for many laboratories for PGD/HLA-matching. However, the protocols used frequently demand tailoring to individual patients, a process requiring weeks or months to complete. The development of a universal protocol would be highly beneficial to patients, allowing more rapid treatment and thereby minimising the deterioration of the ill sibling requiring stem cell transplantation.
Study design, size, duration: Tests were prepared using the two developed methods for 19 couples, either seeking preimplantation HLA-matching alone or in combination with PGD for a single gene disorder (SGD). A total of 15 PGD cycles have been carried out to date and 100 embryos assessed (66 cleavage stage and 34 blastocysts).
Participants/materials, setting, methods: A PCR-based method assessing 19 polymorphic STRs was utilized in eight fresh PGD cycles (day-3 biopsy). Karyomapping, a microarray-based method of linkage analysis was used to assess 524 SNPs for seven frozen cycles (day-5 biopsy). Comprehensive chromosome screening (array-comparative genomic hybridization) was carried out in parallel for 5/7 cycles.
Main results and the role of chance: Using the PCR method, the average test development time (in days ± standard error of the mean) was 78 ± 11 for HLA-matching cases and 180.25 ± 13.1 for HLA-matching with SGD; combined average was 146.2 ± 23.3. Karyomapping preparation required a similar amount of time regardless of whether SGD was included (average 40.2 ± 6.1 days). This was significantly shorter ($p < 0.001$) than the time required for development of the PCR-based test. In total, 6/57 cleavage stage embryos and 4/30 blastocysts were found to be HLA-compatible, unaffected/carrier and euploid (when applicable), leading to transfers in 7/15 cycles. Clinical outcome data are currently available for 4 cycles, with two ongoing clinical pregnancies and one healthy live birth followed by successful transplantation to the sibling.
Limitations, reason for caution: Test development times presented in this study reflect the time needed by scientists working in a busy clinical laboratory, with multiple diagnoses and the development of other PGD tests being carried out simultaneously. If tests were prepared by themselves, preparation times using Karyomapping could theoretically be reduced to ~2 days.
Wider implications of the findings: Utilization of a PCR-based universal test still requires extensive test preparation times. Karyomapping offers significantly reduced test development times in addition to more comprehensive assessment of the MHC region. This increases the chances of patients initiating an IVF cycle and achieving pregnancy with a healthy, HLA-compatible embryo in a shorter time period. This is a critical advantage, since any delay to transplantation could have adverse effects on the deteriorating health of the ill child.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Institutional funding.
Trial registration number: NA.
Keywords: human leukocyte antigen matching, reimplantation genetic diagnosis, ingle nucleotide polymorphism arrays, karyomapping

P-658 Low responders older than 37 have comparable success rates to normo/high-responders of same age using a novel embryo-banking strategy including PGS at blastocyst stage

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Study question: To evaluate whether an embryo-banking (EB) strategy with Preimplantational Genetic Screening (PGS) can be a reasonable option for low responders in terms of drop out rate, transfer rate, efficiency and satisfaction. We will compare them to normo- and high-responders of same age which needed only one IVF-PGS cycle (IP).
Summary answer: Banking a mean of 2.8 subsequent cycles for low responders equalizes chances for an euploid embryo transfer and pregnancy rate compared to normo/high-responders of same age. Drop out, transfer cancellation rate and satisfaction were similar in both groups, thus low response is not an impediment for a successful outcome.
What is known already: Low response at a certain age (>38) is associated with poor results per cycle and often an indication for egg donation. Usually oocyte quality is not concomitantly decreased, then fertilization and blastocyst rates are proven to be similar. With newest technologies (trophectoderm biopsy, PGS with aCGH and blastocyst vitrification) the accuracy has arisen dramatically. PGS allows eSET contributing to lower twin rates and pregnancy complications. EB of blastocyst is more cost-effective than oocyte banking.
Study design, size, duration: Comparative and retrospective (01/2013–12/2014). 48 patients (mean age 39.3 ± 2.7) were included and divided into two groups: 19 low responder (5.2 ± 2.1 mean follicles) to EB strategy and 29 normo/high-responder patients (9.3 ± 3.1 mean follicles) to single IVF-PGS.
Participants/materials, setting, methods: All patients used the same antagonist protocol with 300 IU of FSH/LH and agonist triggering. Embryos were cultured, biopsied and vitrified on days 5 to 7. aCGH was used for genetic screening. Euploid embryos were transferred in HRT cycle. Severe male and uterine factors were excluded.
Main results and the role of chance: Both groups were comparable in terms of age, male factor and previous cycles. Comparing EB vs. IP groups there were significant differences in average number of oocytes per cycle (7.36 ± 0.74 vs. 12.24 ± 2.08, $p = 0.034$). The average cycle number per patient in EB group was 2.84 ± 0.29. There were no significant differences in biopsied embryos (6.16 ± 1.23 vs. 4.45 ± 0.92; $p = 0.263$) and euploidy rate (35.90% vs. 36.43%; $p = 1.000$). Transfer cancellation rate was 31.58% in EB group, whereas 48.28% in IP group ($p = 0.370$). Pregnancy rate was slightly increased in EB group even though no significant differences were found (69.23% vs 66.67%; $p = 1.0$). Drop out and satisfaction were comparable in both groups. If we assume up to 3 stimulation as a treatment unit for low responders it turns a reasonable option.
Limitations, reason for caution: Previous research suggest that cryopreservation influences embryo quality, but does not increase aneuploidy. In order not to compromise the cycle outcome, an optimized cryopreservation technique must be established at the IVF Laboratory. Further studies are necessary and patient number should be increased to achieve more significant results.
Wider implications of the findings: More than 50% of patients included in the EB group asked primarily for egg donation. With this approach we have contributed to rescue a success using own gametes. PGS on blastocyst and deferred transfers combination eliminates risks of missing endometrial implantation window, associated with premature luteinization that may be observed in this patient group. Another reason for the EB eligibility would be the cost–effectiveness improvement of accumulating all embryos for just one genetic test.
Study funding/competing interest(s): Funding by hospital/clinic(s) – University associated private clinic and its Fundation.
Trial registration number: NA.
Keywords: PGS, embryo-banking, low responders, euploid

P-659 IVF outcomes for patients that elect to undergo preimplantation genetic screening (PGS)

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Study question: For patients of all ages, what is the expected rate of having blastocysts to biopsy, the average number biopsied and the rate of aneuploidy? What is the average number of oocytes needed to result in one euploid embryo and what is the live birth rate following frozen embryo transfer (FET)?
Summary answer: The probabilities of having a sufficient number of embryos to biopsy, the number of biopsied embryos, the likelihood of having ≥ 1 euploid embryo, and a live birth outcome all decrease with increasing maternal age.
What is known already: Older patients produce fewer oocytes and therefore generate fewer blastocysts during IVF. The rate of aneuploidy is higher and the live birth rate is lower in older mothers.
Study design, size, duration: Patients that started an IVF cycle with the intention of performing PGS between June 2010 and July 2013 were reviewed for this study. Outcomes were analyzed for 433 patients that initiated cycles with the intention of having trophectoderm biopsy on Day 5 or 6 post-retrieval.
Participants/materials, setting, methods: Private U.S. IVF clinic with data separated into groups based on patient age at IVF stimulation start. Blastocysts were subjected to trophectoderm biopsy and immediately vitrified. Biopsy samples were analyzed using SNP microarray for all 24 chromosomes. Patients transferred a euploid embryo on progesterone day 6 in an FET cycle.
Main results and the role of chance: The age groups for analysis were: oocyte donor (age 21–29), < 35, 35–37, 38–40, 41–42, and 43–45. Euploidy rates by group were 73.2% (331/452), 62.7% (185/295), 59.5% (154/259), 40.7% (154/378), 23.4% (31/132), and 15.1% (11/73), respectively. The rates at which initiated cycles had a sufficient response to undergo biopsy were 96.7% (58/60), 79.0% (49/62), 73.9% (51/69), 73.7% (87/118), 48.3% (43/89), and 53.3% (24/45), respectively. The average number of blastocysts biopsied were 7.8(452/58), 6.0(295/49), 5.1(259/51), 4.3(378/87), 3.1(132/43), and 3.0(73/24) and the average number of oocytes necessary to result in one euploid embryo were 4.5(1495/331), 6(1114/185), 6.6(1018/154), 10.7(1644/154), 29.1(901/31), and 41.6(458/11) respectively. Live birth rates (LBR) per cycle start were 53.3% (32/60), 58.1% (36/62), 44.9% (31/69), 35.6% (42/118), 7.9% (7/89), and 6.7% (3/45) but the LBR per transfer were 66.7% (32/48), 83.7% (36/43), 79.5% (31/39), 75.0% (42/56), 41.2% (7/17), and 42.9% (3/7), respectively.
Limitations, reason for caution: Criteria for foregoing embryo biopsy may vary by patients' and clinicians' preferences. PGS may not benefit all patients and embryos could be harmed in the biopsy procedure.
Wider implications of the findings: In analyzing a large number of cycles and the resulting embryos, we can confirm that the chances of having euploid embryos decreases with maternal age. However, we can now advise patients on the likelihood of having any euploid embryos and how many eggs it may require to achieve a euploid embryo based on the patient age. This data may help patients in their decision to undergo IVF. Based on these data, patients in the older age groups can expect a reasonable chance of live birth if they can get a euploid embryo from one or more IVF cycle.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Pacific Fertility Center.
Trial registration number: NA.
Keywords: IVF, aneuploid, PGS, euploid, blastocyst

P-660 Clinical experience using single nucleotide polymorphism (SNP) arrays for preimplantation genetic diagnosis (PGD) of chromosomal translocations

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Study question: Can the use of SNP arrays be used in a clinical setting to perform PGD for couples affected by chromosomal translocations, detecting chromosomally abnormal embryos and also allowing karyotypically normal embryos to be distinguished from those carrying a balanced translocation?
Summary answer: SNP arrays provided a reliable means of detecting the inheritance of rearranged chromosomes, based upon linkage analysis. Normal, balanced and abnormal embryos were revealed. Additionally, this approach permits a simultaneous analysis of the copy number of the structurally normal chromosomes, thus improving the likelihood of transferring a viable euploid embryo.
What is known already: Traditional methods of PGD used for chromosome rearrangements successfully detect unbalanced embryos, but cannot distinguish balanced rearrangement carriers from completely normal embryos. Recently, a new technology known as karyomapping has been developed. The method involves tracking the inheritance of chromosomes from the parents to their embryos, achieved by assessing the transmission of thousands of polymorphisms situated on each chromosome. Although predominantly used for PGD of gene disorders, karyomapping might also be applicable to chromosome rearrangements.
Study design, size, duration: PGD was performed for three patients carrying different balanced translocations between June and September, 2014. A total of 29 embryos were analysed in three cycles: 9 and 10 with biopsy at the cleavage stage; 10 with biopsy at the blastocyst stage.
Participants/materials, setting, methods: The biopsied cells were subjected to whole genome amplification and genotyped with the HumanKaryomap-12 Bead Chip array (Illumina). DNA samples from the parents and from another relative carrying the translocation were genotyped in the same way. Transmission of specific chromosomal regions from parents to embryos was determined using BlueFuse software (Illumina).
Main results and the role of chance: 28 out of the 29 (96.6%) embryos analysed gave analysable results. 8 embryos were found to be euploid of which 3 presented the parental balanced translocation and 5 had an entirely normal karyotype. Out of the 20 chromosomally abnormal embryos, 15 had anomalies related to the translocation, while the other 5 had aneuploidies affecting unrelated, structurally normal chromosomes. Each of the three couples underwent transfer of a single embryo to the uterus, resulting in a positive pregnancy in all the three cases.
Limitations, reason for caution: Due to technical limitations, karyomapping is unable to detect 100% of spontaneously arising aneuploidies. However, we estimate that at least three quarters of chromosome abnormalities present in preimplantation embryos will be detected. Detection of inherited abnormalities (e.g. due to a translocation) are predicted to be detected with higher accuracy >95%.
Wider implications of the findings: Karyomapping is primarily used for PGD of gene disorders, with diagnosis of embryos via linkage analysis. This study confirms that karyomapping can also serve as a widely applicable method for PGD of chromosome rearrangements. Work-up time can be as little as a few days. In many cases, rearrangements that could not be assessed using standard PGD methods due to their complexity, or the small size of the chromosomal regions involved, can be diagnosed using karyomapping.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Reprogenetics UK.
Trial registration number: NA.
Keywords: PGD, chromosome, translocation, SNP array, karyomapping

P-661 Preimplantation genetic screening in combination with cumulus cells gene expression profile: use in assessment of embryo potential

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Study question: The aim of this work was to evaluate the embryo quality and potential for implantation combining two methods: (1) Preimplantation Genetic Screening (PGS) applied with a more reliable biopsy technique (S-biopsy), and (2) egg quality valuation via cumulus cell (CC) qRT-PCR gene expression analysis (GEA).
Summary answer: The simultaneous detection of PTGS2/VCAN index in CC from individual oocytes (expression level generated via qRT-PCR) combined to PGS (24sure array/BlueGnome), became a suitable system that can be added for embryo selection in IVF.
What is known already: PGS is a proven reliable tool to improve clinical outcomes in ART by selecting chromosomally integral embryos for transfer.

Although the day three biopsy required to obtain nucleated cells should not adversely impact embryo viability, this biopsy is technically challenging and requires extensive experience. Egg quality can be assessed by profiling the expression of cumula cells from individual oocytes via qRT-PCR and several potential markers have been described.
Study design, size, duration: Cohort study, one year frame. All 211 embryos assigned to PGS (maternal age or recurrent pregnancy lost), were biopsied with a less invasive, day-three stripper-based method (S-biopsy), using a laser to create a thin funnel in the zona pellucida next to the desired blastomere, then extracted by aspiration and release of the embryo with a 140 micrometer stripper capillary (Maldonado et al 2013).
Participants/materials, setting, methods: PGS was performed with BlueGnome 24sure arrays. CC collection from individual eggs was also performed. To assess egg quality, one step qRT-PCR for PTGS2 (Prostaglandin endo peroxidase synthase) and VCAN (Versican), generating an expression index associated with good embryo quality (cut-off value 57). Clinical pregnancy was recorded after transfer.
Main results and the role of chance: S-biopsy applied prior to PGS considerably reduced invasiveness, performance time, embryo-manipulation and need for extensive technician experience. Biopsied embryos reached blastocyst stage with 80% success rate. Successful chromosome analysis was obtained for all 211 blastomeres. From these, 143 blastomeres were abnormal and not transferred; 68 blastomeres were normal and PTSG2/VCAN index was obtained from their CCs via qRT-PCR. PTSG2/VCAN index (cutoff value 57) accurately predicted pregnancy result in 90% of cases (either negative or positive depending on index). When pregnancy was only assayed by bHCG test, 80% of cases predicted as non-pregnancy were negative while a 100% of cases predicted as positive pregnancy were positive. Of these, 80% of cases were confirmed as clinical pregnancies (week 12, heart beat).
Limitations, reason for caution: Large case number is needed combining normal PGS result and PTSG2/VCAN index to support largely the rutine use of CC analysis. S-biopsy may not be completely risk free as the embryo or the blastomere can be damaged if the technique is not performed properly.
Wider implications of the findings: Particularly in cases when more than 3 normal embryos are available, combination of these techniques can help assess embryo selection, reduce the number of transferred embryos and allow to freeze only valuable embryos. S-biopsy for PGS is cheaper, less time consuming and accessible to a wider array of IVF programs.
Study funding/competing interest(s): Funding by national/international organization(s), Funding by commercial/corporate company(ies) – CONACyT/PROINNOVA, Instituto Ingenes. Biociencias FYA SA de CV.
Trial registration number: NA.
Keywords: PGS, cumula cells, egg quality

P-662 Decrease in complex aneuploidies during embryo development from day-3 to blastocyst stage

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Study question: Is there any difference in the number of aneuploid chromosomes diagnosed per embryo from day-3 (D3b) to trophoectoderm biopsies (D5b)?
Summary answer: There is a significantly higher incidence of abnormal embryos at day-3 compared to blastocyst stage mostly due to the decrease of complex abnormalities and embryos with chaotic pattern at blastocyst stage. There is no selection against segmental aneuploidies from cleavage to blastocyst stage.
What is known already: Preimplantation Genetic Screening (PGS) is commonly indicated for the selection of euploid embryos to improve clinical outcome in specific indications. Different strategies have been applied to analyze the embryos either at day-3 biopsies or blastocyst stage. Aneuploidy impairs embryo development at a different degree, resulting in embryo arrest for certain chromosomal abnormalities. Complex abnormal embryo are defined as having more than 3 aneuploidies and chaotic pattern all chromosomes show abnormal profile.
Study design, size, duration: Retrospective cohort study of 296 embryos analyzed at day-3 and 863 at the blastocyst stage between April to December 2014. Patients' age ranged between 25 and 46 years and were divided into two groups: Group A (25–37 years) and Group B (38–46 years). Welch and Fisher tests were applied for statistical comparisons.
Participants/materials, setting, methods: Patients undergoing PGS for advanced maternal age, repetitive implantation failure, male factor, recurrent miscarriage and mixed causes. Chromosomal analysis for all 24-chromosomes was performed using array Comparative Genomic Hybridization (BlueGnome, Cambridge, UK). A single blastomere was obtained at day-3 or 4–5 cells in trophectoderm biopsies.
Main results and the role of chance: D3b resulted in 81.08% aneuploidies (19.26% chaotic, 22.97% complex abnormalities [CA] and 6.08% segmental aneuploidies [SA]). The mean number of abnormal chromosomes per embryo was 2.6 ± 1.5. In D5b aneuploidy was identified in 62.46% (4.40% chaotic, 8.92% CA and 3.36% SA). The mean number of abnormal chromosomes per embryo was 1.8 ± 1.2. Statistical comparisons showed significant differences for above described parameters except for SA. Regardless of age, there was a significant decrease in the mean number of aneuploid chromosomes per embryo in D5b compare to D3b. In Group A (1.5 ± 0.9 versus 2.2 ± 1.4) ($p < 0.001$) and in Group B (1.9 ± 1.3 versus 2.8 ± 1.6) ($p < 0.0001$).
Limitations, reason for caution: Retrospective study and heterogeneity of patients included.
Wider implications of the findings: These results would help to understand the mechanisms that produce embryo development arrest and the chromosomal abnormalities involved. Also, could help to evaluate what stage of the embryo must be analyzed in each patient.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – None.
Trial registration number: NA.
Keywords: embryo biopsy, aneuploidies, PGS, embryo development

P-663 Clinical, obstetric and neonatal outcomes of Hungarian preimplantation genetic diagnosis for aneuploidy (PGD-A) cycles of the year 2013

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Study question: The aim of this study was to determine the success rate and neonatal outcomes of in vitro fertilization (IVF) combined with PGD-A in Hungary in 2013.
Summary answer: Here we present data on clinical, obstetric and neonatal outcomes and success rates of the fresh PGD-A cycles done in Hungary in 2013. A live birth rate of 42,47% was achieved with 31,03% pregnancies showing complications. Neonatal malformation was noted in 6,4% of the cases.
What is known already: Aneuploidy is the leading cause of the failure of IVF cycles. PGD-A is a widely used technique to examine the chromosomal status of early embryos and provide a useful information for embryo selection for transfer. There is a growing body of evidence about the advantages of the technique (e.g. higher pregnancy and implantation rates), but data is lacking about the clinical outcomes – live birth rates and birth data of neonates are only scarcely reported.
Study design, size, duration: Data was retrospectively collected from patients went through IVF combined with PGD-A in the year of 2013. Indication for PGD-A was established by clinical genetisits. One hundred and thirty nine fresh cycles were carried out during this period analysing 431 embryos.
Participants/materials, setting, methods: Patients where aneuploidy screening was indicated and carried out were included in the study. Embryo biopsy was done at cleavage-stage. For aneuploidy screening of embryos array based comparative genetic hybridization was used. Where fetal heart beat was detected, patients were requested to provide data about their pregnancy and birth.
Main results and the role of chance: A total of 155 embryos were diagnosed as euploid (35,96%). Non-diagnostic rate was 4,64% ($N = 20$). Seventy six patients had at least one euploid embryo (54.68%) and 73 fresh embryo transfers were carried out. Chemical pregnancy rate was 56,16% (41/73), clinical pregnancy rate was 42,21% (33/73) and implantation rate was 40,38%

(42/104). Complications were present in 31,03% of pregnancies (e.g. bleeding, gestational diabetes, pre-eclampsia). Live birth rate was 42,47% (31/73). The mean gestational age was 38,31 ± 2.23 weeks with an average birth weight of 2893,5 ± 679.55 g. The sex ratio of newborns was 0,59: 0,41 in favor of males. Neonatal malformations were noted in two cases (6,4%) (aorta vascular ring and hydronephrosis).

Limitations, reason for caution: The number of examined and followed-up cycles are low. Also, caution has to be taken when neonatal malformations are interpreted because of the peculiar patient population where PGD-A is indicated and performed.

Wider implications of the findings: Collection of obstetrical and neonatal data of PGD-A cycles are very important. Only limited data set is available in the literature. The follow-up and analysis of these cycles is very important in order to properly advise patients about their treatment options.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Versys Clinics Human Reproduction Institute.

Trial registration number: NA.

Keywords: preimplantation genetic diagnosis for aneuploidy, live birth rate, birth outcomes

REPRODUCTIVE ENDOCRINOLOGY

P-664 Body-mass-index and weight gain in early adulthood are associated with polycystic ovary syndrome (PCOS): prospective population-based cohort study

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Study question: Does BMI or its change from adolescence to late adulthood associate with the presence of self-reported PCOS symptoms [oligo- or amenorrhea (OA) and/or hirsutism (H)] at age 31 or self-reported diagnosis of PCOS by age 46?

Summary answer: Women with self-reported OA + H at age 31 or diagnosis of PCOS by age 46 had significantly greater BMI in adolescence, early and late adulthood and showed significantly greater weight gain in early, but not in late adulthood, compared to the reference population.

What is known already: Obesity affects more than half of the women with PCOS and is known to affect negatively many aspects of their life. Our previous study showed the importance of obesity in adolescence and in early adulthood, and of weight gain in early adulthood, for reporting PCOS symptoms at age 31. Previous studies, however, have been inconsistent about the association of weight and weight gain with PCOS *per se*.

Study design, size, duration: In the prospective follow-up Northern Finland Birth Cohort 1966 (n = 5889) postal questionnaires were sent at ages 14 (95% answered), 31 (81% answered) and 46 (72% answered), and clinical examination and blood sampling were performed at ages 31 (n = 3115) and 46 (n = 3280).

Participants/materials, setting, methods: At age 31 participants answered to questions on the presence of OA and H (women with both OA + H n = 125, referents n = 2182), and PCOS diagnosis by age 46 (women with PCOS n = 180, referents n = 3466). Pregnant women, users of hormonal preparations, and women with only one symptom were excluded. Odds ratios (ORs) were calculated by logistic regression analysis.

Main results and the role of chance: Women with OA + H at age 31 or self-reported diagnosis of PCOS by age 46 had significantly greater BMI compared to the reference group at ages 14, 31 and 46. Increase of BMI between ages 14 and 31, but not later, was greater in women with isolated OA ($p < 0.05$), isolated H ($p < 0.05$), OA + H ($p < 0.001$) and self-reported PCOS ($p = 0.003$) compared to the reference group. In the univariate logistic regression analysis OA + H (OR = 20.7 [95% CI 11.65–36.80]), isolated OA (OR = 9.32 [95% CI 5.75–15.12], isolated H (OR = 7.80 [95% CI 4.69–12.97]) and WHR (OR = 14.11 [95% CI 2.39–83.34]) and to a lesser degree BMI at age 14, 31 (OR = 1.09 [95% CI 1.06–1.11]) and 46, serum levels of insulin, testosterone, LDL and FAI (OR = 1.11 [95% CI 1.05–1.16] were associated with PCOS by age 46.

Limitations, reason for caution: The symptoms and the diagnosis of PCOS were self-reported. The questionnaire at 46 years did not distinguish between polycystic ovaries on ultrasound and the syndrome. Ovarian ultrasonography was not available to aid the diagnosis of PCOS.

Wider implications of the findings: Isolated OA or H, or both of them screened by a simple questionnaire in early adulthood, are good predictor of PCOS in later adulthood. Weight gain in early adulthood plays a crucial role for the emergence of PCOS, suggesting that, with the dramatic progress of obesity, an increasing prevalence of PCOS can be expected in the future. Therefore, weight gain prevention from early on is crucial in order to prevent the development of PCOS and associated health burdens.

Study funding/competing interest(s): Funding by University(ies). Hungarian, Funding by hospital/clinic(s), Funding by national/international organization(s) – European Commission and Medical Research Council, National Institute for Health Research (UK), Finnish Medical Society Duodecim, North Ostrobothnia Regional Fund, Academy of Finland,, Sigrid Juselius Foundation.

Trial registration number: NA.

Keywords: polycystic ovary syndrome (PCOS), body mass index (BMI), weight gain, hyperandrogenism, free androgen index (FAI)

P-665 Investigation of the effect of isotretionine, leuprolid acetate and cetrorelix on ovarian reserve : an experimental study

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Study question: Does GnRH agonist, GnRH antagonist and retinoic acid derivatives have any adverse effect on ovarian reserve (such as follicle number and serum AMH level)? Is there any difference between the effect of three agents on ovarian follicle reserve pool?

Summary answer: Isotretionine, leuprolid acetate and cetrorelix, all have positive effect on ovarian reserve and preserve ovarian follicle number. The best agent on ovarian follicle number preservation may be isotretionin, since it decreased the atretic follicle number.

What is known already: It has been known that GnRH agonists, GnRH antagonists may preserve ovarian reserve especially in patients having ovarian toxicity agents. However the effect of isotretionine on ovarian reserve had not been well known. Also the comparison of such effect of isotretionine with GnRH has not been known.

Study design, size, duration: Prospective randomized placebo controlled experimental study, totally on 32 mature, female rats were used, one month of duration.

Participants/materials, setting, methods: This randomised, placebo–controlled single blind study was carried out on 32 mature, female rats of the Sprague-Dawley weighing between 180 and 220 per gram at the Karadeniz Technical University Surgery Research Laboratory. Rats' serum AMH levels were measured using tail vein blood samples before the treatment in all groups.

I. The control group (8 rats); no-medical care control group
II. Cetrorelix group (8 rats): injected 0.001 mg/day cetrorelix subcutaneous for 30 days.
III. Isotretinoin group (8 rats) : injected orally 10mg/kg/day isotretionin orogastric gavage for 30 days
IV. Leuprolide acetate group (8 rats): injected 0.075 mg/kg/day leuprolide acetate for 30 days.

After ten days of finishing the treatment protochol, both ovaries were excised via laparotomy and histopathologic examination was done for follicle count to assess the ovarion reserve. AMH levels were measured again with the blood taken from abdominal aorta after medical care.
Main results and the role of chance: When compared with the control group, a statistically significant increase was seen in GnRH agonist (leuprolide acetate), GnRH antagonist (retrorelix) and isotretinoin groups in serum AMH levels.(0,59 ± 0,63; 1,04 ± 0,41; 1,19 ± 0,51; and 1,63 ± 1,12 ng/mL; $P < 0.05$, respectively). At the same time, in all three groups, significant increase was seen in primordial and primary follicles number compared to the control group ($P < 0.05$) Unlike other groups, the atretic follicles number was significantly lower in isotretinoin group, compared to control group ($P < 0.05$).
Limitations, reason for caution: The main limitations of the study were the low sample size.
Wider implications of the findings: Isotretionin may have role in preservation of ovarian function especially in poor ovarian responders.
Study funding/competing interest(s): Funding by University(ies) – The authors had no study funding and competing interests to report.
Trial registration number: NA.
Keywords: ovarian reserve tests, isotretionine, GnRH agonist, GnRH antagonist

P-666 FMR6 (a long non coding RNA) may play a role in the pathogenesis of fragile X associated premature ovarian insufficiency (FXPOI)

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Study question: Does long no coding (lnc) RNA accumulation in granulosa cells of FMR1 premutation carriers play a role in the pathophysiology of fragile X-associated premature ovarian insufficiency (FXPOI).
Summary answer: *FMR6* but not *FMR4* lnc-RNA accumulation in granulosa cells of FMR1 premutation carriers may lead to RNA toxic gain-of-function causing FXPOI.
What is known already: Amplification of CGG triplet number above the normal range (n = 5–44 repeats) towards the premutation status (n = 55–200 repeats) is associated with increased risk for FXPOI in females and fragile X-associated tremor/ataxia syndrome (FXTAS) in males. Both male and female premutation carriers have elevated *FMR1* transcript levels suggesting RNA toxic gain of-function as a possible common mechanism for FXPOI and FXTAS. Recently, *FMR4* and *FMR6* lnc-RNA's were also suggested to play a role in the pathogenesis of FXTAS.
Study design, size, duration: Study population consisted of all 18 consecutive FMR1 premutation carriers referred to our IVF unit for IVF-PGD treatment, during a 12 month period. The control group consists of 12 patients, with less than 55 CGG repeats, undergoing IVF-ICSI for male factor infertility, matched by age, who were treated in the same period.
Participants/materials, setting, methods: After oocyte retrieval granulosa cells from follicular fluid were washed and stored at -80°C until RNA extraction. RNA was transcribed to generate cDNA and the genes RNA levels were measured using RT-PCR.
Main results and the role of chance: In FMR1 premutation carriers there was a trend for a non-linear association between the number of CGG repeats and the *FMR6* RNA levels ($p = 0.07$), but not *FMR4* in granulosa cells. The highest level of *FMR6* was seen in granulosa of women with a mid-size CGG repeats (80–120). In addition, a significant negative linear correlation was observed between the number of oocytes retrieved and the granulosa cells *FMR6* RNA levels ($p < 0.007$) but not *FMR4*.
Limitations, reason for caution: The study sample should be increased in future larger studies are.
Wider implications of the findings: We support previous findings suggesting RNA toxic gain of-function as a possible mechanism underlying FXPOI. As previously shown for *FMR1* mRNA, a non-linear association was observed between the number of CGG repeats and *FMR6* levels in granulosa cells from FMR1 premutation carriers in the mid-range (80–120 CGG repeats). Increased accumulation of both *FMR1* mRNA and *FMR6* lnc-RNA may lead to impaired ovarian function in FMR1 premutation carriers in the mid-range (80–120 CGG repeats).
Study funding/competing interest(s): Funding by University(ies) – Tel Aviv University, Sackler School of Medicine.
Trial registration number: NA.
Keywords: FMR1 premutation carriers, POF, long non coding RNA, FXPOI

P-667 MicroRNA-93 promotes ovarian granulosa cells proliferation through targeting CDKN1A in polycystic ovarian syndrome

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Study question: We want to explore whether different miRNAs expression in polycystic ovarian syndrome (PCOS) ovaries contributes to promoting granulosa cell proliferation in PCOS disease.
Summary answer: MiR-93 was increased in PCOS granulosa cells and through targeting CDKN1A to promote the proliferation and cell cycle progression.
What is known already: Granulosa cells have higher proliferation rates in PCOS ovaries and secreted more AMH to participate in the PCOS ovarian dysfunction.
Study design, size, duration: Experimental study.
Participants/materials, setting, methods: Expression of miRNAs was measured using RT-PCR in cortex of ovaries including 16 PCOS patients and 8 control. KGN granulosa cells were cultured for proliferation assay including MTS, flow cytometric analysis and EdU after overexpreesion or inhibition of miR-93. CDKN1A espression was examined using RT-PCR and Western blot analyses.
Main results and the role of chance: MiR-93 expression was higher in PCOS ovarian cortex and high concentration of insulin induced up-regulation of miR-93 and down-regulation of CDKN1A in granulosa cells. The overexpression of miR-93 dramatically promoted cell proliferation and G1 to S transition. Moreover, we identified CDKN1A gene as a direct target of miR-93. In consistent with the overexpression of miR-93, the down-knocking experiments of CDKN1A promoted cell growth and cell cycle progression in granulosa cells.
Limitations, reason for caution: We used KGN cells but not the granulosa cells from the growing follicles, which might be the best cell model for proliferation research.
Wider implications of the findings: Our finding identified that high concentration of insulin could increase the expression of miR-93 and promote granulosa cells proliferation in PCOS disease. This might offer new insight of why the ovarian production of AMH was excessive in PCOS and influenced the ovarian dysfunction.
Study funding/competing interest(s): Funding by national/international organization(s) – National Natural Science Foundation of China.
Trial registration number: NA.
Keywords: polycystic ovarian syndrome, miR-93, granulosa cell proliferation

P-668 Women with PCOS report decreased health status and present with several BMI-independent comorbidities from reproductive age on – a 15-year population based cohort study

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Study question: The aim of the study was to investigate self-estimated health status, comorbidities, smoking and alcohol consumption in women with polycystic ovary syndrome (PCOS) at fertile age (31 years) and perimenopause (46 years), in a population based birth cohort, the Northern Finland Birth Cohort 1966 (NFBC66).
Summary answer: The PCOS women experienced impaired health already at fertile age compared with asymptomatic women and also reported non-metabolic comorbidities (migraine, arthrosis, gastric/duodenal ulcer) by perimenopausal age independently of BMI while the smoking and alcohol consumption was similar between the groups.
What is known already: Women with PCOS have been shown to be in a higher risk for several metabolic (hypertension, dyslipidemia, metabolic

syndrome, T2DM) and non-metabolic comorbidities (infertility, depression and anxiety). Even though we have already previously shown increased risk for mental disorders in women with PCOS in this cohort, a comprehensive analysis of comorbidities in a larger population based analysis with a prospective follow-up, adequate adjustments and reference population is still lacking.

Study design, size, duration: Questions on oligo-amenorrhea (OA), hirsutism (H), self-estimated current health status, self-reported physician diagnosed or treated sicknesses, smoking and alcohol abuse were included in the postal questionnaires at ages 31 and/or 46. At age 31, 4427 (79%) women and at age 46, 3706 (72%) in the NFBC66 answered the questionnaire.

Participants/materials, setting, methods: At age 31 2188 asymptomatic and 125 PCOS women (OA + H) were identified. Of these 1576 asymptomatic and 85 PCOS women participated to the follow-up at age 46. The associations were calculated using logistic regression model and Pearson's Chi-square test. The data were adjusted for BMI, social and marital status.

Main results and the role of chance: Already from age 31 the PCOS women reported considerably impaired health status compared to asymptomatic women (31 years 8.8% vs. 2.60%, $p < 0.001$; 46 years 11.9% vs. 3.40%, $p < 0.001$). After BMI-adjustment PCOS women had increased risks of self-reported hypertension [31 years: OR 1.93, (95% CI 1.23–3.01); 46 years; OR 2.14 (1.29–3.54)] and migraine [31 years: OR 1.71, (1.13–2.59); 46 years: OR 1.60 (0.98–2.62)] already at age 31. At age 31, but not 46, PCOS women reported more gastric/duodenal ulcer [OR 3.85 (1.52–9.76)]. At perimenopause PCOS women reported more T2DM [OR 3.79, (1.62–8.88)] and knee arthrosis [OR 2.55 (1.31–4.94)]. No differences were found as regards genital infections, epilepsy, asthma/allergies, thyroid, gallstone or inflammatory bowel disease. Also the smoking and alcohol consumption was similar between the groups.

Limitations, reason for caution: The health-related diagnosis and the diagnosis of PCOS were based on self-reporting, which may bias the results. However, the self-reported PCOS diagnosis has already been well validated in our previous publications.

Wider implications of the findings: This study provides a unique population based follow-up data and demonstrates that the unfavourable effect of the syndrome on health is complex where also non-metabolic comorbidities developed independently of obesity. Given that PCOS heavily affects women's health from as early as puberty on to older ages it is important to take into consideration all the health risks when treating these women.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s) – The North Ostrobothnia Regional Found, the Academy of Finland, the European Commission and Medical Research Council, the National Institute for Health Research, Sigrid Juselius Foundation and Finnish Medical Society Duodecim.

Trial registration number: NA.

Keywords: PCOS, comorbidity, health status, migraine, smoking

P-669 The long-term impact of controlled ovarian hyperstimulation on thyroid function

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Study question: What is the long-term impact of controlled ovarian hyperstimulation (COH) on serum thyroid stimulating hormone (TSH) levels in euthyroid and hypothyroid women undergoing *in vitro* fertilization (IVF)?

Summary answer: Three months after the end of COH, serum TSH concentration exceeds the recommended threshold of 2.5 mIU/L in one out of two adequately treated hypothyroid women and in one out of six euthyroid women.

What is known already: It has been shown that in approximately one out of three euthyroid women and in two out of three hypothyroid women, TSH concentration exceeds the recommended threshold of 2.5 mIU/L during IVF cycles suggesting a reduced ability of the thyroid to adapt to the increased demand resulting from COH. However, whether thyroid function is restored at the end of the IVF cycle or whether COH results in a long-term impairment has not yet been investigated.

Study design, size, duration: We selected women who underwent IVF and did not become pregnant. Cases were women with treated hypothyroidism and serum TSH <2.5 mIU/L prior to initiate the cycle. Controls were euthyroid women matched to cases by age (±1 year) and basal serum TSH (± 0.1 mIU/L).

Participants/materials, setting, methods: Serum TSH was tested prior to initiate COH (Time 1), at the time of human chorionic gonadotropin (hCG) administration (Time 2), 16 days after hCG administration (Time 3) and three months after the end of the IVF cycle (Time 4). Thirty seven matched case-control pairs were selected.

Main results and the role of chance: Serum TSH at Times 1, 2, 3 and 4 was 1.7 ± 0.6, 3.1 ± 1.4, 3.1 ± 1.3, 2.7 ± 1.7 mIU/L, respectively among cases and 1.7 ± 0.6, 2.9 ± 1.0, 2.7 ± 1.0, 1.9 ± 0.7 mIU/L, respectively among controls. A statistically significant difference emerged at Time 4 ($p < 0.001$). In both groups, serum TSH was higher at time 4 compared to time 1. The rate of cases in which serum TSH exceeded the recommended threshold of 2.5 mIU/L at Time 4 was significantly higher in cases (51%, 95% CI: 35–68%) compared to controls (16%, 95% CI: 4–28%) ($p = 0.003$). In the entire population the only predictive factor of TSH >2.5 mIU/L at Time 4 was a diagnosis of hypothyroidism (adjusted OR = 4.3, 95% CI: 1.1–17.7, $p = 0.04$).

Limitations, reason for caution: Albeit unlikely, we cannot exclude that thyroid function may have deteriorated on its own (thus independently from COH) during the latency period of three months.

Wider implications of the findings: COH seems to have not only a short-term but also a long-term impact on TSH levels. The magnitude of this effect is particularly pronounced among hypothyroid patients. In a clinical perspective, we suggest to systematically retest thyroid function in women who underwent IVF few months after the end of COH, or in any case before a subsequent IVF cycle. Levothyroxine initiation or adjustment should then be considered in women overcoming the threshold of 2.5 mIU/L.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.

Trial registration number: NA.

Keywords: thyroid, controlled ovarian hyperstimulation, TSH

P-670 Comparison of the prevalence and characteristics of the metabolic syndrome in Greek women with polycystic ovary syndrome in relation with the general population

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Study question: Are there differences in the prevalence and characteristics of the metabolic syndrome (MetS) between the Greek women with polycystic ovary syndrome (PCOS) and the general population? Is there association between the body mass index (BMI) and MetS?

Summary answer: The prevalence of the MetS was nearly 7-fold higher in Greek PCOS women compared with the control group. The increased BMI was much more common in Greek PCOS women than in the age-matched healthy controls, and the prevalence of MetS in obese and overweight PCOS women was significantly higher in comparison with the controls.

What is known already: PCOS is the most common endocrinopathy affecting 4–12% of women in the reproductive age group. Multiple studies indicate that women with PCOS are at increased risk for developing of glucose intolerance or type 2 diabetes mellitus (DM2) and therefore at risk of having MetS as well. The prevalence of the MetS is high in women PCOS. The prevalence shows a marked variation between countries and ethnic groups, probably due to differences in diet, lifestyle and genetics factors.

Study design, size, duration: Greek women, during their reproductive age, were participated. The PCOS women were outpatients at the Reproductive Endocrinology Outpatient Clinic of the University General hospital 'Attikon' during the last five years, and they have been diagnosed according to the criteria of Rotterdam. The control group consisted of healthy volunteer females. Both groups were evaluated for MetS based on the International Diabetes Foundation (IDF).

Participants/materials, setting, methods: 385 Greek women; 230 PCOS women and 155 age matched healthy females as controls. All subjects completed a detailed personal and family history, and they underwent to ultrasound scans, clinical examination and biochemical measurements in order to estimate the prevalence, to evaluate the characteristics of the MetS in Greek women with PCOS and to investigate the correlation of MetS with BMI.
Main results and the role of chance: The proportion of participants with MetS was 12.6% in the PCOS group, significantly higher as compared with the correspondence proportion (1.9%) in the control group ($p < 0.001$).. The most frequent MetS component in both study groups was central obesity, followed by low HDL-C and elevated blood pressure. Elevated fasting plasma glucose (7.0% vs. 1.9%) and elevated triglycerides (10.4% vs. 3.2%) were more frequent in the PCOS group as compared with the controls ($p < 0.05$). Of the abnormalities present in affected women with PCOS, BMI occurred significantly higher between two study groups (42.7% vs 21.9%, $p < 0.001$). The proportion of obese and overweight subjects with MetS was greater in the PCOS group in comparison with controls (24.5% vs. 8.8%, $p = 0.050$).
Limitations, reason for caution: The limitation of this study was the relatively small number of women in both study groups, although a ratio of PCOS women and age-matched healthy controls of about 1: 1 was achieved.
Wider implications of the findings: The prevalence of the MetS differs from country to country depending on the habits of diet, lifestyle, and many other factors that increase the risk of incidence of the syndrome. The results of present data indicate that Greek PCOS women have an increased risk of MetS. The lipid abnormalities, the fast plasma glucose abnormality and elevated blood pressure places them an increased risk of long-term cardiovascular disease and DM2. It is important to emphasize that in obese and overweight subjects presented greater prevalence and much more metabolic abnormalities. The results of this study call attention to the need for comprehensive screening and education of women with PCOS regarding appropriate diet and exercise program.
Study funding/competing interest(s): Funding by hospital/clinic(s) – No funding was needed.
Trial registration number: NA.
Keywords: polycystic ovary syndrome, prevalence, characteristics, metabolic syndrome, international diabetes foundation

P-671 The relation between variation in size of the primordial follicle pool and menopause: a cohort comparison for observed and predicted distribution of age at menopause

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Study question: This study aims to investigate the hypothesis that the size of the primordial follicle pool is the main determinant for the length of the individual ovarian lifespan.
Summary answer: Predictions of age at menopause based upon the primordial follicle pool show a close conformity with observed ages at menopause.
What is known already: The value of ovarian reserve tests (ORTs), such as anti- Müllerian hormone (AMH) or the antral follicle count (AFC) in the prediction of age at natural menopause has been extensively researched and promising predictions can be made using these ORTs. Interestingly though, there is no research available assessing age at menopause based upon what is generally considered as the true ovarian reserve, i.e. the non- growing (primordial) follicle pool.
Study design, size, duration: Age at menopause (ANM) was modelled based on the declining number of primordial follicles, after identifying a critical threshold for cycle cessation. The distribution of predicted age at menopause based on this model was then compared to an observed distribution of age at menopause.
Participants/materials, setting, methods: 4 papers provided histologically derived non- growing follicle (NGF) counts obtained from single ovaries from women over ≥16 years. ANM data was obtained from the Prospect-EPIC cohort containing women ≥58 years with a natural menopause. Exclusion criteria were hormone use, ovarian abnormalities/surgery and uterus surgery prohibiting a menstrual cycle.
Main results and the role of chance: The decline in NGF numbers was plotted against age and a decline model was constructed. Predicted distribution of ANM was derived from this decline model, assuming menopause occurs when NGF numbers fall below a critical threshold. Derived distribution of ANM was fitted to observed menopausal ages provided by the Prospect-EPIC database. Maximum likelihood was used to estimate models parameters and predicted distributions of ANM were visually compared to observed ANM. Individual predictions of ANM were obtained combining percentile bands for age specific NGF numbers (i.e. low age specific NGF counts place women in the lower 5%, whereas high age specific NGF counts place women in the high 95%). Predictions of ANM based upon the primordial follicle pool showed a close conformity with observed ANM.
Limitations, reason for caution: There was more residual variation present in NGF counts than needed to describe variation in menopausal ages. This most likely stems from slightly different NGF counting methods present in the papers contributing to the NGF database.
Wider implications of the findings: The close conformity observed between NGFs and age at natural menopause supports the hypothesis that that the size of the primordial follicle pool is an important determinant for the length of the individual ovarian lifespan and supports the concept of menopause prediction using ORTs, such as AMH and the AFC, as derivatives of the true ovarian reserve.
Study funding/competing interest(s): Funding by University(ies) – University Medical Center Utrecht.
Trial registration number: NA.
Keywords: non-growing follicles, menopause, prediction, AMH, AFC

P-672 Oscillation expression of circadian gene PER2 and steroidogenesis related gene STAR following human chorionic gonadotropin stimulation in human luteinized granulosa cells

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Study question: Are circadian genes expressed in human ovary? Can oscillation expression of circadian genes in human granulosa cells be induced? How are the variation patterns of expression of steroidogenesis related genes in human granulosa cells after hCG stimulation?
Summary answer: Circadian genes were expressed in dominant antral follicles of human ovaries; hCG can induce oscillation expression of PER2 and STAR in cultured human granulosa cells.
What is known already: Expression of circadian genes has been observed in ovary of chicken, quail, rat, mice and ruminants. Both follicle stimulating hormone and luteinizing hormone can induce oscillation expression of circadian genes in cultured granulosa cells.
Study design, size, duration: Paraffin sections of normal ovarian tissue were obtained from five women who had undergone bilateral salpingo-oophorectomy for gynecologic malignancies. Human luteinized granulosa cells were obtained from follicle fluid of ten patients during ovum aspiration undergoing IVF for each experiment.
Participants/materials, setting, methods: Distribution of circadian genes expression in human ovaries was observed by immunohistochemistry. Accumulation patterns of circadian genes mRNAs and steroidogenesis related genes were observed in human luteinized granulosa cells during 48 hours after treatment of hCG.
Main results and the role of chance: Immunostaining of CLOCK and PER2 were observed in in dominant antral follicles in human ovaries. After hCG stimulation, expression of PER2 in human granulosa cells oscillated peaking at 24th and 40th hour; expression of BMAL1 decreased to the bottom at 20th hour; expression of CLOCK increased without statistical significance; expression of STAR displayed a mild oscillation pattern with two peaks at 24th and 40th hour;

expression of HSD3B2, CYP11A1, CYP19A1 increased not displaying oscillation pattern.
Limitations, reason for caution: Present results cannot illuminate the association between expression rhythms of PER2 and STAR.
Wider implications of the findings: Circadian genes expression may be involved in steroidogenesis of human ovary; rhythms of circadian gene expression and steroidogenesis function might exist in granulosa cells of human ovary.
Study funding/competing interest(s): Funding by national/international organization(s) – This work was supported by grants from National Basic Research Program of China (973 program, 2012CB947600), Scientific Project of Health Industry (201002013) and Science and Technology Planning Project of-Guangdong Province, China (2008A030201028).
Trial registration number: Not RCT.
Keywords: circadian, human chorionic gonadotropin, granulosa cell, ovary

P-673 Use of dual trigger with gonadotropin-releasing hormone agonist (GnRH-a) and human chorionic gonadotropin (hCG) to optimize oocyte recovery rates and IVF results

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Study question: Does dual trigger of final oocyte maturation improve the oocyte recovery and pregnancy rates in IVF cycles?
Summary answer: Co-administration of GnRH-a and hCG for final oocyte maturation significantly improves oocyte recovery rate in IVF cycles, however, pregnancy rates are similar in dual trigger and hCG trigger groups.
What is known already: Oocyte maturation for IVF cycles is commonly induced by hCG as a surrogate for the natural LH surge. In the last years, the use of GnRH-a for final follicular maturation has been shown to significantly reduce the occurrence of ovarian hyperstimulation syndrome compared with hCG triggering, however, a poor reproductive outcome was reported after GnRHa triggering. More recently, the so-called "dual trigger" that combines GnRH-a with hCG has been investigated in IVF with promising results.
Study design, size, duration: Prospective randomized study. The population under study consisted of 73 consecutive patients treated by IVF or ICSI at Hospital Donostia Assisted Reproduction Unit, from January to March 2014. Participants were randomly assigned to two groups: (1) hCG trigger group ($n = 39$) and (2) dual trigger (GnRH-a + hCG) group ($n = 34$).
Participants/materials, setting, methods: Final oocyte maturation was triggered by either 250 mg of recombinant hCG (Ovitrelle; Merck Serono) alone, or by 250 µg of recombinant hCG plus 0.2 µg of triptorelin (Decapeptyl; Ipsen Pharma), depending on the assigned group. All oocyte retrievals were performed under transvaginal ultrasound guidance 36 h after triggering. All embryo transfers were performed 48 hours after oocyte retrieval. Statistical analysis was performed using SPSS 21.0.
Main results and the role of chance: Female mean age, corporal mass index, basal FSH levels, number of antral follicles and AMH levels, as well as cycle stimulation characteristics (estradiol, progesterone) were similar in both groups. We found a significantly higher oocyte recovery rate (proportion of the number of oocytes retrieved per number of preovulatory follicles punctioned) in patients who received the double trigger compared to hCG trigger group (82.5% vs 66.9% respectively, $p = 0.013$). However, there were no statistically significant differences in the percentage of MII oocytes retrieved (84% vs 81.1%), fertilization rate (72.5% vs 63.3%) and pregnancy rate per transfer (47.5% vs 41.9%), between the hCG trigger and dual trigger groups respectively.
Limitations, reason for caution: Our results are limited by the small sample size; therefore, it was not possible to analyze the effects of dual triggering in IVF results of different subgroups of patients. A larger data set is needed to provide more information about dual triggering potential use. This is not a blinded study.
Wider implications of the findings: The results from the study indicate that dual trigger of final oocyte maturation with GnRH-a and the standard dosage of hCG significantly improves oocyte recovery rates in IVF. Although we don't find any relation with pregnancy rates, the co-administration of GnRH-a and hCG could be an effective strategy to optimize pregnancy outcome for poor ovarian responders. However, further large prospective studies are needed to elucidate the aforementioned recommendation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hospital Donostia.
Trial registration number: NA.
Keywords: dual trigger, hCG, GnRH agonist, oocyte recovery rate, pregnancy rate

P-674 Serum and follicular hormone levels do not correlate in different age groups, questioning the impact of LH and androgen substitution on the follicular endocrine milieu

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Study question: Do serum and follicular fluid endocrine profiles change with age, indicating that endocrine substitution with LH and androgens might improve the follicular endocrine milieu?
Summary answer: As several endocrine parameters such as LH and testosterone decrease with age in serum but not in follicular fluid and as serum and follicular fluid hormone parameters do not correlate, endocrine substitution to improve the endocrine milieu is subject to debate.
What is known already: Serum androgen concentrations decrease with age. The endocrine milieu has been found to be predictive for the implantation potential of oocytes. In "IVF low responders" treatment strategies are based on the administration of LH and androgens to increase the ovarian response and the oocyte quality.
Study design, size, duration: Cross-sectional study involving 97 women undergoing each one Natural Cycle (NC)-IVF cycle without any hormone stimulation apart from ovulation induction with hCG (5,000 IU) and performed between 2011 and 2014.
Participants/materials, setting, methods: Ninety-seven women undergoing NC-IVF were divided into three age groups: 28–35 years ($N = 30$), 35–39 years ($N = 46$), and 39–42 years ($N = 21$). Serum and follicular fluid were collected at the time of follicle aspiration and the concentrations of LH, testosterone (T), estradiol (E_2) and anti-Mullerian hormone (AMH) were determined by immunoassay and their concentrations correlated.
Main results and the role of chance: Serum LH (28–35 years group = 49.2, 39–42 years group = 26.4 mIU/mL, $P = 0.0178$), T (28–35 years group = 1.26, 39–42 years group = 1.01 nM, $P = 0.2394$), and AMH (28–35 years group = 22.3, 39–42 years group = 12.5 pM, $P = 0.1380$) concentrations decreased with increasing age, E_2 did not. However, none of the hormones decreased with age in the follicular fluid. Furthermore, neither serum LH nor testosterone correlated with the follicular fluid concentrations of T, E_2 and AMH. In follicular fluid significant correlations of T vs. LH,, E_2 vs. T, AMH vs. T and AMH vs. E_2 were observed in women aged 35–39 years. The correlation between E_2 and T was found in all three age groups ($P \leq 0.0011$).
Limitations, reason for caution: A direct correlation between the hormone concentrations and the implantation potential of the oocytes could not be investigated as the oocytes were not treated individually in the IVF laboratory.
Wider implications of the findings: As serum concentrations of LH and androgens do not correlate with the concentration of androgens, E_2 and AMH in follicular fluid, it is questionable whether the substitution with LH or androgens would improve the endocrine milieu in follicles. Therefore such substitution in women with advanced age and undergoing IVF therapy should not be encouraged without further clinical studies.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Public university.
Trial registration number: NA.
Keywords: androgens, AMH, LH, follicle, maternal age

P-675 Are AMH type II receptor and AMH gene polymorphisms associated with ovarian reserve, ovarian response or outcomes of ovarian stimulation?

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Study question: Is there any effect of Anti-Mullerian Hormone (AMH) gene and AMH type II receptor polymorphisms on ovarian response/treatment outcomes and/or current markers of ovarian reserve in patients undergoing in vitro fertilization (IVF) treatment?
Summary answer: No significant associations of *AMH* Ile49Serand AMHR2 -482a > g genotypes with ovarian response (number of oocytes retrieved) and/or markers of ovarian reserve (FSH, AFC, and AMH) were detected in our cohort of women undergoing IVF treatment ($p = 0.136$, and $p = 0.208$).
What is known already: Single nucleotide polymorphisms (SNPs) are increasingly being investigated for a possible association with abnormal responses to ovarian stimulation. Serum AMH is used in clinical practice as a biomarker of ovarian reserve and to predict ovarian response. The AMH signalling pathway is thought to regulate FSH sensitivity in the ovary and follicular recruitment and selection. Therefore, polymorphisms of AMH and AMH receptor genes may have a role in influencing ovarian reserve and ovarian response to stimulation.
Study design, size, duration: In this prospective observational study, we genotyped the *AMH* Ile49Ser and AMHR2 -482a > g SNPs in 579 unrelated women undergoing their first cycle of controlled ovarian stimulation for IVF and ICSI (intracytoplasmic sperm injection) using gonadotrophins at a tertiary referral centre for reproductive medicine between March 2009 and August 2010.
Participants/materials, setting, methods: Pelvic ultrasound and blood samples were taken on day 2–3 of the cycle for baseline hormones/DNA extraction. Genotypes were determined using TaqMan allelic discrimination assay. Regression analysis was performed to assess the effect of the two SNPs on the ovarian reserve markers, the primary outcomes of response, and treatment outcomes.
Main results and the role of chance: There was no evidence of any statistically significant ($p < 0.05$) difference in basal FSH, AMH and AFC between individuals with different *AMH* Ile49Ser and AMHR2 -482a > g genotypes. The number of oocytes retrieved and gonadotropin dose used was also comparable between the individuals with different genotypes.
Limitations, reason for caution: A larger sample size would be required in order to determine if the *AMH* Ile49Ser and AMHR2 -482a > g genotypes had a smaller effect on ovarian reserve or response.
Wider implications of the findings: When considering the development of integrative clinical algorithms for individual FSH doses, our analysis suggests that the genotyping of *AMH* Ile49Ser and AMHR2 -482a > g polymorphisms does not provide additional useful information as a predictor of ovarian reserve or response to ovarian stimulation.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – The study was funded by the Manchester Biomedical Research Centre.
Trial registration number: South Manchester Research Ethics Committee. REC ref no. 08/81003/212.
Keywords: ovarian response, SNP, AMH, polymorphism, ovarian stimulation

P-676 The effect of isotretinoin on ovarian reserve based on hormonal parameters, ovarian volume, and antral follicle count in women with acne

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Study question: Does oral isotretinoin impact on the ovarian reserve as evaluated with hormonal parameters, anti-Mullerian hormone (AMH), ovarian volume (OV), and antral follicle count (AFC) in reproductive aged women with acne?
Summary answer: This prospective study demonstrated that oral isotretinoin had a significant negative effect on ovarian reserve as evaluated by AMH, AFC, and OV in patients with acne.
What is known already: Widely prescribed in routine practice, isotretinoin has several side effects in many organ systems; however, the effect of isotretinoin on female gonads is not clear. Oral isotretinoin significantly affects ovarian reserve evaluated with follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and AMH.
Study design, size, duration: This prospective study was conducted between March 2013 and March 2014 at a tertiary referral center in Turkey. We investigated the impact of oral isotretinoin on ovarian reserve in 82 reproductive aged women with acne who were treated with oral isotretinoin.
Participants/materials, setting, methods: Of the 121 reproductive aged women evaluated for eligibility criteria, 82 were included. Patients were evaluated for ovarian reserve status prior to therapy and reevaluated 6 months after isotretinoin treatment. Their ovarian reserve status was identified according to hormonal parameters, AMH, OV and AFC.
Main results and the role of chance: Significant differences were found between the pretreatment and posttreatment for AMH (2.20 (1.14–4.07) vs. 1.31 (0.32–2.28), $p < 0.001$), AFC (16 (14–18.25) vs 12.5 (10 -15), $p < 0.001$) and OV (23 (18–29) vs 15 (13–18), $p < 0.001$). We found a significant decrease in AFC, OV, AMH, and E2 levels after 6 months of isotretinoin treatment.
Limitations, reason for caution: Data collection from a single clinic is a potential limitation, and the generalizability of our findings is limited. The absence of longitudinal data as well as control data after recovery and treatment is another limitation of this study; hence further prospective, multicenter studies with longer follow-up times are required.
Wider implications of the findings: The findings of our study indicate that oral isotretinoin significantly decreased ovarian reserve evaluated with AMH, AFC and OV. This is the first study which included all these reliable markers of ovarian reserve, such as FSH, LH, E2, AMH, AFC, and OV together. Based on the present findings, widely and commonly prescribed in routine clinical practice, oral isotretinoin treatment should be considered deleterious for ovarian function and reserve.
Study funding/competing interest(s): Funding by hospital/clinic(s) – There were no external funding and financial support for this study and only departmentally available equipment and drugs were used for the study. There are no conflicts of interest to declare.
Trial registration number: Registration number has not been received.
Keywords: acne, anti-mullerian hormone, antral follicle count, isotretinoin, ovarian reserve

P-677 Granulin levels in patients with premature ovarian failure

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Study question: Are Granulin levels associated with premature ovarian failure (POF)?
Summary answer: Granulin levels in patients diagnosed with POF were significantly lower than ones of fertile women.
What is known already: As a crucial health problem in a woman's life, Premature Ovarian Failure (POF) has gained significance with the prolongation of expected lifetime. Etiology has a diverse spectrum, but no exact etiologic factor was found to-date.
Study design, size, duration: We aimed to investigate the diagnostic value of Granulin which were showed to have oocyte-specific expression in the pathogenesis of POF as a growth factor. 31 women diagnosed with POF and 57 fertile women as a control group were recruited in this cross-sectional study, from August 2014 to December 2014.
Participants/materials, setting, methods: Recruited patients were younger than 40 years and had no systemic disease or drug use. Antecubital venous samples were obtained from each woman in the morning, after 8-h fasting period. Serum samples were separated and Granulin levels were determined with ELISA method.
Main results and the role of chance: Ages of the patients were similar between the POF group (32.06 ± 4.37 years) and the control group (31.89 ± 4.25)

($p = 0.86$). Likewise, body mass indices of the patients were similar between the POF group (23.67 ± 3.05 kg/m^2) and the control group (23.89 ± 3.0 kg/m^2) ($p = 0.747$). Obstetric history characteristics (gravidity, parity, living child and abortus numbers) were significantly different between two groups ($p < 0.001$, for each one). FSH levels were significantly higher in the POF group, as it was expected ($p < 0.001$). Granulin levels in the POF group (2.94 ± 1.91 ng/mL) were significantly lower than ones in the control group (4.76 ± 1.62 ng/mL) ($p < 0.001$). No association found between Granulin levels and FSH levels.

Limitations, reason for caution: A fully evaluation of ovarian reserve (Anti-mullerian hormone, antral follicle count) and its interaction with Granulin levels could not be performed because of insufficient data about it. This was a limitation in our study.

Wider implications of the findings: This is the first study on Granulin levels in POF. Granulin is a candidate biomolecule to shed light on the pathogenesis of POF, but we could not set forth its exact mechanism whether it's a cause or a result. Further large studies are warranted to figure out molecular role of the Granulin about cognitive problems and ovarian reserve in patients with POF and a lifelong follow-up may provide much more reliable results on these inferences.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Zekai Tahir Burak Women's Health Training and Research Hospital.

Trial registration number: NA.

Keywords: growth factor, granulin, marker, ovarian reserve, premature ovarian failure

P-678 Exploring microRNA expression profile in follicular fluid to classify women with polycystic ovary syndrome

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Study question: Can microRNAs (miRNAs) in follicular fluid (FF) from women with polycystic ovary syndrome (PCOS) be used to improve classification of women with PCOS?

Summary answer: The miRNA expression profile in FF between subsets of PCOS women is significantly different ($p < 0.05$) from healthy controls.

What is known already: Few studies have shown that miRNAs can be isolated from FF from women with PCOS and that the expression level of certain miRNAs is altered in PCOS. Furthermore, insulin resistance, a clinical feature of PCOS, has been associated with an altered miRNA profile in type 2 diabetic patients but the involvement of miRNAs in patients with PCOS is unclear.

Study design, size, duration: A case-control study of forty-nine women with PCOS (mean ± SD; age 27.98 ± 3.87, BMI 25.88 ± 5.25) divided into groups based on their PCOS phenotypes, and twenty-one healthy, regularly cycling women (mean ± SD; age 27.76 ± 3.79, BMI 24.24 ± 3.82) undergoing in vitro fertilization from January 2010 to February 2013.

Participants/materials, setting, methods: FF from the first punctured follicle on each ovary was collected at the time of oocyte pick-up. MiRNAs were extracted from the FF and reverse transcribed into cDNA. TaqMan miRNA arrays (748 different miRNAs) on four representative individuals from each phenotypic group were analyzed and individual selected miRNAs were validated using quantitative real-time PCR in the entire study population.

Main results and the role of chance: Several miRNAs showed a significantly and differently expression in subgroups of PCOS patients compared to healthy controls with miR-24, miR-29a and miR-151 being decreased in PCOS patients. Correlation analysis revealed that markers of metabolic syndrome correlated with the expression levels of miR-24, miR-29, miR-132 and miR-320a in PCOS patients. Furthermore, miR-151 was associated with androgen status in PCOS patients. The number of antral follicles correlated with the expression of miR-574 in the controls. Target gene analyses suggest that steroid biosynthesis signaling and metabolic pathways could be over-represented.

Limitations, reason for caution: Only known miRNAs were investigated. The functional targets of the validated miRNAs as well as the consequences of the miRNA expression profile remains to be determined. Since PCOS is a very heterogeneous disorder, it will be relevant to perform sub-phenotype investigations in relation to miRNA expression levels.

Wider implications of the findings: The usage of miRNAs as a minimal non-invasive biomarker holds great potential. This study suggest that women with PCOS have an altered miRNA profile and that miRNAs can be used to further sub-classify women with PCOS. Moreover, miRNAs may be involved in the pathogenic mechanisms underlying decreased fertility among PCOS patients.

Study funding/competing interest(s): Funding by national/international organization(s) – The study was partly funded by grants from The Danish Diabetes Academy supported by the Novo Nordisk Foundation and a Merck Serono Grant for Fertility Innovation (GFI).

Trial registration number: NA.

Keywords: polycystic ovary syndrome, microRNA, insulin resistance, infertility

P-679 Obstetric and neonatal outcome in oocyte donated 46,XY compared to 45,X and 46,XX women: a nationwide controlled cohort study

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Study question: What are the obstetric and neonatal outcomes after oocyte donation (OD) in 46,XY women compared to women with 45,X or 46,XX karyotype?

Summary answer: The obstetric outcomes were reassuring and neonatal outcomes were good for both singleton and twin deliveries in OD women with 46,XY gonadal dysgenesis.

What is known already: There is an increased rate of pre-eclampsia, failed labor induction, and ceasarean section (CS) as delivery method in women with 46,XY gonadal dysgenesis. Numerous reasons have been suggested including deficient uterus receptors for prostaglandin and oxytocin, that causes failure to induce labor and uterus contractions, a weak uterine wall and a small pelvis. Few deliveries are described in literature and they all lack an appropriate control group.

Study design, size, duration: This retrospective controlled cohort study included all OD women nationwide who gave birth between 1994 and 2013 in Denmark. A total of 404 OD women were enrolled in the study with 346 singleton and 92 twin deliveries.

Participants/materials, setting, methods: Women were identified and data collected using the Cytogenetic Central Register, the National Patient Registry, the IVF registry and the Medical Birth Register. Six OD women with 46,XY gonadal dysgenesis were enrolled in the study and delivered eight babies including one set of twins. 29 OD women with 45,X gonadal dysgenesis and 369 OD women with 46,XX karyotype were enrolled in two different control cohorts and delivered 38 babies (including four twin deliveries) and 484 babies (including 87 twin deliveries) respectively.

Main results and the role of chance: The obstetric outcomes were reassuring for both singleton and twin deliveries. There was an increased ceasarean section (CS) rate of 71.4%, similar to previous literature, and an increased elective CS rate of 80% of all CS, the reason for that is still unknown. Additional an increased rate of postpartum haemorrhage of 43%, but the difference was insignificant. The neonatal outcomes were good for both singleton and twin deliveries with Apgar scores = 10, no stillborns, neonatal mortality or serious birth defects.

Limitations, reason for caution: This study was performed over two decades and is the first controlled cohort study of OD 46,XY women, however larger cohorts are needed to assess rare events.

Wider implications of the findings: Obstetric and neonatal data was collected for all OD women nationwide, supporting generalizability to similar populations. Neonatal outcomes were in agreement with the literature, while obstetric outcomes were generally better than previously reported. We suggest evaluation of the uterus and pelvis size before pregnancy and delivery. In addition we recommend cross-matched blood available for delivery. We remind clinicians that OD women with 46,XY gonadal dysgenesis is not in itself an indication for CS.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Odense University Hospital.

Trial registration number: Clinical trial registration number was not necessary as it is a register study. Approved by the Danish Data Protection Agency. Journal number: 2014-41-2694.

Keywords: gonadal dysgenesis, oocyte donation, neonatal outcome, 46,XY women, pregnancy outcome

P-680 The kisspeptin-GnRH pathway and the expression of kisspeptin, GnRH and their receptors in human endometrial stromal cells: possible involvement in embryo implantation and early pregnancy

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Study question: We try to investigate the presence of GnRH, kisspeptin and their receptors in the human endometrial decidual stromal cells, thus establishing an autocrine/paracrine kisspeptin-GnRH pathway in the regulation of embryo implantation and early pregnancy.
Summary answer: These results demonstrate that GnRH, kisspeptin, GnRH receptor, kisspeptin receptor, and kisspeptin-GnRH signaling are present in human endometrial decidual tissues and stromal cells. Our findings represent a new concept regarding the potential modulatory role of kisspeptin and GnRH on embryo implantation and decidual programming of human pregnancy.
What is known already: Endometrial decidualization plays an important role on the implantation of the embryo and human pregnancy. Invasion of the maternal decidua by extravillous trophoblast is an important process for embryo implantation and placentation.
Study design, size, duration: In this in vitro study, we examined the expression of GnRH, kisspeptin, GnRH receptor and kisspeptin receptor in human endometrial decidual stromal cells, indicating the role of GnRH, kisspeptin, and kisspeptin-GnRH pathway in embryo implantation and early pregnancy.
Participants/materials, setting, methods: Endometrial decidual stromal cells were isolated from women undergoing elective pregnancy termination of pregnancy at 6- to 12-week gestation, after informed consent. Immunoblot analysis, immunohistochemistry, kinase array and RT-PCR were performed to investigate the GnRH-kisspeptin signaling following the treatment with steroid hormones and GnRH analoges in human endometrial decidual stromal cellls.
Main results and the role of chance: The mRNA expression of GnRH, kisspeptin, GnRH receptor and kisspeptin receptor was found in human endometrial decidual stromal cells. The protein expression of GnRH, kisspeptin, GnRH receptor and kisspeptin receptor was detected in human endometrial decidual stromal cells. GnRH, kisspeptin, GnRH receptor and kisspeptin receptor immunoreactivity was detected in human endometrial decidual tissue. The expression of kisspeptin and kisspeptin receptor was regulated following treatment with estradiol, progesterone or GnRH analoges in human endometrial decidual stromal cells.
Limitations, reason for caution: Hard to detect the kisspeptin, GnRH and their receptors expression in human endometrial decidual stromal cells by immunoblot analysis.
Wider implications of the findings: Our findings represent a new concept regarding the potential modulatory role of kisspeptin and GnRH on embryo implantation and decidual programming of human pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Chang Gung Memorial Hospital.
Trial registration number: NA.
Keywords: kisspeptin, GnRH, endometrium, implantation

P-681 A single-centre evaluation of elecsys roche automated anti-mullerian hormone assay and comparison with the current clinical standard assay

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Study question: What are the concordance and the repeatability of serum AMH measurements performed by the well established Beckman Coulter Gen II AMH ELISA assay and the novel Roche Elecsys Automated AMH assay?
Summary answer: The Elecsys Roche Automated Assay has different calibration to the Gen II assay, but exhibits greater sensitivity and repeatability consistent with its automated methodology making it superior for clinical use.
What is known already: The Beckman Coulter Gen II assay is the most commonly used AMH assay in routine biochemistry at present, but persistent calibration/interference problems have been reported
Study design, size, duration: Prospective single centre study performed between September and December 2014. Samples from 277 patients submitted for AMH evaluation were determined with both Gen II and Elecys assays. We randomly selected 66 samples for analysis of repeatability
Participants/materials, setting, methods: All serum samples from patients referred for AMH at Instituto Valenciano de Infertilidad during the study period were eligible. Methods were compared using Lin's concordance correlation coefficient (using log transformed data to satisfy assumptions of normal distribution), Passing-Bablok regression and Bland–Altman plots using Pitman's test to assess likelihood of bias.
Main results and the role of chance: Samples which were below the Limit of Detection (n = 35, 5 of which were for both assays) were excluded from the analysis. Median [interquartile range (IQR)] AMH was 13.0 pmol/l in the Gen II (interquartile range 6.8 –29.0) and 10.8 pmol/l (5.4 –22.4) in the Elecsys ($P < 0.0001$). The concordance between log-transformed values was rho = 0.96 (95% CI 0.96 –0.97). The Passing–Bablok regression equation was: y (Roche) = 0.51 + 0.75 x Gen II. Bland–Altman analysis showed evidence of bias in absolute measurements, such that samples with higher AMH had higher estimates on the Gen II assay. The correlation between the difference and the mean was r = 0.51, $P < 0.001$. The R^2 coefficient for repeatability was 0.999 for the Elecsys and 0.993 for the Gen II.
Limitations, reason for caution: The present study is a pragmatic assessment of the new assay under ideal conditions. Lot to lot variation could not be assessed. Demographics and outcomes of patients referred for AMH measurement were not known.
Wider implications of the findings: The new Elecsys Roche Automated assay exhibits different calibration to the Gen II assay such that new reference ranges will be required to be established. That the Elecsys assay exhibits superior sensitivity, precision and repeatability suggests that it would be preferable to the Gen II assay for clinical and epidemiological use.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Roche Diagnostics provided kits for this study free of charge. The manufacturer played no part in conducting assays or data analysis. SM Nelson has received honoraria from Beckman Coulter and Roche Diagnostics. Ernesto Bosch has received honoraria from Roche Diagnostics.
Trial registration number: NA.
Keywords: AMH, diagnostic test

P-682 Serum progesterone elevation in early follicular phase has an adverse effect on cycle cancelation and ongoing pregnancy rates of in vitro fertilization treatment

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Study question: It is well known that progesterone elevation (PE) on hCG trigger day has a detrimental effect on the pregnancy rate in IVF cycles. If the progesterone concentration in early follicular phase (EPE) remains elevated, would the pregnancy rate of IVF treatment be also affected?
Summary answer: EPE was associated with a similar number of oocytes and embryos, a higher cycle cancelation rate (CCR) due to no or poor ovarian response and a lower ongoing pregnancy rate (OPR). Women with PE at early and late follicular phases performed worst with the highest CCR and the OPR.
What is known already: PE on hCG trigger day has a detrimental effect on the pregnancy rate in IVF cycles. It may adversely affect the endometrial receptivity, but not the embryos quality, as shown by gene expression study and studies showing comparable pregnancy rate in subsequent frozen-thawed embryo transfer cycles. Only few studies looked into the effect of EPE. A prospective study showed cycles with EPE, even if progesterone concentration normalised within two days, had a significantly lower OPR.
Study design, size, duration: This is a retrospective study. All first IVF cycles carried out between January 2011 and December 2013 at the Centre of Assisted Reproduction and Embryology, The University of Hong Kong were retrieved and 1,122 cycles were included in the final analysis.
Participants/materials, setting, methods: Clinical details of treatment cycles were prospectively entered into a computerized database, which were retrieved for analysis. Cycles with pre-implantation genetic diagnosis, or using donated gametes were excluded. The primary outcome was OPR and

secondary outcomes included the number of oocytes and embryos and CCR due to no ovarian response.

Main results and the role of chance: The cut-off level of EPE was defined as 4.5 nmol/L, above which the OPR dropped abruptly. The incidence of EPE was 4.9% (55/1122, 95% CI 3.9–6.9%). Women with and without EPE had a similar number of oocytes and embryos. The EPE group had a significantly higher CCR than the non-EPE group (9.1% vs 3.7%, RR 0.408 95% C.I.0.171–0.973, $p = 0.043$). The OPR per cycle initiated was significantly lower in the EPE group, when compared with that of the non-PE group (16.4% vs 28.8%, RR 0.971 95% C.I.0.946–0.996, $p = 0.046$). In the subgroup analysis, those women with normal progesterone concentration both at early and late follicular phases performed best with the lowest CCR and the highest OPR, while those with PE on both occasions had the highest CCR and the lowest OPR

Limitations, reason for caution: This is a retrospective analysis but the serum progesterone concentration from the achieved samples did not alter the clinical management of the treatment cycles during the study period. There may be confounding factors that were not controlled during the statistical analysis. There were various stimulation regimen used.

Wider implications of the findings: The findings are important for counselling of women undergoing IVF treatments. Though there were various protocols used and the data look heterogenous, it resembled the real situation in fertility centres. It actually reinforced the generalization and applicability of our data in the real clinical situations. Strategies to deal with the EPE should be studied as it seems that postponing the starting day till progesterone concentration normalised in the same cycle does not help.

Study funding/competing interest(s): Funding by University(ies) – This study was funded by the Department of Obstetric and Gynaecology, the University of Hong Kong.

Trial registration number: This study was registered on the HK clinical trial registry with the register number HKCTR-1820.

Keywords: progesterone elevation, early follicular phase, cycle cancellation, pregnancy rate, IVF

P-683 Effects of Korean red ginseng extracts on endometrium in postmenopausal women

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Study question: Does Korean red ginseng (RG) extract affect human endometrium of postmenopausal women?

Summary answer: RG extract seems to not influence on serum estradiol and endometrial thickness. Anti-proliferative effect on endometrial stromal and epithelial cells has been shown by our study.

What is known already: Ginseng extract has been a representative tonic, which may exert estrogenic effect. Studies have shown various effects of ginseng in postmenopausal women. Ginseng has been shown positive effect as anti-oxidant, by increasing enzyme activity in postmenopausal women. However, discrepant results have been reported about effect of ginseng on menopausal symptoms. Our previous RCT that evaluated RG effect on menopausal symptoms has shown significant improvement, which may suggest to have favorable effects to postmenopausal women.

Study design, size, duration: A randomized, placebo-controlled, double-blinded clinical trial was conducted for 12 weeks. Total 72 postmenopausal women were enrolled, and analyzed changes in serum estradiol and endometrial thickness on ultrasonography from baseline to 12 weeks. Additional experimental study was conducted to see whether KRG extract affects endometrial cell proliferation/apoptosis.

Participants/materials, setting, methods: Postmenopausal women between ages 45 to 60 years were eligible. Participants were assigned to either RG or placebo group. Experimental study was conducted using isolated endometrial stromal cell from human endometrium, and Ishikawa cell from endometrial cancer cell line. Cell proliferation/apoptosis was assessed after treating RG.

Main results and the role of chance: Serum estradiol level and endometrial thickness were not influenced by KRG supplementations. Moreover, RG extracts were found to inhibit growth of both endometrial stromal cells and Ishikawa cells in a dose-dependent manner, and to induce apoptosis in Ishikawa cells by dose increase as well. Concurrently, western blotting & caspase-3 activity assay revealed that RG extracts lead to activation of caspase-3 and PARP cleavage, indicating caspase-3 mediated PARP cleavage which results in apoptosis. Since RG has shown anti-proliferative, pro-apoptotic effect on endometrium, it may regard as little potential danger of developing endometrial pathology as adverse event associated with taking RG.

Limitations, reason for caution: Limitations for the study are small sample size and lack of detailed adverse events including bleeding pattern.

Wider implications of the findings: Our result may provide a further understanding on ginseng extract as a complementary medication that can be used for postmenopausal women.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – This work was supported by a grant from the Korean Society of Ginseng, funded by Korea Ginseng Corporation, 2010.

Trial registration number: NA.

Keywords: ginseng, endometrium, apoptosis, menopause

P-684 GnRH agonist trigger with intensive luteal phase support versus hCG trigger with standard luteal phase support in a population of high responders

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Study question: Is there a difference in clinical pregnancy rate, live birth rate and the incidence of ovarian hyperstimulation syndrome when a GnRH agonist (GnRHa) trigger with intensive luteal phase support is compared to hCG trigger with standard luteal phase support in high-risk patients undergoing IVF treatment?

Summary answer: GnRHa trigger is associated with similar pregnancy rates with hCG trigger and a significant reduction in hospitalisation for severe OHSS after an intention-to treat analysis was performed.

What is known already: The GnRHa-induced LH surge has a relatively short duration of 24–36 h. To counteract the resulting luteal phase deficiency, the use of intensive or modified luteal phase support protocols was introduced. The largest study so far comparing GnRHa triggering with intensive luteal phase support to hCG triggering after a GnRH antagonist protocol reported no difference in pregnancy rates and a reduction in OHSS in high responders having a triple embryo transfer on day 2.

Study design, size, duration: A retrospective, cohort study of high-risk patients having a GnRH antagonist protocol with a GnRHa trigger (October 2011–June 2014) was performed. The control group consisted of high-risk patients having a GnRH antagonist protocol with hCG trigger during the transitional period of the introduction of the GnRHa trigger protocol.

Participants/materials, setting, methods: 382 patients had GnRHa triggering and 194 controls had hCG triggering. All patients had ≥18 follicles ≥11 mm and/or serum estradiol >18,000 pmol/l on the day of trigger. Patients had a single or double embryo transfer at cleavage or blastocyst stage. Logistic regression was used to adjust for differences between the groups.

Main results and the role of chance: An intention-to-treat analysis of all started cycles was performed (GnRHa group $n = 382$ vs. hCG group $n = 194$). No statistically significant differences were observed in terms of positive pregnancy test rate (48.9% vs. 48.9%, OR: 0.92, 95% CI: 0.63–1.35, $p = 0.94$), clinical pregnancy rate (35% vs. 41.2%, OR: 0.75, 95% CI: 0.52–1.10, $p = 0.15$), implantation rate (31.2% vs. 36.6%, OR: 0.76, 95% CI: 0.57–1.07) and live birth rate (30% vs. 35.4%, OR: 0.78, 95% CI: 0.51–1.18, $p = 0.24$). Significantly fewer patients had an embryo transfer cancellation in the GnRHa trigger group (3.9% vs. 8.7%, OR: 0.43, 95% CI 0.21–0.87, $p = 0.02$). Only one patient (0.3%) was hospitalised due to severe OHSS in the GnRHa group, compared to 26 patients (13%) in the hCG group (OR: 0.01, 95% CI: 0.00–0.06, $p < 0.001$).

Limitations, reason for caution: No monitoring of serum progesterone or estradiol was performed during the luteal phase. The decision to proceed to a cancellation of the embryo transfer procedure and to an elective cryopreservation of embryos was based on clinical judgment rather than any set clinical or biochemical criteria.

Wider implications of the findings: To our knowledge this is the largest, observational study comparing the outcomes of IVF cycles using a GnRHa or hCG trigger. GnRHa trigger with the use of intensive luteal phase support appears to be as effective and safer than hCG trigger. A large randomised trial comparing the intensive and modified luteal phase support protocols with an

elective embryo cryopreservation protocol is needed to establish the best approach after the use of a GnRHa trigger.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Hammersmith, London, UK.
Trial registration number: NA.
Keywords: GnRHa trigger, hCG, ovarian hyperstimulation syndrome, GnRH antagonist

P-685 Serum levels of 25-hydroxyvitamin D and time to natural pregnancy

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Study question: Are serum 25-hydroxyvitamin D (25-OH-D) levels, which is the form of vitamin D reflecting the store of the vitamin, higher in fertile compared to subfertile women?
Summary answer: We did not find differences in serum levels of 25-OH-D between subfertile and fertile patients. Our study does not support a crucial role of 25-OH-D in natural fertility.
What is known already: Studies in animal models support a critical role of Vitamin D in reproductive mechanisms. However, data in human is inconsistent. Some authors reported a detrimental effect of vitamin D deficiency in IVF but others did not. Noteworthy, IVF is a valuable model to investigate several factors affecting fertility in women but it may not properly reveal all the potential effects of vitamin D on natural fertility.
Study design, size, duration: Women referring for 1st-trimester screening for aneuploidies between January 2012 and June 2013 were evaluated to participate to this nested case-control study. The sample size (73 cases and 73 controls) was calculated based on a paired study design and assuming as relevant a 15% reduction in serum 25-OH-D in cases.
Participants/materials, setting, methods: Cases and controls were defined as women conceiving in 12–24 and ≤12 months, respectively. They were matched by age and study period. Exclusion criteria included: known cause of subfertility; BMI > 25 Kg/m^2; pregnancy achieved using ovarian stimulation or ART. 25-OH-D levels were tested using a chemiluminescence kit.
Main results and the role of chance: Baseline characteristics of the studied groups were similar with the exception of men age and parity. Serum 25-OH-D did not differ between cases and controls (21.2 ± 6.8 and 19.7 ± 7.3 ng/ml, respectively; paired *t*-test, $p = 0.16$; unpaired *t*-test $p = 0.20$). The number of women with serum levels <20 ng/ml was 34 (47%) and 37 (51%), respectively (McNemar test, $p = 0.73$; Fisher Exact test, $p = 0.74$). The OR of subfertility in women with 25-OH-D insufficiency (<20 ng/ml) was 0.85 (95% CI: 0.44–1.62). The OR adjusted for men age and parity was 0.84 (95% CI: 0.42–1.66). When considering women with vitamin D deficiency (<10 ng/ml), statistical analyses showed similar results.
Limitations, reason for caution: We could not assess whether appropriate serum 25-OH-D would shorten time to pregnancy since only a minority of studied women had levels >30 ng/ml. Even if time to pregnancy is commonly used to investigate causes of subfertility, we lack results from basic infertility investigations (semen analysis, basal hormones …).
Wider implications of the findings: Our results contrast with the available emerging evidence suggesting a detrimental effect of vitamin D deficiency on woman fertility. However, given that the vast majority of studied women have sub-optimal levels of 25-OH-D, interventional studies are warranted to draw definite conclusion.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Fondazione IRCCS Ca' Granda.
Trial registration number: NA.
Keywords: vitamin D, subfertility, pregnancy, time to pregnancy

P-686 Comparison of the efficacy of cabergoline and bromocriptine in a rat model of ovarian hyperstimulation syndrome

Abstract withdrawn by the author

P-687 Assessment of insulin signaling pathways gene expression in granulosa cells from eutrophic and obese women with polycystic ovary syndrome after in vitro fertilization cycles

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Study question: Determine if insulin signaling pathway gene expression in granulosa cells of obese Polycystic Ovarian Syndrome (PCOS) patients is altered compared to eutrophic PCOS patients undergoing *in vitro* fertilization (IVF) cycles.
Summary answer: Among the 84 genes analyzed, 09 genes were statistically significant over-expressed in obese patients compared to eutrophic. The up-regulated genes were BCL2L1, BRAF, CBL, DOK1, FBP1, FRS2, PCK2, RPS6KA1, and SORBS1.
What is known already: Insulin plays a central role on obese PCOS women. It acts through its own receptor or the IGF 1-receptor to enhance ovarian and adrenal steroidogenesis, activating tyrosine kinase phosphorylation intracellular signaling cascades. However the precise mechanism of insulin action on granulosa cells and the consequences to oocyte maturation is not completely elucidated in obese or even in non-obese PCOS patients.
Study design, size, duration: Cross-sectional study to evaluate granulosa gene expression in patients submitted to IVF treatment between January 2013 and October 2014. Fifteen PCOS patients (Rotterdam criteria) were subdivided: 09 eutrophic (Control group) and 06 obese (Obese group). In both groups, a normal insulin resistance were assessed according to *homeostasis model assessment insulin resistance* (HOMA IR).
Participants/materials, setting, methods: PCOS infertile patients were submitted to IVF with standard ovulation induction, oocytes were recovered to IVF and the granulosa cumulus cells were removed to RNA extraction by quantitative PCR array analysis of gene expression profile (RT² Profiler™ PCR Array Human Insulin Signaling Pathway- PAHS-030ZC-Quiagen, USA).
Main results and the role of chance: The results were expressed by fold up (³3) or fold down (≤3) values comparing obese patients gene expression over eutrophic patients. The results were considered statistically significant when $p \leq 0.05$ over gene expression. Among the 84 genes analyzed, 09 genes were statistically significant over expressed in obese patients. The up-regulated genes were BCL2L1 (fold = 4.7; $p = 0.021$), BRAF (fold = 3.8; $p = 0.031$), CBL (fold = 6.2; $p = 0.019$), DOK1 (fold = 4.6; $p = 0.040$), FBP1 (fold = 5.3; $p = 0.011$), FRS2 (fold = 4.1; $p = 0.044$), PCK2 (fold = 4.4; $p = 0.041$), RPS6KA1 (fold 4.2; $p = 0.044$), and SORBS1 (fold = 3.6; $p = 0.014$). These genes are involved in inhibition pathway of follicle development, insulin resistance, glucose uptake, granulosa cell growth and proliferation.
Limitations, reason for caution: The study was developed in a small sample size, as it is a screening for 84 genes. However, the sample power was calculated based on the difference of gene expression between groups and it showed to be higher than 80%. Besides that, the ovulation induction might produce some detrimental effects in the oocyte competence, regardless the insulin action. Hence, the gene profile expression of insulin pathways in the granulosa cells is not well elucidated and can not be generalized for IVF patients.
Wider implications of the findings: PCOS patients submitted to IVF show a large number of immature oocytes, poor embryos, miscarriages and a higher incidence on ovarian hyperstimulation syndrome, mainly in obese if compared to non-obese PCOS infertile patients. Also the pregnancy rate is lower in obese compared to non-obese patients. The knowledge of specific genes that modulate the granulosa-cumulus complex can expand the possibilities of new therapies and individualized approaches to PCOS patients seeking for IVF treatments.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Federal University of Sao Paulo/Huntington Reproductive Medicine.

Trial registration number: NA.
Keywords: polycystic ovary syndrome, obesity, insulin signaling pathways, gene expression

P-688 The performance power of the revised antimullerian hormone Generation II assay and reference range: population based comparison study

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Study question: Dose the revised Beckman-Coulter second generation (Gen II) antimullerian hormone (AMH) assay provides more reliable AMH results than the original Gen II assay and what are the age-specific reference AMH ranges for the revised Gen II assay?
Summary answer: Population data suggest a systematic shift between AMH distributions measured by the original and revised Gen II assay. The Immunotech (IOT) and revised Gen II assay are comparable in assay reliability from the point of variability. The quadratic model is fit for describing the decline in AMH measured by all the IOT, original and revised Gen II assay with age.
What is known already: AMH is accepted as a reliable and important predictor of ovarian response in IVF and ovarian reserve in other clinical settings. The original Gen II replaced the Diagnostic Systems Laboratory (DSL) & IOT assays but has higher instability than previous two assays and kit manufacturer revised assay method. There is no study comparing performance power among the IOT, original Gen II and revised Gen II assay in a large cohort.
Study design, size, duration: We recruited AMH data obtained from unselected women aged 25 to 45 years old examined for infertility work-up between November 2008 and June 2014. AMH samples were subject to the same handling procedures and were analyzed by the same laboratory.
Participants/materials, setting, methods: AMH values were measured using IOT assay (from November 2008 to August 2012, Cohort 1: n = 32,824), subsequently using the original Gen II assay (from September 2012 to July 2013, Cohort 2: n = 13,445) and lastly using the revised Gen II assay (from June 2013 to June 2014, Cohort 3: n = 15,801). Three cohorts were comparable in age characteristic. Each cohort was randomly divided into a training and validation cohort to set up and validate optimal age-AMH model.
Main results and the role of chance: AMH values measured with the original Gen II were 40% lower than those with the IOT and 36% lower than with revised Gen II assay in clinical practice. The distribution of AMH values obtained by the revised Gen II is similar with that obtained by the IOT assay. The quadratic model was the most appropriate to describe the decline in AMH obtained using the revised Gen II assay according to age.
Limitations, reason for caution: We can not exclusively rule out the existence of differences among the three cohorts.
Wider implications of the findings: The systematic shift between AMH distributions measured by the original and revised Gen II assay may imply the presence of assay-specific preanalytical instability. The revised Gen II assay may settle a controversy of appreciable variability in the original Gen II assay. Further studies are consistently required to minimize variabilities of AMH measurement.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Hamchoon Women's Clinic.
Trial registration number: NA.
Keywords: anti-Mu¨llerian hormone, AMH Gen II ELISA, AMH ELISA, reference values

P-689 Cumulus cells DFI as indicator of good response in different protocols of ovulation induction

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Study question: To validate the relationship between cumulus cells (CC) apoptosis rates revealed through the analysis of DNA fragmentation (DFI) and oocyte quality and to determine the effects of different stimulation protocols on apoptosis rate of the cumulus cells.
Summary answer: Cumulus cells DFI correlates with oocyte and embryo quality and fertilization rates. Addition of LH in the early follicular phase in ovulation induction protocols modify the CC apoptotic index; in particular we observed that the LH addition in the early follicular phase reduces DFI rates only in women over 35.
What is known already: A correct oogenesis requires a corresponding development of follicular cell component and an adequate dialogue between cumulus ooforus cells and oocyte is essential for developing oocyte competence. Data from literature have shown that the CC apoptosis rates are inversely correlated with mature oocyte rate, fertilization rates and success rates in IVF cycles. Furthermore it has been observed that different stimulation protocols may affect in different ways on CC apoptosis rates.
Study design, size, duration: Prospective observational study.
Participants/materials, setting, methods: 70 women candidates to an ICSI cycle. Exclusion criteria were age >38 years, BMI >25, smoking status, basal FSH >10 mIU/mL, severe endometriosis and PCOS. At the time of pick-up were isolated CC for laboratory evaluation. The apoptotic index assessment was performed by TUNEL TEST.
Main results and the role of chance: Samples were divided according to the levels of DFI into three groups: A-DFI <25, B-DFI 25–35, C-DFI >35. It is observed that in group C compared to group A (M2 55% vs 85% $p < 0.05$, 2PN 62% vs 93%, $p < 0.05$, embryo 1 45% vs 85%, $p < 0.05$) rate of the analyzed parametres were statistically significantly lower. It was also compared the CC DFI of women treated with LH and FSH together from 2 to 3 of the cycle of stimulation (36 pz) and CC DFI of women treated with FSH alone (29 pz); whereas there is no differences on the CC DFI in women younger than 35, was observed an apoptotic index lower in women of advanced reproductive age treated with LH (31% vs 42%, $p < 0,05$).
Limitations, reason for caution: The number of cases analyzed is still small to draw conclusions; further validation will be required. The small number of patients did not make it possible to rule out other potentially confounding factors.
Wider implications of the findings: Our results can represent a valid support of the already present literature for the use of apoptosis rate of cumulus cells as a possible indicator of oocyte quality and good response to stimulation. Furthermore, our results have suggested that the beneficial effects of the addition of LH from the initial stages of stimulation in women over 35 can be successfully mediated also by the improvement of the apoptotic index of cumulus cells.
Study funding/competing interest(s): Funding by University(ies) – Second University of Naples.
Trial registration number: The study was not registered because it is an observational study and the assignment of the medical intervention is not at the discretion of the investigator.
Keywords: cumulus cells, DFI, Ovulation induction

P-690 Nesfatin-1 level in follicular fluid (FF) is associated with the number of retrieved oocyte in IVF cycle

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Study question: Recently, nesfatin-1 is known as a regulator in hypothalamus-pituitary-ovary axis of animal model. What are the roles of nesfatin-1 in human IVF cycles undergoing a GnRH-antagonist protocol?
Summary answer: Nesfatin-1 was expressed in placenta, uterus, ovary, and testis. Furthermore, the expression of nesfatin-1 in ovarian granulosa cells of preantral follicles was higher than those in the other ovarian cells. In addition, higher nesfatin-1 levels in FF were significantly associated with higher number of retrieval oocytes.
What is known already: Nesfatin-1 is an anorexigenic peptide and regulates feeding behavior and energy homeostasis. Also, nesfatin-1 is known to participate in the activation of the hypothalamic-pituitary-adrenal axis in rat and to regulate the hypothalamic-pituitary-ovarian axis of fish.
Study design, size, duration: A total of 104 infertile women without PCOS undergoing IVF were studies from June 1, 2014 to October 31, 2014. The patients were divided to three groups according to the tertile of nesfatin-1 level in FF [Group I (<0.1 ng/ml): 37 cases, Group II (≤0.1–<0.2 ng/ml): 37 cases, Group III (≥0.2 ng/ml); 24 cases)]. The data were analyzed with age, number of retrieved oocyte, maturation rate, fertilization rate, pregnancy rate and BMI.

Participants/materials, setting, methods: Nesfatin-1 mRNA expression was tested by real-time PCR using total RNA of human placenta, uterus, ovary, and testis. Also, the localization of nesfatin-1 protein in human ovarian tissue was performed by immunocytochemistry. Follicular fluid was collected from the first follicle at time of oocyte retrieval. Nesfatin-1 levels were measured by ELISA.

Main results and the role of chance: Real-time PCR results showed that nesfatin-1 mRNA was generally expressed in placenta, uterus, ovary, and testis. In comparison with testis and ovary as reproductive organs, nesfatin-1 in ovary is expressed in about 30 times more than in testis. Nesfatin-1 protein was mainly localized in granulosa cells of primordial follicles and preantral follicles. By analyzing the results of age, maturation rate, fertilization rate, pregnancy rate and BMI, there were not significantly different in comparison of three groups. However, in data analysis of retrieval oocyte number, mean number (18.5 ± 6.8) of retrieved oocyte in group III (> 0.12 ng/ml) were significantly higher than mean number (12.4 ± 7) of retrieved oocyte in group I (< 0.1 ng/ml) ($p < 0.005$). Also we can freeze surplus embryos for cryopreservation cycle in 30 cases (66.7%) of group III,, but only 14 cases (37.8%) of group I are available for cryopreservation cycle.

	Group I ($n = 37$)	Group II ($n = 37$)	Group III ($n = 30$)
Range of nesfatin-1 concentration in FF	<0.1	≤0.1–<0.2	≤0.2
Nesfatin-1 concentration in FF	0.067 ± 0.02	0.133 ± 0.026	0.317 ± 0.139
Age	35.4 ± 4.4	35.8 ± 3.9	36.9 ± 3.8
Number of IVF cycles	1.8 ± 1	2.2 ± 2	2.3 ± 2
BMI	20.3 ± 2.2	20.9 ± 2.4	22.0 ± 3.0
Number of retrieval oocyte	12.4 ± 7.2[a]	16.9 ± 9.0	18.5 ± 6.8[a]
Maturation rate	70.40%	73.30%	73.20%
Fertilization rate	71.90%	81%	81.40%
Chemical pregnancy rate	48.6% (18/37)	45.9% (17/37)	43.3% (12/30)
Ongoing pregnancy rate	40% (15/37)	43% (16/37)	43.3% (12/30)
Percentage of cryopreservation available cycle	37.8% (14/37)[b]	54.1% (20/37)	66.7% (20/30)[b]

Mean ± SD; [a]$p < 0.005$; [b]$p < 0.01$ by *t*-test.

Limitations, reason for caution: There were some limitations in this study, including variable size of follicles that FF was collected from, and possibility of blood contamination during FF collection procedure. Additionally, to consolidate our conclusion, more ART cycles should be studied.

Wider implications of the findings: Finally, our data showed that there are positive correlation between nesfatin-1 and the number of retrieval oocyte, suggesting that nesfatin-1 could play a role in early folliculogenesis in human ovary. Also, it would be another interesting suggestion to study the correlation between nesfatin-1 level in blood and oocyte retrieval number

Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: NESFATIN, oocyte retrieval number, hypothalamus-pituitary-ovary axis, ART

P-691 Ovarian surgery for symptom relief in women with polycystic ovary syndrome: a meta-analysis and systematic review

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Study question: To assess the effectiveness and harms of laparoscopic ovarian drilling (LOD) as a treatment for symptomatic relief of hirsutism, acne and menstrual disturbances in women with polycystic ovary syndrome (PCOS).

Summary answer: Regarding the improvement of menstrual disturbances in women with PCOS, there is no evidence of benefit of LOD over medical or surgical treatment; 4–5 punctures per ovary is more effective than 2 or less. There is insufficient evidence that LOD improves androgenic profiles, BMI or quality of life.

What is known already: PCOS is a common female endocrine condition affecting 1 in 10 women worldwide. Many women with sub-fertility and PCOS also suffer from non-fertility related symptoms such as hirsutism, acne, metabolic derangement and menstrual irregularities. LOD is known to improve fertility in women with PCOS but its impact on the menstrual disturbances, androgenic and metabolic symptoms is yet to be determined.

Study design, size, duration: RCTs on surgery for PCOS were sought using standard search strategy via electronic databases, trials registers and websites. Data was extracted, assessed, synthesised and analysed by two authors (Rev Man 5). The outcome measures were improvement in menstrual and androgenic disturbances, adverse effects, metabolic effects and quality of life.

Participants/materials, setting, methods: 19 RCTs were identified out of 1041 papers extracted. A total of 1811 women were included; 10/19 studies compared LOD vs medical treatment, 6/19 compared LOD vs surgical treatment and 3/19 were head-to-head comparisons between different dosages of diathermy energy or number of punctures per ovary.

Main results and the role of chance: There is no evidence of benefit of LOD over metformin in menstrual regulation of women with PCOS (OR 1.00 95% CI 0.71–1.43, $I^2 = 67\%$, $n = 566$, 4 studies). 4–5 punctures per ovary is more beneficial in regulating the menstrual cycle compared to 2 or fewer (OR 13.77, 95% CI 3.58–52.92, $I^2 = 0$, $n = 70$, 2 studies).There is no evidence that alternative surgical techniques such as unilateral LOD, dosing according to ovarian volume, ultrasound guided LOD, or the use of different energy modalities is superior to traditional LOD. Single studies suggest benefit of ultrasound guided transvaginal LOD and thermally-adjusted dosage LOD on lowering testosterone levels when compared to LOD alone. Metformin was associated with more side effects compared to LOD (OR 20.61, 95% CI 2.67–158.84, $I^2 = 0$, $n = 338$, 2 studies).

Limitations, reason for caution: The results of this review may be influenced by small sample sizes and increased clinical heterogeneity amongst the studies included.

Wider implications of the findings: Whilst there is no evidence that LOD is more beneficial over existing medical and surgical treatments for the improvement of PCOS related symptoms, it should still be considered as a complimentary option for the regulation of the menstrual cycle in women with PCOS. Studies investigating the impact of LOD on symptom relief of PCOS should include economic costings and patient reported outcome measures. The exploration of the biological mechanisms behind LOD is long overdue.

Study funding/competing interest(s): Funding by University(ies) – University of Southampton.
Trial registration number: NA.
Keywords: PCOS, LOD

P-692 Association of serum levels of typical organic pollutants with the risk of polycystic ovary syndrome (PCOS)

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Study question: What is the relationship between typical organic pollutants exposure and the development of polycystic ovary syndrome (PCOS)?

Summary answer: There is a significant association between the serum levels of pollutants including polychlorinated biphenyls (PCBs), organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs) and the risk of PCOS in females.

What is known already: Polycystic ovary syndrome (PCOS) is arguably the most common endocrinopathy in females of reproductive age. The etiology of PCOS is thought to be multifactorial.

Study design, size, duration: This was a preliminary case-control study undertaken at the Division of Reproductive Center, Peking University Third Hospital. A total of 50 subjects affected by PCOS and 30 normal controls were recruited between August and October 2012.

Participants/materials, setting, methods: PCOS were diagnosed according to the 2003 Rotterdam criteria. The controls were non-pregnant females unable to conceive due to male azoospermia. Serum levels of a wide range of organic pollutants were analyzed using gas chromatographic mass spectrometry, including PCBs, organochlorine pesticides, PAHs, and more than 20 phenolic pollutants.

Main results and the role of chance: Serum levels of PCBs, pesticides, and PAHs were significantly higher in the PCOS group than the control group. Concentrations of PCBs, *p,p'*-DDE, and PAHs in serum above median levels

were associated with 3.81-fold [95% confidence interval (CI), 1.45–10.0], 4.89-fold (95% CI, 1.81–13.2), and 2.39-fold (95% CI, 0.94–6.05), respectively, increased risks of PCOS. Partial least-squares-discriminant analysis (PLS-DA) confirmed that serum levels of organic pollutants were risk factors of PCOS, especially for *p,p'*-DDE and PCBs.

Limitations, reason for caution: Some other possible covariates (e.g. dietary and income) were missed in this study, although the education and occupation has been considered as an indicator of personal income.

Wider implications of the findings: Our study identified bodily retention of environmental organic pollutants—including PCBs, pesticides (especially *p,p'*-DDE), PAHs—as a risk factor of PCOS.

Study funding/competing interest(s): Funding by national/international organization(s) – This research was supported by the Ministry of Science and Technology of China Grants (973 program; 2014CB943203and 2015CB553401), National Natural Science Foundation of China (21322705, 21190051, 41121004and 81170538), National Key Technology R&D Program in the Twelve Five-Year Plan (2012BAI32B01), and the Collaborative Innovation Center for Regional Environmental Quality.

Trial registration number: NA.

Keywords: PCOS, persistent organic pollutants, endocrine disrupting chemicals

P-693 GnRHa trigger in Asian oocyte donors co-treated with a GnRH antagonist – the first randomized GnRHa trigger dose finding study

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Study question: What is the most optimal dose of GnRH agonist (GnRHa) used for triggering of final oocyte maturation in oocyte donors who have GnRHa trigger as a standard trigger concept?

Summary answer: The use of either 0.2 mg, 0.3 mg or 0.4 mg of Triptorelin leads to the retrieval of comparable numbers of mature oocytes (MII) and top-quality embryos. Although any of the three doses may be successfully used for trigger in oocyte donors, 0.2 mg sufficiently secures an optimal outcome.

What is known already: GnRH antagonist protocols allow the use of GnRHa to trigger final oocyte maturation. GnRHa displaces the GnRH antagonist from the GnRH receptor in the pituitary, eliciting a surge of LH and FSH, similar to that of the natural mid-cycle surge. Importantly, GnRHa trigger significantly reduces, if not eliminates the risk of OHSS and, thus, GnRHa trigger should be considered the gold standard trigger in oocyte donors. Until now no dose finding study was performed.

Study design, size, duration: A randomized single center study. One-hundred twenty donors (120) were consecutively enrolled and randomized to receive either 0.2 mg, 0.3 mg or 0.4 mg Triptorelin for trigger. Block randomization was performed on cycle day 2, using a computer generated list. The study was performed between August and November 2014.

Participants/materials, setting, methods: A total of 120 oocyte donors, aged 18–35 years, having a BMI < 28kg/m^2, AMH > 1.25ng/ml and AFC ≥ 6 were included. Primary endpoint was the number of MII oocytes. Secondary endpoints were fertilization and cleavage rates, number of embryos and top-quality embryos, steroid levels and ovarian volume.

Main results and the role of chance: No significant difference was found between the 0.2mg, 0.3mg, and 0.4mg trigger groups regarding number of MII oocytes (17.0 ± 8.9, 17.5 ± 7.8, and 14 ± 7.5, respectively). Moreover, no difference was seen regarding cleavage rates (13.5 ± 8.1, 13.0 ± 7.2, 11.3 ± 6.6), and number of top-quality embryos (3.7 ± 2.8, 3.8 ± 2.9, 4.0 ± 2.7), all $p > 0.05$. LH, FSH, and estradiol levels peaked at 39.1IU/L, 42.6IU/L, and 10,985pg/ml, respectively 4 hours after trigger, whereas progesterone peaked at 34.7ng/ml, 36 hours after trigger. LH at 36 hours after trigger was significantly higher in the 0.4mg-group than the 0.2mg-group (4.8 ± 3.4 vs 3.1 ± 2.2IU/L). No difference was found regarding ovarian volume measured on the day of trigger, day of OPU, and day of OPU + 6 between the three groups. A full data analysis will be presented at the conference.

Limitations, reason for caution: This is the first GnRHa trigger dose finding study performed until now. Being performed in Asian oocyte donors, however, the present findings do not necessarily apply to all ethnic groups, as ethnic differences in response to GnRHa trigger might exist.

Wider implications of the findings: Although higher doses may be used, a bolus 0.2 mg GnRHa successfully secures oocyte maturation and the development of competent embryos in oocyte donors. With the increasing number of donor cycles performed worldwide, this finding may have significant economical implications. The knowledge obtained from the early luteal steroid profiles related to the GnRHa trigger dose may be used to further improve the luteal phases of IVF patients who receive GnRHa trigger followed by fresh transfer.

Study funding/competing interest(s): Funding by commercial/corporate company(ies) – MSD.

Trial registration number: ClinicalTrials.gov identifier: NCT02208986.

Keywords: IVF, GnRHa trigger, oocyte donor, dose-finding, luteal phase

P-694 Dehydroepiandrosterone (DHEA) treatment for poor responders in IVF patients: a prospective randomised controlled trial

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Study question: Can Dehydroepiandrosterone (DHEA) supplementation improve in vitro fertilisation (IVF) outcomes of ovarian reserve biomarkers, number of oocytes retrieved, embryos generated and clinical pregnancy rates (CPR) in patients with diminished ovarian reserve (DOR) or poor ovarian response (POR)?

Summary answer: No difference was found between the two groups in any of the outcomes. DHEA treatment resulted in an increase in follicular phase serum free-testosterone, DHEA-S and estradiol that did not translate into an improvement in the number of oocytes retrieved and embryos formed.

What is known already: Androgen levels have been suggested to impact upon ovarian response in patients with DOR/POR through enhanced steroidogenic activity in the developing follicle. Besides the use of testosterone or androgen-modulating agents, DHEA treatment has been proposed as a way to increase follicular androgen levels, with reports of improved reproductive outcomes in DOR/POR patients noted in small observational trials.

Study design, size, duration: This is a prospective randomised controlled trial in a tertiary referral academic hospital. It recruited 60 patients with DOR or POR undergoing IVF treatment between February 2012 and October 2014.

Participants/materials, setting, methods: Block randomisation was implemented to allocate 60 women with DOR/POR to DHEA treatment (75mg/day for 4 months) or control, followed by controlled ovarian stimulation (COH) with recFSH 300 IU and hpHMG 150 IU per day and initiation of Antagonist on Day 5 of COH. A Day 2/3 embryo transfer was done.

Main results and the role of chance: 15 patients dropped-out. The baseline characteristics between the groups (25 DHEA and 20 controls) were comparable. DHEA treatment over a median duration of 20 weeks (range 13–30) did not improve AMH or AFC values in the treated group, but increased serum free testosterone (RR = 2.25 [95% CI: 1.74–2.91]), DHEA-S (RR = 3.35 [95% CI: 2.42–4.63]) and estradiol (RR = 1.50 [95% CI: 1.24–1.83]) significantly ($p < 0.001$). There were no difference in IVF outcomes between the groups in terms of number of oocytes retrieved (5.2 ± 2.9 vs. 4.5 ± 3.6), number of embryos generated (2.7 ± 2.4 vs. 2.3 ± 1.8) and CPR (4.0% vs 10.0%, $p = 0.557$). Follicular fluid aspirated from lead follicles showed significantly higher levels of DHEA-S (RR = 4.77 [95% CI: 2.83–8.04]) and free testosterone (RR = 3.44 [95% CI: 2.12–5.59]) in the treatment as compared to the control group, but not in estradiol, AMH or IGF-1 levels.

Limitations, reason for caution: The number recruited was small.

Wider implications of the findings: Enhancement of androgen levels in the follicular compartment may be advantageous in the setting of DOR/POR.

Study funding/competing interest(s): Funding by national/international organization(s) – SingHealth Foundation.

Trial registration number: NCT01535872.

Keywords: dehydroepiandrosterone, ICSI, poor ovarian responders, diminished ovarian reserve, outcomes

P-695 Human immunodeficiency virus infected women and ovarian reserve: a matched case-control study examining serum anti-mullerian hormone

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Study question: To evaluate Human Immunodeficiency Virus (HIV) directly or indirectly related altered ovarian function, using serum anti-mullerian hormone (AMH) levels, in a large cohort of HIV infected women and disease free controls. In addition, we looked for correlations between AMH and the characteristics of HIV infection and/or treatment.
Summary answer: Serum AMH levels were lower in the HIV infected group than in the seronegative control group. Age, BMI, CD4 count and viral load were the independent contributors affecting serum AMH levels amongst HIV infected women.
What is known already: Several research groups have reported higher infertility rates and impaired ovarian function in HIV-positive as compared to HIV-negative women. Yet, it is unclear whether it is the virus itself, HIV-related immunodeficiency status, or Highly Active Antiretroviral Treatment (HAART) that primarily hamper(s) the ovarian reserve.
Study design, size, duration: We conducted an age-matched cohort study from January 2008 to December 2013 at our designated viral infection infertility center, in Paris at the Cochin tertiary university center. Two hundred and one HIV infected women and 603 HIV age-matched seronegative women, with male infertility, were enrolled in this study
Participants/materials, setting, methods: All women underwent clinical and laboratory evaluations at the screening visit, using a semistructured questionnaire. All data were collected prospectively. Serum AMH levels in HIV-infected women and age-matched controls were compared. To find out the contributing factors to increased serum AMH levels, a backward multiple linear regression was performed
Main results and the role of chance: Serum AMH levels were significantly lower in HIV infected group as compared to seronegative controls (3.0 ± 2.6 vs 3.3 ± 3.0 ng/ml; respectively, $p = 0.020$). Looking for factors associated with altered AMH in HIV infected women, we show for the first time that tubal disease is associated with a further decrease in serum AMH (2.7 ± 3.4 vs 3.6 ± 3.8 ng/ml; respectively, $p = 0.014$). After multivariate linear regression analysis we showed among HIV infected women that an increase in age, BMI and viral load was associated with a decrease in serum AMH levels whereas in striking contrast higher CD4 count are associated with elevated AMH levels.
Limitations, reason for caution: Our study suffers from the limitations of its design. The nature of the issue studied precludes other approaches however. Aware of these limitations, great care was taken to minimize the possibility of biases. In particular, the most important AMH confounder – women's age– was controlled for.
Wider implications of the findings: The link found between decreased ovarian reserve highlighted by lower serum AMH levels in HIV infected women and CD4 count and viral load stresses that HIV-infected women carefully follow their treatment. If need be that treatment ought to be adjusted prior to attempting ART.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – Department 'Development, Reproduction and Cancer', Institut Cochin, INSERM U1016, Université Paris Descartes, Sorbonne Paris Cité, Paris, France.
Trial registration number: NA.
Keywords: AMH, HIV, HAART, ovarian reserve

P-696 Polyglycine-containing protein (FMRpolyG) may play a role in the pathogenesis of fragile X associated premature ovarian insufficiency (FXPOI) in FMR1 premutation carriers

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Study question: Does repeat associated non-AUG initiated (RAN) translation of a cryptic polyglycine-containing protein, named FMRpolyG occur in granulosa cells of RMR1 premutation carriers (CGGn = 55–200 repeats)?
Summary answer: Inclusion bodies containing FMRpolyG protein accumulate in granulosa cells of RMR1 premutation carriers but not in granulosa cells from women with less than 55 CGG repeats.
What is known already: Amplification of CGG triplet number is associated with increased risk for FXPOI in women and fragile X-associated tremor/ataxia syndrome (FXTAS) in males. Both male and female premutation carriers have elevated *FMR1* transcript levels suggesting a possible common toxic mechanism for FXPOI and FXTAS. Recently, it has been shown that CGG repeats trigger RAN translation of FMRpolyG. FMRpolyG accumulates in ubiquitin-positive inclusions in neuronal cells from brain tissue of FXTAS patients and may lead to protein-mediated neurodegeneration.
Study design, size, duration: Study population consisted of 5 FMR1 premutation carriers referred to our IVF unit for IVF-PGD treatment. The control group consists of 3 patients, with less than 55 CGG repeats, undergoing IVF-ICSI for male factor infertility, matched by age and treated during the same period.
Participants/materials, setting, methods: After oocyte retrieval, mural granulosa cells were collected from follicular fluid and washed, grown on coverslips and then fixed and permeabilized. Then the cells were immunostained with a primary antibody (mouse anti-polyglycin) and incubated with a secondary fluorescent antibody (labeled goat anti-mouse-IgG antibody).
Main results and the role of chance: Granulosa cells from all 5 FMR1 premutation carriers stained positively to large cytoplasmic inclusion bodies containing FMRpolyG. FMRpolyG was not identified in the granulosa cells from control women.
Limitations, reason for caution: these preliminary results should be validated in larger studies.
Wider implications of the findings: As was previously shown in neuronal cells from brain tissue of FXTAS patients, we observed accumulation of inclusion bodies containing FMRpolyG protein in granulosa cells from FMR1 premutation carriers. These findings support previous findings suggesting a possible common protein-mediated toxic mechanism for both FXPOI and FXTAS.
Study funding/competing interest(s): Funding by University(ies) – Tel Aviv university, Sackler school of medicine.
Trial registration number: NA.
Keywords: FMR1 premutation carriers, RAN translation, FMR polyglycin A, FXPOI

P-697 Hormonal profile of follicular fluid from mature follicles in mild stimulation and controlled ovarian hyperstimulation IVF cycles in POR patients

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Study question: Is there any difference in the hormonal milieu from pre-ovulatory follicles between mild stimulation and controlled ovarian hyperstimulation (COH) in-vitro fertilization (IVF) cycles in poor ovarian response (POR) patients?
Summary answer: Comparing with COH, the intra-follicular LH and testosterone (T) levels were significantly higher, while FSH was lower in mild stimulation group. No significant difference was observed in estradiol (E_2), progesterone (P) and anti-mullerian hormone (AMH) levels between groups. No hormone was found to be correlated with IVF laboratory parameters.
What is known already: Previous studies showed that the follicular hormonal profiles from different ovarian stimulation protocols were varied, while the intra-follicular hormone level was found to be associated with oocyte developmental potential and even can predict embryo development. However, no researches compared the follicular hormonal profiles between mild stimulation and conventional COH in POR patients undergoing IVF treatment and investigate the potential influence of these distinct hormonal characteristics on the yield of oocytes and embryos.
Study design, size, duration: From Jan to Nov. 2014, follicular fluid was prospectively collected at the time of oocyte retrieval from 51 POR patients undergoing IVF treatment stimulated with mild stimulation ($n = 23$) or COH (GnRH

agonist long 'stop' protocol, $n = 28$), and intra-follicular FSH, LH, E_2, P, T and AMH levels were assayed.

Participants/materials, setting, methods: Follicular fluid was collected from one follicle larger than 16mm and analyzed to quantify the concentrations of hormones. The differences in intra-follicular hormonal profile between two groups were compared and the correlation between each hormonal level and the number of oocytes retrieved, transferrable embryos or good-quality embryos was evaluated.

Main results and the role of chance: The differences in basic characteristics, such as age, AFC and AMH levels between two groups had no significant meaning. The dose of Gn consumption was significantly higher in COH group (858.7 ± 223.9 vs.2833.0 ± 580.7, $P < 0.001$), while the number of oocyte retrieved (2.93 ± 2.13 vs. 2.87 ± 2.01), transferrable embryo (1.54 ± 1.48 vs. 1.27 ± .098) and good quality embryo (1.14 ± 1.35 vs. 1.09 ± 0.97) were same in two groups (all $P > 0.05$). The follicular hormonal profiles were listed below.

	Mild stimulation	COH	*P*
FSH (IU/L)	6.77 ± 2,57	10.00 ± 4.96	< 0.01
LH (LH/L)	7.05 ± 4.30	1.81 ± 2.64	< 0.01
Estradiol (ng/ml)	52.29 ± 27.35	54.98 ± 18.79	0.97
Progesterone (μg/ml)	16.36 ± 4.93	15.61 ± 5.01	0.59
Testosterone(ng/ml)	36.70 ± 18.79	5.50 ± 2.93	< 0.01
AMH(ng/ml)	1.41 ± 0.79	1.70 ± 1.24	0.23

None of these hormones was found to be correlated with the number of oocyte retrieved, transferrable embryos, or good quality embryos (all $p > 0.05$).

Limitations, reason for caution: This study was part of an ongoing prospective randomized controlled research on the comparison of mild stimulation and COH in POR patients, therefore, the sample size was limited and conclusion need to be interpreted cautiously. In addition, the impact of the difference in hormonal profiles needs further investigation.

Wider implications of the findings: The distinction of intra-follicular hormone profiles indicated that follicular microenvironment was varied between mild stimulation and COH, which might consequently have impact on the growth and maturation of oocyte. Further study on the comparison of gene expression of granulosa cells between mild stimulation and COH should be continued not only in POR patients but also in normal ovarian reserve patients.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproductive medicine center of Sun Yat-Sen University.

Trial registration number: ChiCTR-TRC-13003454.

Keywords: follicular fluid, hormonal profile, mild stimulation, controlled ovarian hyperstimulation, poor ovarian response

P-698 Validation of a new prognostic biomarker to enhance overall implantation rates in an egg donation (ED) program through cancellation of bad prognosis stimulations before triggering

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Study question: To determine whether a progesterone-to-estradiol (P4/E2) ratio at triggering could be a predictor in ED to identify stimulations with good or bad prognosis in terms of blastocyst and implantation rate. Which impact could have cancellation of bad prognosis cases in the overall success rates of an egg donation program?

Summary answer: Using a specific algorithm with P4 and E2 levels/follicle at triggering we defined clearly retrospectively two prognosis groups, which also could get validated prospectively. Cancelling those cycles with bad prognosis before triggering we estimated an increase in overall pregnancy from 63.8 to 72.8% and also the implantation rates.

What is known already: Antagonist protocol with agonist triggering is the golden standard in OD programs, as it eliminates OHSS risk. The premature rise of progesterone in the late folicular phase has been reported to be involved in worsening the clinical outcome, but it still remains unclear if it also applies for egg donation recipients cycles, as it may be affecting separately the embryo viability. Linear cut off limits for hormonal levels may be not strong enough biomarkers.

Study design, size, duration: Retrospective study with 1069 ED cycles studied to determine by multivariate curvilinear regression analysis a new predictive biomarker of outcome. Subsequent prospective validation of the algorithm with 141 fresh ED cycles performed between September and December 2013, generating 2 groups: good prognosis and bad prognosis group.

Participants/materials, setting, methods: Prospective validation study of 298 ED as intent to treat resulting in 141 analyzed cycles, resulting good prognosis group-A ($n = 51$) and bad prognosis group-B ($n = 43$). Donors aged 21–31, stimulated with rFSH 225IU/day in antagonist protocol and agonist triggered. Recipients aged 40.6 + /-2.3 balanced through inclusion/exclusion criteria, in synchronized substituted cycle.

Main results and the role of chance: The algorithm was designed by two curvilinear limits based on our retrospective analysis of more than 1000 cycles; defining an upper limit at: $P4/n^o\ ovo = 0.022*e^{0.013*E2/n^o ovo}$; and a lower limit at : $P4/n^o\ ovo = 0.01*e^{0.006*E2/n^o ovo}$). Patients inside both limits were considered as good prognosis, outside as bad prognosis. No significant differences found between both groups regarding fertilization, number of transferred embryos, transfer quality and abortion rates. Significant differences ($p < 0.05$) found regarding number of mature oocytes (17.1 + /-SD), final Estradiol (2388 + /-SD vs 3400 + /-SD pg/ml) and Progesterone (1.53 + /-SD vs 2.50 + /-SD ng/ml) levels. Overall pregnancy rate was 63.8%, being 72.8% in group-A and 51.6% in group-B, showing statistical significance ($p = 0.001$). We also observed statistically significant differences regarding blastocyst (68.3 ± 10.6 vs. 48.1 ± 18.2; $p < 0.01$) and implantation rates (47.5 ± 7.2 vs. 35.2 ± 10.2; $p < 0.01$).

Limitations, reason for caution: The statistical algorithm described applies for our ED programm. To extrapolate this data to other centers probably an adaptation of the algorithm will be needed. Similarly it is still open if a similar algorithm could also help to predict the outcome in regular IVF patients with own eggs.

Wider implications of the findings: The proposed regression algorithm appears helpful as prognostic indicator to decide when to cancel a donor cycle. Attending to our results we could improve the overall success of our program up to 9–12% just by cancelling those donors with a bad prognosis profile at late folicular phase. There are very few tools available capable of increasing success rates like this, may be PGS, but none of them in a comparable efficient and smooth way.

Study funding/competing interest(s): Funding by hospital/clinic(s) – University-Associated Fertility Private Clinic and its Foundation.

Trial registration number: NA.

Keywords: estradiol, progesterone, biomarker, egg donation, implantation

P-699 A prospective randomised controlled trial (RCT) on the role of AMH tailored stimulation protocols (Agonist or Antagonist),in improving IVF outcome in previous failed cycles

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Study question: Does AMH tailored stimulation protocol (Agonist or Antagonist) improve IVF outcome in previous failed cycles?

Summary answer: Yes, AMH tailored stimulation protocol (Agonist or Antagonist) improves IVF outcome in previous failed IVF cycles.

What is known already: The relative stability and consistency of AMH serum levels indicate that AMH could be used as a predictor of ovarian response in controlled ovarian stimulation. In this application, the predictive power of AMH for ovarian response to FSH appears to be similar to that demonstrated by the Antral follicular count. One main advantage of AMH measurement is that it is a cycle-independent test. The exact role of AMH measurement in the IVF setting should be clarified by a cost-benefit analysis. With high probability, the most useful clinical application of AMH measurement may be in the individualization of treatment strategies for COS-protocols (Agonist or Antagonist).

Study design, size, duration: This study involved 286 infertile patients below 42 years with previous failed IVF cycles, who were treated at our hospital between 2010 to 2014. In the study Group of AMH-tailored COH – the different ovarian stimulation treatments stratified according to the basal AMH level. Patients whose AMH levels fell within the acceptable range were divided into three strata (optimal, satisfactory or low ovarian reserve), which determined their stimulation protocol. Essentially, those with higher levels received lower doses of gonadotrophins and vice versa. Women in the satisfactory ovarian fertility group received an long protocol with a GnRH agonist, whereas those in the optimal or low ovarian fertility groups underwent a GnRH antagonist cycle. In the control group of patients: Conventional stimulation protocol was given (untailored AMH).

Participants/materials, setting, methods:
This is a prospective randomised control study (RCT) of 286 women with failed previous IVF-ICSI cycles. The study group included 143 patients who used AMH Tailored COH prior to entry into another repeat IVF cycle. The control group was composed of 143 women who received Conventional (AMH-Untailored) COH treatment,

- The primary outcome was implantation rate, cumulative pregnancy rate and total cost of cycle.
- The secondary outcomes were cancellation rates and OHSS rates.

Main results and the role of chance: Cumulative pregnancy rates were significantly higher in the AMH tailored study group (39% vs. 23%) with lower cost, less cancellation rates and less OHSS.
Limitations, reason for caution: A more contentious point is whether AMH measurements should be used to deny IVF treatment to couples shown by such a test to have a poor prognosis. This is an issue which may arise with other predictors of ovarian response to the IVF treatment. Although a number of markers, including AMH, may be predictive of ovarian response, none are 100% reliable. Moreover, AMH, as with other markers of ovarian reserve, is a poor predictor of who will achieve a pregnancy after IVF. Indeed, it has been widely demonstrated that many poor responders, in particular young ones, achieve pregnancy and live birth. This indicates that AMH measurement, similarly to the other ovarian reserve markers, should not be offered with the aim of withholding IVF, in particular to women undergoing their first IVF cycle.
Wider implications of the findings: Very good correlation exist between basal AMH and the number of retrieved oocytes, indicating that circulating AMH may definitely be considered a better marker for quantitative aspects of ART.A new interesting field of application is the individualization of treatment strategy on the basis of the AMH-based ovarian reserve assessment, in order to possibly reduce the incidence of cycle cancellation and OHSS. Since low and high AMH values are predictive of poor and high-response to gonadotrophins, respectively, it has been proposed that COC protocol and FSH dose may be adjusted according to the basal AMH levels and independently of the Age, FSH and BMI.
Study funding/competing interest(s): Funding by hospital/clinic(s) – BTTB CENTRE.
Trial registration number: BTTBC/2010/14.
Keywords: AMH, agonist-antagonist-protocol, IVF-ICSI, COS, cost-effectiveness

P-700 Ovarian reserve and IVF outcome in different ethnic groups

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Study question: The aim of the study was to establish if ovarian reserve and IVF outcomes may differ in multi-ethnic populations.
Summary answer: This data demonstrated no difference in ovarian reserve or live birth rate between different ethnic groups of women undergoing IVF, after adjustment to independent variables.
What is known already: Ethnic difference has been previously reported for different reproductive outcomes such as age of menarche, menopause or prevalence of reproductive system pathology. However, the difference of reproductive outcomes within assisted conception in multiethnic-ethnic groups remains uncertain.
Study design, size, duration: Observational Cohort study of 1502 women undergoing cycles of ART between January 2012 and December 2013. Among those patients 983 were white, 213 were black, 173 were Asian and remaining 133 were Chinese, Mixed or Others.
Participants/materials, setting, methods: Data for 1502 women were analysed for AMH, AFC, retrieved oocyte number and IVF outcome. Chi-square tests were performed analysing the relationship between categorical variables. Logistic regression analysis was used to assess the association of ethnicity and other demographic variables with parameters of ovarian reserve and live birth rates.
Main results and the role of chance: The mean age of patients was 35.8 ± 4.5. There was no significant difference in AFC or AMH between different ethnic groups after adjusting for age, smoking, BMI and previous pelvic or ovarian surgeries ($P > 0.05$). The number of oocytes retrieved was significantly different between ethnic groups ($p = 0.005$). On logistic regression analysis, black ethnicity was an independent predictor of low ovarian response (OR 1.7; 95% CI 1.8–2.4) in comparison with white when adjusted to FSH dose, age, BMI and previous surgery. The further analysis demonstrated lower live birth in black people in comparison with white (OR 0.35; 95% CI0.35–0.74). However once adjusted for previous uterine and pelvic surgeries, the odd ration became non significant (OR 1.1; 95% CI 0.2–5.1).
Limitations, reason for caution: This is a retrospective cohort study. In spite of relatively large size of initial cohort, when multiple confounders were taken in consideration, the numbers within individual subgroups were relatively small.
Wider implications of the findings: Analysis of this dataset did not confirm difference in ovarian response or live birth between ethnic groups. Ethnicity does not seem to play as great a role in ART outcome as previously reported when surgical history is taken into account. Larger prospective studies would improve our understanding of effects ethnic factors in ART outcome.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Guys and St Thomas' NHS Foundation Trust
Trial registration number: NA.
Keywords: ovarian reserve, ethnic difference, outcome, live birth

P-701 Androgen synthesis in peripheral adipose tissue: the role of insulin and luteinizing hormone in women with polycystic ovarian syndrome

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Study question: Is there a role for insulin and/or luteinising hormone (LH) in excess androgen production by peripheral adipose tissue in women with PCOS?
Summary answer: Insulin appears to stimulate adipocyte testosterone secretion, but neither insulin nor LH alone or in combination seems to influence the main steroidegenic enzyme activity.
What is known already: Epidemiological studies in premenopausal women found a positive correlation between circulating androgen, insulin resistance and central obesity. In addition, recent research has provided evidence of androgen synthesis in peripheral adipose tissue, which could be a potential source of hyperandrogenism in PCOS. In-vitro studies provided evidence of increased 17-Beta hydroxysteriod dehydrogenase type 5 (17B-HSD5, AKR1C3) expression and increased testosterone secretion from peripheral adipocytes in PCOS women. The role of insulin ± LH in this excess androgen production remains uncertain.
Study design, size, duration: This laboratory based study involved an in-vitro differentiated serum free monolayer cell culture of adipocytes harvested from subcutaneous adipose tissue obtained during gynaecological surgery from five women with and five without PCOS. All participants were of reproductive age (20–45) with a BMI of 20–35 kg/m^2.
Participants/materials, setting, methods: Pre-adipocytes were isolated from adipose tissue samples and differentiated to mature adipocytes, which were cultured in FCS-free medium. Different concentrations of insulin ± LH ± LY294002 were added to the cell culture. The supernatant was collected to measure testosterone using ELIZA. CYP17A1 and AKR1C3 mRNA expression were determined in cultured cells using qRT-PCR.
Main results and the role of chance: CYP17A1, AKR1C3 and testosterone were significantly higher in PCOS versus the control group in treated and untreated cultures. Insulin increased testosterone concentration in the control, but not the PCOS group, Insulin ± LY294002 reduced testosterone concentrations in both groups. Insulin did not alter CYP17A1 or AKR1C3 mRNA expression in PCOS group. In the control group, AKR1C3 significantly ($p < .05$) increased only with high insulin concentrations (100nm). LH ± insulin did not alter the expression of either of the enzymes. LY294002 + insulin resulted in a significant ($p < 0.0001$) rise in CYP17A1 in the PCOS, but not the control group. LY294002 + insulin resulted in a significant ($p = 0.012$) rise in AKR1C3 mRNA expression in the control, but not the PCOS group.
Limitations, reason for caution: The number of biopsies was relatively small in this study and the results will therefore need to be verified by other larger studies.

Wider implications of the findings: The data in this study revealed a markedly higher CYP17A1, AKR1C3 and testosterone in peripheral adipose tissue of PCOS vs. non-PCOS women. This supports the hypothesis that peripheral adipose tissue plays an important role in the pathogenesis of hyperandrogenaemia and PCOS. The study suggests that insulin, but not LH seems to play a role in excess testosterone secretion by adipocytes in PCOS women as evidenced by reduction of testosterone by inhibition of insulin. However, the effect of insulin on steroidogenic enzymes is less clear. Surprisingly, inhibition of insulin seems to enhance the activity of these enzymes. Further research is therefore required to better understand peripheral adipose tissue dynamics in PCOS and the role of insulin and LH.

Study funding/competing interest(s): Funding by national/international organization(s) – Scholarship from Libyan Government. No conflict of interest to declare.

Trial registration number: NA.

Keywords: PCOS, peripheral adipocytes

P-702 The connections between serum anti-mullerian hormone and metabolic parameters in polycystic ovary syndrome patients vary depending on body mass index category

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Study question: Is there an influence of body mass index (BMI) on the anti-mullerian hormone (AMH) levels and its relationship with the hormonal and metabolic parameters in polycystic ovary syndrome (PCOS)?

Summary answer: In the PCOS patients the relationship between AMH and hormonal and metabolic parameters vary depending on BMI category and excess body weight is associated with lower serum AMH levels, but this association does not seems to be mediated by adipokines (adiponectin and leptin).

What is known already: It was suggested that obesity could influence the circulating level of AMH. Although in PCOS adiposity play a central pathogenic role, the impact of adiposity on AMH levels and on the connections of AMH with the other pathogenic factors is incompletely understood.

Study design, size, duration: We performed a cross sectional study which included 150 PCOS patients consecutively referred for endocrinological evaluation between January 2011 and January 2014.

Participants/materials, setting, methods: The participants were patients consecutively diagnosed with PCOS (Rotterdam criteria) in an out patients clinic of a public University Hospital. All the patients were evaluated by a clinical exam and hormonal tests. AMH was measured with an ELISA kit (Beckmann Coulter). Analyzed variables were submitted to principal component analysis (PCA).

Main results and the role of chance: AMH serum levels were significantly lower in PCOS patients with overweight and obesity in comparison to those with normal weight ($p < 0,05$). PCA showed that in normal weight PCOS AMH aggregated in the first component (correlation coefficient 0,907) with abdominal circumference, waist-hip ratio, total testosterone and SHBG. In overweight/obese group PCA revealed that AMH correlated with the first component (correlation coefficient 0,829) together with HOMA-IR, insulinemia and free androgen index. In both groups of patients leptin and adiponectin aggregated in a distinct principal component, without any correlation with AMH.

Limitations, reason for caution: Our findings are limited to PCOS patients and probably can not be extended to other populations.

Wider implications of the findings: Our study is the first demonstrating a different relation between AMH and metabolic and hormonal parameters depending on BMI category. Our data suggest that the modality to reduce the increased AMH levels found in some of the patients with PCOS may be different according to adiposity status.

Study funding/competing interest(s): Funding by hospital/clinic(s) – Elias Hospital, Bucharest, Romania, Endocrinology Department.

Trial registration number: NA.

Keywords: polycystic ovary syndrome, AMH

P-703 Identification of altered serum metabolites in women with PCOS using gas chromatography-mass spectrometry (GC-MS)

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Study question: What are the differently expressed serum metabolites in women with PCOS? Using gas chromatography-mass spectrometry (GC-MS) based metabolomics, is it possible to identify metabolites other than those already known to be altered in PCOS? Whether these altered metabolites hold merit to be considered as supportive diagnostic marker(s) for PCOS?

Summary answer: Using GC-MS based metabolomics, several new metabolites were found to be significantly altered in serum of women with PCOS when compared with controls. Amongst these metabolites, phosphoric acid, oleic acid, β-sitosterol, α-D-glucose and propanoic acid exhibited highest predictive values in discriminating the two groups.

What is known already: PCOS is a metabolic disorder and is known for metabolic dysregulation including dyslipidemia. Few studies arguably suggest differences in expression level of different metabolites. Metabolomic studies involve reliable and reproducible quantification of wide variety of intracellular metabolites. GC-MS based metabolomics has been successfully used in several diseases to assess the alterations in metabolomic profile and has proved to be helpful in the identification of promising diagnostic and predictive biomarkers.

Study design, size, duration: 41 women with PCOS (according to the Rotterdam criteria 2006) and age ≥18 years to ≤40 years diagnosed with hyperandrogenism and ovarian dysfunction were recruited from February 2014 to November 2014. Age and parity matched proven fertile women in good health condition reporting for tubal ligation were considered as the control group ($n = 38$).

Participants/materials, setting, methods: Serum samples were collected from PCOS and controls. Briefly, metabolites were extracted and dried metabolic extract was derivatized. Samples were injected into a GC-MS system with temperature gradient for metabolite separation and helium used as carrier gas. Multivariate and univariate statistical analyses were performed for group-discrimination and marker(s) identification, respectively.

Main results and the role of chance: Principal component analysis, partial least squares discriminant analysis (PLS-DA) and orthogonal-PLS-DA showed strong classification between PCOS and controls based on the differently expressed metabolites. The up-regulated metabolites, phosphoric acid, oleic acid, α-D-glucose along with down-regulated β-sitosterol and propanoic acid were found to contribute most towards the differentiation between PCOS and controls ($p < 0.05$). High levels of phosphoric acid and glucose have been linked to an imbalance in insulin pathway, which is implicated in PCOS. The increase in long-chain fatty acid, such as oleic acid and decrease in propanoic acid suggests increased lipolysis, possibly secondary to impaired insulin action at adipose tissue. Increased rate of visceral fat-cell lipolysis is believed to play a pathophysiological role in PCOS and seems to be associated with lower levels of β-sitosterol.

Limitations, reason for caution: This study needs to be extended to a larger sample size for data reproducibility and accurate predictability. Differences in metabolites expression can also be checked using other analytical platforms. The alterations in expression of these metabolites need further validation in a larger cohort of samples using biochemical tests.

Wider implications of the findings: These metabolites hold merit to be considered as supportive diagnostic marker(s) for PCOS along with the presently existing clinical diagnostic method. This may help in a more precise diagnosis of PCOS along with conventional diagnostic methods.

Study funding/competing interest(s): Funding by national/international organization(s) – Department of Biotechnology, Government of India (BT/PR5063/BRB/10/1058/2012).

Trial registration number: NA.

Keywords: metabolomics, PCOS, gas chromatography-mass spectrometry, biomarker

P-704 Effect of testosterone administration on ovarian morphology, determined by transvaginal (3D) ultrasound in female-to-male transsexuals

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Study question: Does exogenous (long-term) testosterone administration result in polycystic ovarian morphology, determined by transvaginal (3D) ultrasound?
Summary answer: Long-term testosterone administration in female-to-male transsexuals does not result in polycystic ovarian morphology determined by transvaginal (3D) ultrasound.
What is known already: The role of androgens in the pathophysiology of polycystic ovary syndrome (PCOS) is still unclear. Small scale uncontrolled ovarian histomorphologic studies indicated that androgens in female-to-male transsexuals can cause PCO-like changes. Ultrasound morphology is an established criterion for PCOS but data on this after prolonged androgen exposure are lacking. The female-to-male transsexual can serve as a model to help clarifying the role of androgens in the development of ultrasound morphology and pathophysiology of PCOS.
Study design, size, duration: Prospective, observational Dutch cohort study, based in an academic setting, performed from 2014 to 2015, included 57 female-to-male transsexuals and 124 controls. The subjects were healthy, native females treated with testosterone for at least 12 months, as part of their cross-sex hormone treatment, scheduled for sex-reassignment surgery (hysterosalpingo-oophorectomy).
Participants/materials, setting, methods: Just before surgery under anaesthesia a transvaginal ovarian ultrasound (3D 3 to 9 MHz probe; Envisor HD, Philips Medical Systems) was performed and stored. Afterwards using 3D analysis-software, antral follicles were counted. Random healthy controls recruited through general practitioners office underwent exactly the same ultrasound evaluation.
Main results and the role of chance: 39 subjects and 77 controls were eligible for 3D-analysis of both ovaries and were under 40 years of age. Polycystic ovarian morphology was defined as: antral follicle count of 12 or more follicles (2–10 mm), in at least one ovary. In the female-to-male transsexuals 35, 9% (14/39) had polycystic ovaries, compared to 27, 3% (21/77) in the control group ($p = 0.34$), OR 0.67 (95% CI 0.294–1.52, $p = 0.34$). After correcting for age: OR 1.46 (95% CI 0.453–4.71), $p = 0.53$). There is no statistical significant difference between the prevalence of polycystic ovarian morphology in long-term testosterone treated females, compared to the control group. The preset power of the study was to substantiate a difference of 28% between the androgen pretreated subjects and controls.
Limitations, reason for caution: Testosterone levels in female-to-male transsexuals are supraphysiological, and may not be comparable to the testosterone levels in women with PCOS. Vaginal ultrasound in the female-to-male transsexuals is usually only allowed under general anaesthesia conditions. Therefore longitudinal data from before throughout androgen treatment are unfortunately not available.
Wider implications of the findings: This study shows that long-term testosterone administration in women does not substantially induce polycystic ovarian morphology, based on transvaginal (3D) ultrasound. To our knowledge this is the first study reporting data on transvaginal ultrasound in female-to-male transsexuals evaluating the effects of long-term androgen treatment on ovaries. Sufficiently powered studies comparing histopathological cross-sections of the ovaries removed from these women compared to ovaries from well defined PCOS patients and controls are justified and underway.
Study funding/competing interest(s): Funding by University(ies) – VU University Medical Center, Amsterdam, The Netherlands.
Trial registration number: Dutch Trial Register, NTR 4784.
Keywords: androgens, polycystic ovary syndrome, polycystic ovarian mophology, transsexuals

P-705 Prenatal hyperandrogenaemia in female babies of mothers with polycystic ovary syndrome (PCOS)

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Study question: Are the neonatal hormone levels of newborns of mothers with PCOS different to newborns of healthy controls?
Summary answer: Neonatal testosterone in girls of mothers with PCOS was raised compared to controls.
What is known already: Polycystic ovary syndrome (PCOS) affects approximately 15% of women of reproductive age. Androgen excess is one of the characteristic features of the syndrome, but the potential effects of this on the developing fetus remain poorly understood. Animal studies have demonstrated that androgen exposure in utero induces insulin resistance, hypertension and changes in behavioural and neurological development in the offspring. There are few studies of elevated prenatal androgens in humans however.
Study design, size, duration: *Design:* Cross-sectional study. *Size:* Four groups were recruited: women with PCOS who delivered a female baby ($n = 17$) or a male baby ($n = 10$); control women (without PCOS) who delivered a female baby ($n = 16$) or a male baby ($n = 17$). *Duration:* 2012–2014.
Participants/materials, setting, methods: *Participants:* Women with singleton pregnancies were recruited. *Setting:* All patients were recruited from four London hospital maternity units. *Methods:* Serum (maternal and umbilical arterial and venous) hormone levels were analysed using Tandem Mass Spectrometry and chemiluminescent microparticle immunoassay. Placental enzymes CYP19 and 3β-HSD were assessed using immunohistochemistry.
Main results and the role of chance: *Results:* Median (range) testosterone levels in the umbilical vein of girls of mothers with PCOS was significantly higher than girls of control mothers: 0.54 nmol/l (0.19–2.12) compared to 0.34 (0.10–0.82) ($P < .04$). Median (range) venous DHEA in girls of mothers with PCOS was significantly higher than girls of control mothers: 7.30 nmol/l (3.31–44.08) compared to 5.86 (1.90–8.10), respectively ($P < .03$). Maternal insulin and testosterone in the umbilical vein were significantly correlated in the PCOS group ($r_s = .640$, $n = 14$, $P < .014$, 2-tailed) but not the controls.
Limitations, reason for caution: Small study size.
Wider implications of the findings: Neonatal testosterone in girls of mothers with PCOS was raised. The correlation between maternal insulin and fetal testosterone could be explained by an inhibitory effect of insulin on placental aromatase expression or activity, as has been found in gestational diabetes. This mechanism could be a potential target for reducing the long term morbidity reported in the offspring of mothers with PCOS.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Royal Free Charitable Trust.
Trial registration number: NA.
Keywords: PCOS, testosterone, prenatal

P-706 Two-step consecutive controlled ovarian stimulation and oocyte retrieval in the follicular and luteral phase increases chance of obtaining embryos in patients with diminished ovarian reserve

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Study question: Diminished ovarian reserve is the frequent cause for obtaining low number of embryos in ART. We evaluated efficacy of consecutive oocyte retrievals at the follicular and subsequent luteal phases.
Summary answer: Additional oocyte retrieval during luteal phase increases chance of obtaining embryos in patients with diminished ovarian reserve.
What is known already: The recent wave theory of follicular development is the basis of random start controlled ovarian stimulation for cancer patients with urgent need to cryopreserve embryos for future use. Several reports documented the efficacy of oocyte retrieval during the luteal phase in young population.
Study design, size, duration: A prospective clinical trial of two-step ART in 21 cycles during 12 months period.
Participants/materials, setting, methods: 20 female patients with advance age or diminished ovarian reserve participated in the study. The patients underwent consecutive controlled ovarian stimulation with antagonist and oocyte retrieval (OR) in the follicular and the luteal phase. IVF or ICSI fertilized embryos were vitrified at cleavage stage. The protocol was approved by IRB.
Main results and the role of chance: The average age and serum AMH of the patients were 41.2 ± 2.8 (mean ± SD) year-old and 0.9 ± 0.6 ± ng/mL. After 21 follicular phase OR, 18 underwent luteal phase OR. The average number of oocytes by follicular phase and luteal phase OR were 1.1 ± 0.9, 0.5 ± 0.6, respectively. Fertilization and good morphology rate for IVF and ICSI were 14.3%, 100%, with IVF, and 100%, 33.3% with ICSI in the follicular phase; 100%, 50.0% with IVF and 83.3%, 60.0% with ICSI in the luteal phase. 5 (23.8%) of the follicular OR and 6 (33.3%) of the luteal phase OR resulted in embryo vitrification. Total 9 cycles had vitrified embryos: 3 in the follicular but not in the luteal phase, 4 in the luteal but not in the follicular phase, and 2 in both phases. Therefore, the two-step method increased cycles with embryo vitrification from 5 to 9 (80% increment).
Limitations, reason for caution: Small sample size and non-randomized study without control population.
Wider implications of the findings: Two-step consecutive oocyte retrieval may avert repeating new ART cycle and may shorten time to embryo transfer for patients with diminished ovarian reserve. We observed two clinical pregnancies after transferring luteal phase embryos. Implantation efficacy and pregnancy outcomes of this approach are under investigation.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Aisei Medical Corporation Ohgimachi Ladies' Clinic.
Trial registration number: NA.
Keywords: ART, luteal phase, oocyte retrieval

P-707 Association of LH, AMH, BMP15, GDF9, FSHR, LHR, and AMHR genetic polymorphisms with poor responses in patients undergoing in vitro fertilization

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Study question: The objective of this study was to evaluate the associations of the polymorphisms in the LH, AMH, BMP15 and GDF9 genes, and FSH, LH and AMH receptors genes, with poor or normal response in patients undergoing stimulation for in vitro fertilization (IVF).
Summary answer: We conclude that the GDF9 398C > G and 447C > T polymorphisms exert important influences on oocyte development.
What is known already: Approximately 10% of women seeking fertility treatment have diminished ovarian reserve (DOR). Gene association studies have identified several single nucleotide polymorphisms (SNPs) involved in the ovarian response.
Study design, size, duration: The setting for this study was a fertility center with Sixty-seven women undergoing IVF treatments using r- FSH and recombinant GnRH antagonist protocol. A case-control study was performed with normal and poor responders undergoing IVF.
Participants/materials, setting, methods: We extracted DNA from the peripheral blood and assessed polymorphisms in the LH, AMH, BMP15, GDF9, FSHR, LHR and AMHR genes using polymerase chain reaction (PCR). The presence of these polymorphisms were evaluated in poor and normal responder patients undergoing IVF.
Main results and the role of chance: In the present study, we found that the 398C > G polymorphism in the GDF9 gene was present in 68% of poor responders versus 23% of normal responders to ovarian stimulation for IVF (OR: 4.01, 95% CI: 1.52–10.60). In addition, the homozygous mutant genotype for the 447C > T polymorphism of the GDF9 gene was found in 50% and 19%, respectively, of poor and normal responder patients (OR: 2.88, 95% CI: 1.19–6.04), which provides evidence for the strong association between poor ovarian response and ovulation induction. Only the GDF9 398C > G polymorphism was associated with a poor response to treatment, after controlling for any bias related to the other polymorphisms.
Limitations, reason for caution: The limitation of our results is that we don't realized functional studies to validate the precise genetic mechanism involved in oocyte development.
Wider implications of the findings: These findings represent the basis for future functional studies aimed at elucidating the precise genetic mechanism involved in oocyte development and developing potential treatments to improve the number of oocytes retrieved in patients with GDF9 polymorphisms.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – We are grateful for the financial support provided by Fundo de Incentivo à Pesquisa (FIPE), Hospital de Clínicas de Porto Alegre, Brazil and Clínica Pronatus – Medicina Reprodutiva, Belém Brazil.
Trial registration number: The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre *n*: 14/0070.
Keywords: GDF9, polymorphism, IVF, follicle retrieval, oocyte

P-708 Comparison of FMR1 mutations distribution between Indian and other Asian patients

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Study question: Is the reason why Indian women age their ovaries faster than women of Spanish background (Iglesias et al, Fertil Steril 2014) based on a high prevalence of *low FMR1* alleles ($CGG_{n<26}$) in Indian populations?
Summary answer: Neither age nor distribution of *FMR1* mutations differed between Indian and other Asian patients in a U.S. infertile patient population. Functional ovarian reserve (FOR), assessed by anti-Müllerian hormone (AMH), was however significantly lower than in other Asian women.
What is known already: Women with so-called *low FMR1* alleles ($CGG_{n<26}$) have been reported to prematurely lose FOR. Since women of African descent demonstrate significantly higher distribution prevalence of *low FMR1* alleles than Asian-Chinese and Caucasian women, this finding has been attributed as a possible cause of lower reported in vitro fertilization (IVF) pregnancy chances in African women.
Study design, size, duration: We performed a cross-sectional cohort study of routine infertility patients of Asian descent seen over a 4-year period, comparing Indian women to other Asian women (mostly Han Chinese) for whom *FMR1* results and AMH values were available in our center's anonymized electronic research data base.
Participants/materials, setting, methods: We investigated 99 Indian and 225 patients of other Asian descent for distribution prevalence of *FMR1* mutations at an academically affiliated private IVF center in New York City, U.S., with international patient pool. In addition, their FOR was compared (based on AMH) to women of other Asian backgrounds.
Main results and the role of chance: Mean ages were 35 and 36 years for Indian and other Asian women (N.S.), and they also did not differ in *FMR1* mutation distributions: Among Indian patients 76 (70%) had a normal (*norm*) *FMR1* (both alleles in range $CGG_{n=26–34}$), 29 (17%) were heterozygous (*het*)-normal (*norm*)/high (1 allele in normal range and 1 in abnormal high, $CGG_{n>34}$, range, and 11 (6%) het-norm/low (1 allele in abnormal low range, $CGG_{n<26}$). Other Asian women demonstrated 148 (65%) cases with *norm*, 56 (25%) with *het-norm/high* and 13 (5%) with *het-norm/low FMR1*. Mean AMH values of Indian women were significantly lower than in other Asian women (1.47 vs. 0.83 ng/mL; $P < 0.05$), confirming the report by Iglesias et al.
Limitations, reason for caution: Since only infertility patients were studied, here reported results can not automatically be extrapolated to general populations.
Wider implications of the findings: Though confirming the findings of Iglesias et al, this study failed to establish a cause for the premature decline in FOR in Indian women. By demonstrating a similar *FMR1* mutation distribution with, as previously reported relatively few *het-low* alleles in Indian and

other Asian patients, this study actually eliminates *low FMR1* alleles as a potential cause.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Center for Human Reproduction; Foundation for Reproductive Medicine.
Trial registration number: NA.
Keyword: fragile X mental retardation 1 (FMR1) gene, ovarian reserve, anti-Müllerian hormone (AMH)

P-709 The relationship between serum anti-mullerian hormone and body mass index in a Caucasian population of infertile women is age-dependent

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Study question: Which is the relationship between serum antimullerian hormone (AMH) levels and body mass index (BMI) in a population of infertile Caucasian women?
Summary answer: The relationship between AMH and BMI in infertile patients mainly of normal weight is influenced by age, the two parameters being positively correlated in women aged 25–40 years, but not in the age categories <25 years and >40 years.
What is known already: Although few studies demonstrated a negative influence of obesity on antimullerian hormone circulating levels, not all reports confirmed these data. These discordant results could be due to a different relationship between BMI and AMH depending on the age or the ovarian reserve and probably the severity of excess body weight of the population analyzed.
Study design, size, duration: We performed a cross sectional study which included 686 caucasian female patients evaluated for infertility over two years.
Participants/materials, setting, methods: The participants were selected from the female patients evaluated in a private outpatient clinic for all causes of infertility between January 2012 and January 2014. Inclusion criteria: age 20–45 years and BMI 18,5–40 kg/m^2. Exclusion criteria: PCOS. Age, weight, height, AMH were measured in all patients.
Main results and the role of chance: Mean age of the study group was 34,97 ± 4,2 years (range 23–45 years), mean BMI 22 ± 3,8 kg/m2 (83,8% normal weight, 12,5% overweight, 3,6% obese) and mean AMH 2,51 ± 2,24 ng/ml (range 0,1–10 ng/ml). In a multivariate liniar regression model with AMH as dependent variable, both age and BMI were independent predictors of AMH serum level (beta = -0,329, $p < 0,0001$ for age, beta = 0,126, $p = 0,001$ for BMI). Dividing the patients in groups according to age we found that: after adjustment for age, AMH and BMI were not correlated in patients <25 years and >40 years, were strongly and positively correlated in patients between 30 and 35 years ($p = 0,002$), and marginally correlated in age groups 25–30 years ($p = 0,023$) and 35–40 years ($p = 0,042$).
Limitations, reason for caution: Due to the fact that our study population was represented mainly by normal weight women we can not extent our findings to other BMI-categories.
Wider implications of the findings: Our study is the first demonstrating a positive association between AMH and BMI in mainly normal weight (83,8% of the patients) infertile female patients 25–40 years old. Our findings are contrasting with previously published data probably due to the small number of obese patients in our group since the negative impact of adiposity on AMH values was reported in obese patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Medlife.
Trial registration number: NA.
Keywords: AMH, BMI, ovarian reserve

P-710 Relative progesterone level increases between initiation and end of ovarian stimulation are detrimental for live birth even among cycles with apparently normal late-follicular progesterone levels

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Study question: Can a relative increase in serum progesterone (P) levels between the initiation of ovarian stimulation (early-P) and the day of ovulation triggering (late-P) impair live birth rates (LBR) following in vitro fertilization (IVF)?
Summary answer: Although it is well established that elevated P levels on the day of ovulation triggering significantly impair LBR, we demonstrate, for the first time, that a relative increase in P > 0.25ng/ml between early-P and late-P is detrimental for LBR, even in cycles with apparently normal late-follicular P levels.
What is known already: Accumulating evidence has shown an association between late-P elevation and a reduction in IVF pregnancy rates. However, it has been challenging until now to define a clinically relevant late-P cut-off, since the previous studies present conflicting results and frequently use different limits. Furthermore, late-P elevations are rather common in both younger and high-responding women and their effect on LBR in these populations are a subject of much debate.
Study design, size, duration: This was a retrospective, single-centre cohort analysis including all patients who underwent ovarian stimulation between February-2009 and March-2013 for IVF followed by a fresh embryo transfer in our centre. All cycles ($n = 2703$) were down-regulated using a GnRH antagonist. Ovulation was triggered with hCG 36 hours before oocyte retrieval.
Participants/materials, setting, methods: We calculated the follicular-phase P variation by subtracting late-P (day of hCG administration) and early-P (stimulation day-1). Using multivariable logistic regression, we compared the LBR of cycles with no significant P variation (absolute variation <0.25 ng/mL) with those in the following follicular-phase P increase intervals: 0.25–0.50, 0.50–0.75, 0.75–1.00 and >1.00 ng/mL.
Main results and the role of chance: Overall, 682 cycles (25.2%) resulted in a live birth. The mean ± SD follicular-phase P variation between stimulation day-1 and the day of hCG administration was 0.28 ± 0.38 ng/mL (early-P 0.63 ± 0.27ng/mL, late-P 0.91 ± 0.41 ng/mL). The effect of the relative increase in P on LBR was evaluated by multivariable logistic regression. After adjusting for female age, basal FSH, the number of oocytes retrieved and day and number of embryos transferred, LBR were significantly higher in cycles with no relative increase in P (28.3%) when compared to cycles with a relative increase in P of 0.25–0.50 ng/ml (23.9%, $p = 0.033$), 0.50–0.75ng/ml (21.8%, $p = 0.018$), 0.75–1.00 ng/ml (18.3%, $p = 0.01$) and >1.00 ng/mL (15.5%, $p = 0.001$). Interestingly, our subgroup analysis including only cycles with late-P < 1.5 ng/mL, demonstrated that cycles with no relative increase in P had significantly higher LBR when compared with all other groups.
Limitations, reason for caution: Our results pertain to cycles in which early-P was ≤1.5 ng/mL, given that it is our centre's policy to not initiate ovarian stimulation otherwise. Therefore, we were unable to evaluate whether a relative increase in P might have had a detrimental effect on LBR among patients with higher early-P levels.
Wider implications of the findings: A relative increase in P between the beginning and end of stimulation significantly impairs LBR, even in cycles with apparently normal P levels on the day of ovulation triggering. The hypothesis that patients have different basal exposures to P and that increased follicular-phase P might impair endometrial receptivity requires further validation. Evaluating the difference between late-P and early-P adds insight into the current limitations of isolated assessments of early-P or late-P in predicting IVF outcome.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) –Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel.
Trial registration number: NA.
Keywords: progesterone, follicular phase, ovarian stimulation

P-711 The use of human growth hormone (hGH) in poor prognosis patients increase euploidy rate and improve embryo quality. a patient-controlled trial

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Study question: Use of hGH as co-adjuvant medication prior to and during ovarian stimulation in bad prognosis patients to improve oocyte quality. Impact of hGH treatment in different clinical parameters: fertilization, blastocyst and good quality blastocyst rate as well as embryo euploidy, pregnancy and implantation rate.
Summary answer: The addition of hGH during ovarian stimulation in bad prognosis patients improves the amount of top quality euploid blastocysts and

therefore pregnancy and implantation rate. However, the number of retrieved oocytes, fertilization and blastocyst rate were not different under the influence of hGH during stimulation regimen for in vitro fertilization.

What is known already: We need to increase the chances of poor prognosis patients to deliver a healthy baby. It has been shown that the supplementation of hGH as adjuvant in ovarian stimulation may increase pregnancy and delivery rates, by increasing oocyte quality and subsequent embryo quality. Until now few studies have been published to confirm these findings but none of them has included any genetic data correlated with clinical results, even less with the latest PGS technologies.

Study design, size, duration: Unicentric, comparative, randomized and patient-controlled trial (2013–2014). Twenty-eight poor prognosis patients underwent at least 2 consecutive IVF cycles (same gonadotrophin regimen intra-patient), planed as embryobanking within 6 months, and using hGH supplementation (1 IU/day) in 1 of them selected randomly. Fifty-six cycles were performed with deferred embryo-transfer.

Participants/materials, setting, methods: Poor prognosis patients (age >40 and at least 3 previous IVF attempts) undergoing ovarian stimulation with antagonist protocol and agonist-trigger. hGH (Saizen®) was administered 5 weeks before egg retrieval. Estradiol and Progesterone measured the trigger-day in all cycles. Preimplantational genetic screening (PGS) was performed using trophectoderm biopsy and aCGH.

Main results and the role of chance: Bad prognosis patients were defined according to the criteria of Yovich and colleagues (2010). Serum estradiol was significantly higher in hGH cycles (1780 ± 232 vs. 896 ± 134; $p = 0.01$) although no significant increase in oocytes retrieved was achieved (10.5 ± 4.5 vs 9.4 ± 4.2; $p = 0.5$). Overall blastocyst rate did not differ between groups (31.94 ± 6.3 vs 31.34 ± 8.06; $p = 0.5$), but top quality blastocyst rate was significantly higher in hGH cycles (17.64 ± 4.00 vs 5.92 ± 2.56; $p = 0.04$). Most interestingly, euploid blastocyst rate was significantly higher in hGH cycles (30.75 ± 7.97 vs. 8.75 ± 2.04; $p = 0.02$). Pregnancy rate was significantly higher in the hGH-derived blastocyst (44.5 ± 3.56 vs 23.3 ± 2.23; $p = 0.04$), may be cause to the increased blastocysts quality. Implantation rate was also increased even though with no statistical significant differences observed (36.6 ± 4.56 vs 18.8 ± 3.7; $p = 0.06$).

Limitations, reason for caution: The number of euploid blastocysts obtained is relatively low due to the bad prognosis in this patients group, limiting the application of robust statistics in terms of clinical parameters. Therefore, it would be desirable to increase patient population in order to confirm our results.

Wider implications of the findings: hGH as co-adjuvant in IVF cycles could be an option in patients with bad prognosis. It may improve cytoplasmic maturation by increasing oocyte and blastocyst quality compared to their previous IVF cycles. Moreover, better meiotic competence is suspected as we found an improvement in the euploidy rate. To confirm our findings, we plan a prospective multicenter study across Europe. We would like to present our study design and an invitation to collaborating centers at ESHRE.

Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) – IVF Spain private fertility clinic, IVF Spain Foundation.

Trial registration number: NA.

Keywords: hGH, poor prognosis patient, ovarian stimulation, blastocyst

P-712 Bilateral oophorectomy has detrimental effect on intima-media thickness (IMT) in carotid and coronary arteries and augments estrogen receptors in neuronal cells: randomized-controlled-trial in a rat model

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Study question: The effects of menopause on the cerebral vasculature and brain function remain controversial. Is there any effect of prior bilateral oophorectomy in terms of estrogen hormone deficiency on intima-media-thickness of carotid and coronary arteries and estrogen receptor status of neuronal cells of oophorectomised to those of non-oophorectomised rats?

Summary answer: Prior bilateral oophorectomy triggers intima-media thicknening in carotid and coronary arteries and augments estrogen receptors in neuronal cells in an experimental rat model. This result may account for the magnified risk of cardiovascular and cerebrovascular diseases in postmenopausal women and lightens the enigmaticpathophysiologic pathway which also introduces new therapeutical insights.

What is known already: The previous studies stated that increased carotid IMT may be observed in postmenopausal women via evaluating by B-mode ultrasound exams, however we assessed it by microscopic immune-histological evaluation in real tissue for the first time in hitherto literature. Effect of estrogen deficiency may cause to increase the IMT, being responsible for atherosclerosis. Alteration of microscopic structures of these arteries may have detrimental effect on the brain with increased estrogen receptors via removing bilateral rats' ovaries.

Study design, size, duration: In this experimental randomised-controlled-trial design study, 10 of 25 *Winter* female rats were randomised to undergo menopause by removing their ovaries surgically. Another ten rats were the control group and hadn't undergone any surgical procedure. The other five rats were sham group. After six-months period, all the rats were euthanised.

Participants/materials, setting, methods: Left descending coronary and carotid arteries including brain of the rats were removed after euthanasia and were investigated under microscopic examination by a pathologist who determined the thickest diameter of these specimens. Neuronal cells were assessed with immune-histological examination to determine the estrogen receptors in the nucleus membrane.

Main results and the role of chance: Mean ± standard deviation of carotid intima-media thicknesses for case, control and sham groups were 268,69 ± 53,67 μm, 195,61 ± 47,60 μm and 193,86 ± 75,01 μm, respectively. Intima-media thickness of coronary arteries were 182,40 ± 30,22 μm for the case, 136,00 ± 35,82 μm for the control and 165,24 ± 40,68 μm for the sham groups, respectively. Both intima-media thickness of carotid and coronary arteries in the case group were significantly higher than those of the other two groups and the difference was statistically significant ($p = 0{,}014$; $p = 0{,}022$, respectively). However, according to the microscopic examination of rats' neuron cells by pathologist, estrogen receptors was more promptly established in the case group by immune-histological investigation subjectively. ANOVA with post-hoc Tukey's tests was performed. *p*-value of less than 0.05 was considered to show a statistically significant result.

Limitations, reason for caution: Our study's advantage is that IMT was evaluated via microscopic measurement in pathologic specimen. However; main limitation is investigating only in one species and not using hormone replacement therapy (HRT) after surgical menopause to compare exact changes and understand the alteration's transiency. This was impossible due to the study's nature.

Wider implications of the findings: Our conclusions are consistent with literature and add new insights in cardiovascular diseases in postmenoposal women. Lack of estrogen may facilitate the process of increased IMT of both coronary and carotid arteries, accounting for the magnified risk of cardiovascular and cerebrovascular diseases. We suggest that the effect of estrogen should further be investigated on IMT of both carotid and coronary arteries by comparing oophorectomized groups with or without HRT in terms of new therapeutic potential.

Study funding/competing interest(s): Funding by hospital/clinic(s) – None.

Trial registration number: NA.

Keywords: oophorectomy, intima-media thickness, coronary artery, carotid artery, estrogen receptor

P-713 Endometrial chronic low-grade inflammation disease: evidence of a pro inflammatory activating NK cell receptor expression in the endometrium of PCOS infertile patients

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Study question: In the present study we evaluated whether the systemic chronic inflammatory condition and immune cell activation associated with obesity, dismetabolism, and insulin resistance may affect the endometrial innate immune environment in patients with PCOS (Polycystic Ovary Syndrome)
Summary answer: The endometrium of PCOS patients shows an inflammatory pattern characterized by an increased expression of pro-inflammatory cytokines and of activating NK cell cytotoxic receptor expression. the same alterations, is well Known to be the pheripheral blood of obese women with PCOS, similar to the systemic inflammatory chronic state and the related peripheral immunity imbalance. The endometrial inflammatory state could have detrimental effect on embryo implantation in such patients.
What is known already: Obesity-related inflammation is often considered a disorder of innate immunity. Abdominal obesity is largely prevalent in PCOS women that also have a significantly increased markers of systemic inflammation disease. Also endometrial tissue is characterized by an innate and adaptive immune cells Natural killer cells are a critical component of the innate immunity
Study design, size, duration: A prospective study was performed on 19 PCOS patients and 11 healthy fertile controls with normal menstrual cycles, from January to October 2014. The diagnosis of PCOS was made according to Rotterdam criteria. Controls were selected among women who have undergone surgery for benign disease.
Participants/materials, setting, methods: Endometrial samples were obtained in the proliferative phase from 9 PCOS patients and from 5 controls. Late secretory samples were obtained from 10 PCOS patients a and 6 controls. A fraction of each endometrial sample was prepared for histological examination; a part was stored for PCR.
Main results and the role of chance: A significant increase in the expression of pro-inflammatory cytokine TNF-α (p = 0,04) and of the activating NK cell receptors expression NKG2A, NKG2D, NKp46 was observed in PCOS compared to controls (p = 0,03; p = 0,07;p = 0,02, respectively). A significant increase in the expression of IL-1beta, IL-6, (p = 0,04; p = 0,06, respectively) and of NKG2A, NKG2D and NKp46 (p = 0,006; p = 0,03 and p = 0,01, respectively) was observed in the secretory phase compared with proliferative phase in PCOS group. Approximately 70% of PCOS patients are obese with a central body fat distribution that is associated with insulin resistance, a state of chronic inflammation and a disorder of innate immunity in peripheral blood. Results demonstrated that also endometrial tissue, in PCOS is characterised by an inflammatory status associated with endometrial innate immunity imbalance.
Limitations, reason for caution: Possible biases related to the limited number of cases analysed.
Wider implications of the findings: Our data show for the first time the existence of a chronic inflammatory endometrial disease in PCOS patients, which involves the NK cells and the activating NK cell receptors with interplay between the immune system and abnormal metabolic conditions.
Study funding/competing interest(s): Funding by University(ies) – This research was funded by the University personal research grants of Matteo Maria. The authors have no competing interests to declare.
Trial registration number: 12.
Keywords: PCOS, endometrium, chronic low grade inflammation, innate immunity

P-714 FSH receptor and LH gene polymorphisms in low, intermediate and high responders in elonva controled ovarian hyperstimulation

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Study question: Is the type of response associated with the FSH receptor and LH genes polymorphisms as suggested previous studies?
Summary answer: Type of response is not associated with FSH receptor (FSH-R) Asn680Ser or LH gene polymorphism V-betaLH, but is associated with FSH promotor –29A/G gene polymorphism alone or combined with Asn680 Ser polymorphism.
What is known already: Patients with allele Ser (Asn680 Ser) and with allele A (–29A/G) and V-betaLH (Trp8Arg and Ile15Thr) required higher dose of FSH.
Study design, size, duration: The study was prospective including 212 women of reproductive age for controlled ovarian hyperstimulation (COH) between July and December 2012.
Participants/materials, setting, methods: Eumenorhoic women, normal BMI, FSH, LH, progesterone, E2, AMH, AFC measurement, antagonist protocol 6.4 days, stimulation 10.5 days. In Elonva protocol (150 mg) was 19.8% with low, 66.5% intermediate and 13.7% high response, genotyping by TaqMan Assay and RFLP for LH polymorphisms, statistics by χ^2, Kruskall-Wallis and Man-Whitney tests.
Main results and the role of chance: No association in response and AMH/AFC levels was found for Asn/680 Ser and LH Trp8Arg and Ile15Thr. The GG genotype of –29A/G polymorphism was significantly higher – 64.58% in high responders, than in intermediate and high ones – 48.69% and 32.35% (p = 0.0149), difference between low and high responders was higher (p = 0.0068). The genotype A/G was significantly more frequent in high responders -61.76% versus intermediate and low ones – 41.88% and 33.33% (p = 0.0331), difference between low and high responders was significantly higher (p = 0.0138). The integrated genotypes G/G and Ser/Ser were increased in low responders –20.83%, comparing to the intermediate and high ones 8.38% and 8.82% (p = 0.0407), difference between lower and intermediate types is significantly higher (p = 0.0195).
Limitations, reason for caution: Further population studies within COH are necessary to confirm these findings by increasing the number of low and high responders for association studies of genetic and epigenetic impact on the final type of individual response.
Wider implications of the findings: It is important to study also oocytes' biological quality for fertilization success, because FSH influences the further differentiation of oocytes since small antral follicles contrary to AMH. Our study indicates that alleles –29A/G and Ser influence risk of reduced fertility with increased resistance to COH, alleles –29A/G have defining phonotypical effect.
Study funding/competing interest(s): Funding by national/international organization(s) – FNM grant 64203, OPPK and CZ.2.16/3.1.00/24022, IGA NT 13770. There are no competing interests involved.
Trial registration number: NA.
Keywords: FSH receptors, LH variant, COH response

P-715 Ovarian reserve and response risk categorisation using the new automated AMH assay produced by Roche®

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Study question: The new automated assay for AMH yields different absolute values from those previously available. Critical values determining risks of sub-optimal and excessive responses to ovarian stimulation have been determined for previously available tests, but they require determination for the new assays.
Summary answer: The regression curve comparison of the new assay compared with the previous assay (Gen II, by Beckman Coulter®) shows highly consistent comparative association (r^2 = 0.95). The antral follicle count profiles confirm the differences between the categories and provide supporting evidence of the validity of these revised concentration cut-off values.
What is known already: AMH is a highly effective biomarker of functional ovarian reserve, but different commercial assays yield different absolute values,

due to different standardisation amongst other factors. Correspondingly, care must be taken when interpreting values obtained from different sources and it is important that guideline cut-off values for the various end-points are ascertained.
Study design, size, duration: Prospective serum samples over a 6-week period from 205 women undergoing ovarian assessment by ultrasound evaluation of antral follicle count were measured using two AMH methods simultaneously – Gen II and the new Roche [E411] automated assay. AMH results between the two assays were compared and regression statistics ascertained.
Participants/materials, setting, methods: The women presented prior to undergoing ovarian stimulation for IVF in a single centre. The previously published cut-off values (Nelson et al, 2007) for response risk prediction in the Gen II assay were transformed for the Roche assay, and the AFC profiles for patients in these categories were determined.
Main results and the role of chance: A strong correlation between the two assays ($r^2 = 0.95$) was found. The response category limits previously described were transformed and the AFC values for these categories showed the expected and significantly different ranges of values (ANOVA: $p < 0.0001$). The 'Negligible response' group upper limit transformed to 1.1 pmol/L and the mean AFC for this group was 4.5, (25^{th}–75^{th} centiles: 3–6). The 'Reduced response' group upper limit transformed to 8.1 pmol/L and mean AFC was 7.7 (5–11). The 'Safe response' group upper limit transformed to 18.3 pmol/L and mean AFC was 12.9 (9–16), and the 'Excessive response' group (AMH > 18.3 pmol/L) showed mean AFC values of 20.9 (13.5–26).
Limitations, reason for caution: The Roche values were 27% lower than the Gen II assay [Roche (pmol/L) = 0.733 × Gen II + 0.6]. Age-related normal data and the response category definitions, currently determined by correlation conversion, will require further confirmation from clinical evaluation and experience, but the extremely close correlation observed is reassuring.
Wider implications of the findings: The new automated assays perform well in all validation tests, and ovarian stimulation risks can now be determined with confidence in a wide variety of settings. These assays will allow greater access for more centres, with guideline values for response risk more generally available.
Study funding/competing interest(s): Funding by hospital/clinic(s), Funding by commercial/corporate company(ies) – GCRM Ltd, Roche®.
Trial registration number: NA.
Keywords: AMH, AFC, ovarian reserve, roche, Beckman Coulter

P-716 Luteal-phase ovarian stimulation strategy for assisted reproduction treatment

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Study question: The purpose of this article was to systematically assess this new raised controlled ovarian stimulation strategy by comparing it with conventional follicular-phase ovarian stimulation protocols.
Summary answer: Compared with mild treatment protocol and short-term protocol, the luteal-phase ovarian stimulation (LPS) strategy had good performances in the number of mature oocytes retrieved, pregnancy rate and live birth rate. Neonatal outcomes and congenital malformations of all fetuses and infants conceived after LPS strategy were similar with other two groups.
What is known already: During the past years, we have managed to establish an effective LPS protocol, which mainly includes aromatase inhibitor (letrozole), HMG, and GnRH agonist and this strategy has been proven to be feasible for producing competent oocytes in women for assisted reproduction treatment. However, compared with traditional ovarian stimulation protocols, the efficiency of this new raised LPS strategy has not been determined.
Study design, size, duration: We did a large retrospective cohort study. Strict access standards were set and 2942 ovum pick-up (OPU) cycles (3104 frozen-thawed embryo transfer cycles corresponded) from LPS and the other two ovarian stimulation protocols conducted between April 2012 and September 2013 were enrolled for analysis.
Participants/materials, setting, methods: Primary outcomes were pregnancy rate, live birth rate and neonatal outcomes. Secondary outcomes were number of mature oocytes retrieved, number of top-quality (Grade I + II) embryos and so on. We used the Kruskal-Wallis test to compare continuous variables among the groups of patients or cycles.
Main results and the role of chance: The mean number of mature oocytes retrieved per OPU cycle was 10.9 ± 7.6 in the LPS group, 3.7 ± 3.0 in the mild treatment group, and 9.1 ± 5.5 in the short-term group ($P < 0.001$ for the comparisons of LPS group with the mild treatment group and short-term group). Pregnancy rate in the LPS group did not differ significantly with the mild treatment group ($P > 0.05$), but was higher than the short-term group ($P = 0.038$). Rate of live birth and still in pregnancy per FET cycle was 44.4% in the LPS group, 41.7% in the mild treatment group, and 39.2% in the short-term group ($P > 0.05$ for the LPS group vs. the mild treatment group, $P = 0.012$ for the LPS group vs. the short-term group). Neonatal outcomes and congenital malformations were similar among the groups.
Limitations, reason for caution: Assignment to the treatment groups was arbitrary, and not randomized; thus, there was a possibility for selection bias. Therefore, further large prospective cohort study is still needed to clarify the efficiency and safety of the LPS strategy.
Wider implications of the findings: The LPS strategy owns several advantages: high number of oocytes retrieved, good quality embryos yield and an excellent pregnancy rate; simple, low cost, effective and patient-friendly; also suitable for patients with poor ovarian response. The LPS strategy is not only a quite beneficial complementation to the follicular-phase ovarian stimulation protocols, but also of far-reaching influences on modern assisted reproduction treatment, especially the inherent down-regulation role of progesterone.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Shanghai Ninth People's Hospital, Shanghai.
Trial registration number: NA.
Keywords: ART, infertility, luteal-phase ovarian stimulation strategy

P-717 FSHR genotype is associated with different response to wild type FSH versus recombinant FSH

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Study question: Does the S680N polymorphism of FSHR gene affect the ovarian response using highly purified FSH or recombinant FSH?
Summary answer: For the first time we show in a population of egg donors 680FSHR gene polymorphism affects the efficacy of wtFSH or rFSH. A possible explanation could be different affinity to the FSH receptor. FSHR genotype is an important factor to determine the doses and the gronadotrophin administration in ovarian stimulation.
What is known already: Previous studies have reported conflicting results for the comparative doses of recombinant FSH (rFSH) and highly purified FSH (wtFSH or FSH-HP) required for an adequate ovarian stimulation. Clinical studies have demonstrated that N680S polymorphism determines ovarian response to FSH stimulation in patients undergoing IVF. Patients with the S680 allele need more FSH during the stimulation. Nothing is known about the clinical efficacy of rFSH or FSH-HP depending on the N680S FSHR polymorphism.
Study design, size, duration: This retrospective study includes 382 cycles performed at Instituto Bernabeu (Alicante. Spain) from 191 oocyte donors genotyped for N680S. All donors carried out two cycles: one with rFSH and the other one with FSH-HP (group1), both with FSH-HP (group2) or both with rFSH (group3). We compare the results in pair from each
Participants/materials, setting, methods: We included 191 egg donors genotyped for N680S polymorphism (63 in group 1, 100 in group 2 and 25 in group 3). The ovarian stimulation protocol was GnRH antagonist with starting doses 150, 225, 300 IU/day according to donor age, body mass index, clinical features and antral follicle count.
Main results and the role of chance: The main outcome measures were oocyte yield, MII, days of stimulation and gonadotrophin dosages. No significant differences were reported when we compared the cycles for each donor in group 1. However, according to FSHR polymorphism statistical differences were shown in oocyte yield and MII. For SS genotype more oocytes (18 vs 17) and MII (16 vs 13; $p < 0,05$) were yielded in a FSH-HP cycle. For NS genotype more oocyte (20 vs 16) and MII (17 vs 14; $p < 0,05$) were yielded in a rFSH cycle. For NN genotype no differences were reported. No significant differences were reported when we compared the cycles for each donor in group 2 and 3 regardless of the FSHR polymorphism.
Limitations, reason for caution: Pharmacogenetics applied to measure ovarian reserve and predicting ovarian response is true. However, an individual is

embedded with the context of that individual's entire genome and environment. In fact, some others genes related to follicular growth could also play an important role in determining the response to gonadotrophins.
Wider implications of the findings: This investigation reveals that in a population of fertile egg donors FSHR gene polymorphism at position 680 is associated with different ovarian response according to gonadotrophin used. Genotyping FSHR N680S could help us to choose not only the doses of gonadotrophin but also the form of administration (rFSH vs FSH-HP) particularly in poor responders where the optimization of protocol is very important to achieve a high number of oocyte retrieval.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Conflicts of interest none declared. This work has been supported by a grant from Rafael Bernabeu Foundation.
Trial registration number: NA.
Keywords: ovarian stimulation, rFSH, N680S, FSHR

P-718 GnRH antagonist versus long agonist protocols in IVF. A systematic review and meta-analysis accounting for patient type

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Study question: What is the impact of the patient population on GnRH antagonists compared to standard long agonist protocols for prevention of premature luteinization in IVF? The aim of the current study was to compare GnRH antagonist versus standard long agonist protocols in various patient populations using various treatment schedules.
Summary answer: In a general IVF population antagonist use is associated with a lower ongoing pregnancy rate but also with lower OHSS rates. In couples with PCOS and poor responders there is no evidence that ongoing pregnancy rate is compromised with antagonist use.
What is known already: Currently available systematic analysis comparing GnRH agonist with antagonist strategies insufficiently account for various patient populations, such as normal ovulatory women, women with PCOS or poor ovarian response and include studies in which the agonist versus antagonist was not the only variable between the compared study arms.
Study design, size, duration: We searched the Cochrane Menstrual Disorders and Subfertility Review Group specialised register of controlled trials, Pubmed and Embase databases throughout 2014.
Participants/materials, setting, methods: Eligible trials were those that compared GnRH antagonists and standard long GnRH agonist protocols in IVF/ICSI. The primary outcome was ongoing pregnancy rate. Secondary outcomes were: live birth, clinical pregnancy, number of oocytes retrieved and ovarian hyperstimulation syndrome (OHSS) accounting for the general IVF, PCOS and poor responder populations.
Main results and the role of chance: We included 43 studies: 28 studies in general IVF patients, 9 studies in PCOS patients and 6 studies in poor responders (NN antagonist/agonist: 3231/2467, 484/510 and 410/370, respectively). In general IVF patients, ongoing pregnancy rate was significantly lower in the antagonist group (RR 0.87, 95% CI 0.79–0.96). Antagonists resulted in significantly lower OHSS rates (RR 0.63, 95% CI 0.44–0.88). No differences in ongoing pregnancy between antagonist and agonist were observed in women with PCOS (RR 0.97, 95% CI 0.84–1.11) and in women with poor ovarian response (RR 0.87, 95% CI 0.65–1.17). Antagonists resulted in significantly lower OHSS rates in women with PCOS (RR 0.47, 95% CI 0.24–0.93). No data on OHSS was available for trials in poor responders.
Limitations, reason for caution: We focused on ongoing pregnancy as the primary outcome for these analyses as live birth rates were often not available. OHSS was poorly defined by various studies and it was not a primary endpoint in most studies analyzed.
Wider implications of the findings: These findings provide an evidence based underpinning for a tailored decision making in premature LH surge prevention strategies in IVF, dependent on the patient profile.
Study funding/competing interest(s): Funding by University(ies) – VU University Medical Centre, Amsterdam, The Netherlands; Academic medical Centre, University of Amsterdam, Amsterdam, The Netherlands.
Trial registration number: NA.
Keywords: GnRH, IVF, agonist, antagonist, systematic review

P-719 Molecular investigations on the BMP15 oocyte-derived growth factor activity in human granulosa cells

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Study question: The identification of novel molecular mechanisms and genes involved in the pathogenesis of Primary Ovarian Insufficiency (POI) is mandatory to clarify the unknown etiopathogenesis underlying this fertility disorder. For this purpose, we unravelled pathways and molecular events induced by BMP15 treatment in human Granulosa cells (hGCs) by microarray gene-expression analysis.
Summary answer: This is the first comprehensive panel of transcriptomic effects induced by BMP15 on hGCs, indicating BMP15 as a master regulator of many folliculogenesis processes such as proliferation, apoptosis, maturation and hormonal response. These findings would permit a better comprehension of the pathogenic mechanisms underlying POI.
What is known already: POI is an inheritable disease with a strong genetic component but characterized by a highly variable expressivity and penetrance. To date, several mutations in the X-linked gene *BMP15* have been identified in association with POI and this oocyte-derived growth factor plays an essential role as a local regulator of the ovarian folliculogenesis in animal models and in humans, also controlling the ovulation quota. Nevertheless, the already known genetic alterations may explain only few cases.
Study design, size, duration: Each pool of hGCs ($n = 2$) was obtained from 6 normal responders to ovarian stimulation for IVF. hGCs were plated and stimulated with 100 ng/ml of recombinant human BMP15 (rhBMP15) in triplicates. Stimulated and not-stimulated cells were collected at 0, 2 and 6 hours, to evaluate early and late regulated genes.
Participants/materials, setting, methods: RNAs from each condition were processed for hybridization on beadchips and analyzed by the Illumina BeadArray. A differential statistical analysis was performed by applying the Linear Model for Microarray Data: stimulation, timev and pool variables were considered. Gene Ontology pathways were also tested for differential expression.
Main results and the role of chance: After the quantile normalization, 4 groups of samples emerged, pool and time dependents (6 h vs. 0 h/2 h), by applying the Principal Component Analysis (PCA) of the means of technical replicates. The differential analysis of stimulated vs non-stimulated samples over time shows 34 differentially expressed probes after 2 h and 46 probes after 6 h (Benjamini-Hochberg (BH)-adjusted P-value < 0.05). Moreover 21 probes were differentially expressed both after 2 and 6 h. The following GO gene sets enrichment analysis was performed by ROAST test (Rotation gene set tests for complex microarray experiments). The rhBMP15 stimulus modulates the BMP signaling pathway, cell fate commitment, proliferation, apoptosis and estrogen response.
Limitations, reason for caution: The microarray and validation analyses were performed on pools of hGCs obtained after ovarian stimulation, and may not be fully representative of the hGCs at earlier stages of the folliculogenesis.
Wider implications of the findings: Several of the pathways here identified have already been described in previous studies dissecting the mechanisms of folliculogenesis in models of altered fertility, confirming BMP15 as a master regulator of folliculogenesis processes. These findings should prompt future analysis of BMP15-regulated genes as novel candidates in POI cohorts.

Study funding/competing interest(s): Funding by national/international organization(s) – GGP09126 Telethon Grant and GR-2011-02351636 Italian Ministry of Health Ricerca Finalizzata 2011-2012 Grant.
Trial registration number: NA.
Keywords: POI, BMP15, folliculogenesis

P-720 A randomized controlled trial of luteal phase supplementation with vaginal progesterone in women with polycystic ovary syndrome undergoing ovulation induction with letrozole

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Study question: Is there a benefit to the use of vaginal progesterone (8% Crinone gel) in women with Polycystic Ovary Syndrome (PCOS) and ovulatory dysfunction undergoing ovulation induction (OI) with letrozole?
Summary answer: Interim analysis of 85 cycles demonstrates a trend towards higher pregnancy rates (PR) and live birth rates (LBR) in OI cycles supplemented with vaginal progesterone (19.5,17.1%) versus those that were not supplemented during the luteal phase (6.8, 4.5%).
What is known already: PCOS patients can have impaired granulosa cell function in the ability to synthesize progesterone. Letrozole may be superior to clomiphene citrate and improve pregnancy and live birth rates in subfertile women who are anovulatory and have PCOS. Limited data suggests that vaginal progesterone supplementation of PCOS patients undergoing OI with clomiphene citrate in the luteal phase may result in an improvement in pregnancy rates.
Study design, size, duration: RCT of PCOS subjects (n = 44) (November 2012–December 2014) undergoing OI (85 cycles) with letrozole. Those meeting follicle growth criteria on ultrasound underwent randomization (1:1 with computer generated spreadsheet) to 8% Crinone vaginal progesterone gel or no luteal support following intrauterine insemination. Primary outcome measured is PR per cycle.
Participants/materials, setting, methods: PCOS subjects (51 between 20 and 40 years and BMI 18–40 kg/m^2 with patent tubes) were enrolled and underwent OI. 44 women responded and underwent randomization. PR and LBR per cycle and per subject were compared. Statistics included Student's t-test, Chi-square test and multivariate logistic regression analysis with odds ratio.
Main results and the role of chance: A total of 85 cycles with a target of 110 cycles was completed. Each subject participated in a maximum of 3 OI cycles with Letrozole. There were no significant differences in baseline characteristics of age, cycle day 3 labs, total motile sperm count, body mass index, gravidity, parity, dose of letrozole prescribed, number of dominant follicles and endometrial thickness between the treatment (n = 41), and control (n = 44) groups. The PR and LBR per cycle was higher in the treatment (19.5, 17.1%) versus control cycles (6.8, 4.5%) (P = 0.08, P = 0.06) although the results were not statistically significant. The PR and LBR subject was also higher in the treatment (33.3, 29.41%) vs. control (15.38. 10.2%) P = 0.1, but the results were not statistically significant. Logistic regression showed odds of pregnancy in Group 1 vs. 2 (OR = 0.3, 95% CL: 0.04–1.5).
Limitations, reason for caution: Interim analysis of an ongoing randomized controlled trial with small cycle numbers (n = 85).
Wider implications of the findings: An interim analysis of a randomized controlled trial demonstrates a trend towards higher pregnancy rates in PCOS patients supplemented with vaginal progesterone in the luteal phase, undergoing OI cycles with letrozole. This suggests there may be some benefit to exogenous supplementation with progesterone in the luteal phase in PCOS patients with ovulatory dysfunction.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Partial funding by Actavia.
Trial registration number: NA.
Keywords: PCOS, luteal support, ovulation induction, letrozole

P-721 Adjuvant gonadotropin-releasing hormone agonist trigger with human chorionic gonadotropin to enhance ooplasmic maturity

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Study question: Does an adjuvant gonadotropin-releasing hormone agonist (GnRH-a) ovulatory trigger with human chorionic gonadotropin (hCG) improve fresh intracytoplasmic sperm injection (ICSI)-embryo transfer (ET) cycle outcomes in patients with poor fertilization history after standard hCG trigger alone?
Summary answer: Combined ovulatory trigger with 2 mg of GnRH-a and 1500 IU of hCG increases oocyte maturity, as well as fertilization and clinical pregnancy rates in patients with poor fertilization history in prior ICSI-ET cycles after standard hCG trigger.
What is known already: Combined GnRH-a and hCG triggers have previously been used to prevent ovarian hyperstimulation syndrome. More recently, studies have suggested an increase in the percentage of mature oocytes retrieved with combined GnRH-a and hCG trigger compared to a standard hCG trigger. These findings have been attributed to the endogenous luteinizing hormone (LH) surge induced by the GnRH-a component of the combined ovulatory trigger.
Study design, size, duration: Retrospective cohort study of all patients with a fertilization rate of <40% in a prior fresh ICSI-ET cycle with standard hCG trigger who subsequently underwent another ICSI-ET cycle with a combined GnRH-a and hCG trigger between January 2006 and June 2013.
Participants/materials, setting, methods: 18633 cycles occurred during the study period. Of these, 744 (3.99%) cycles utilized combined GnRH-a and hCG triggers. Based on a previous study showing a 16.6% difference in fertilization rate, a sample size of 118 patients was estimated assuming an a error of 5% and a power of 80%.
Main results and the role of chance: 120 patients with <40% fertilization rate in a prior ICSI-ET cycle with standard hCG trigger received a combined GnRH-a and hCG trigger in the subsequent cycle. All patients underwent ovarian stimulation with an antagonist-based protocol. There was no difference in the demographics or baseline cycle characteristics of the two groups. The mean (±standard deviation) oocytes retrieved in the hCG and dual trigger groups were 13.4 (±1.09) and 12.6 (±0.75), respectively. There was an overall increase in the percentage of mature oocytes retrieved, fertilization and clinical pregnancy rate in the combined trigger group compared to the hCG group: 84.2% vs. 70.2% (P = 0.01); 52.2% vs. 35.3% (P = 0.01); 52.5% vs. 40.8% (P = 0.03). These findings remained unchanged even after controlling for age and number of embryos transferred.
Limitations, reason for caution: Although our study reveals increased oocyte maturity, fertilization and clinical pregnancy rates after combined GnRH-a and hCG trigger compared to standard hCG trigger, the molecular mechanisms by which the GnRH-a induced LH surge promotes nuclear and cytoplasmic maturity in oocytes remain to be elucidated.
Wider implications of the findings: Utilization of a combined GnRH-a and hCG ovulatory trigger can be a reasonable approach to increase oocyte maturity and fertilization rates in patients with a history of poor fertilization after standard hCG trigger alone. Prospective data are required to validate the overall generalizability and efficacy of this management strategy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical Center.
Trial registration number: NA.
Keywords: IVF, oocyte maturity, gonadotropin-releasing hormone agonist trigger

P-722 A review of 100 consecutive new referrals with secondary amenorrhoea: aetiology and presentation

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Study question: To assess the causes of secondary amenorrhoea, as well as patient characteristics (age, BMI and ethnic origin), family history and reproductive history in women presenting with secondary amenorrhoea.
Summary answer: The cause for secondary amenorrhoea in this study was PCOS in 34%, POI in 32%, hypogonadotropic hypogonadism in 22%, mixed PCOS/hypogonadotropic hypogonadism [defined as polycystic ovaries with low gonadotropins and low estradiol] in 5%, hyperprolactinemia in 4%, drug induced amenorrhoea in 2% and idiopathic in 1%.

What is known already: Most cases of secondary amenorrhoea have been attributed to polycystic ovary syndrome [PCOS] (WHO Group II), hypothalamic amenorrhoea (WHO Group I), hyperprolactinaemia and Premature Ovarian Insufficiency [POI] (WHO Group III). We carried out this review to assess the causes of secondary amenorrhoea and patient demographics for this condition in our local population.
Study design, size, duration: A retrospective analysis of 100 consecutive new referrals with secondary amenorrhoea to a UK tertiary referral centre during the period 2012–2014. Secondary amenorrhoea was defined as absence of menstrual periods for at least 3months in women with otherwise normal periods.
Participants/materials, setting, methods: 100 new referrals to the Reproductive Medicine clinic where selected. A retrospective analysis was their electronic medical records was done to obtain all the data for this study.
Main results and the role of chance: The cause for secondary amenorrhoea in this study was PCOS in 34%, POI in 32%, hypogonadotropic hypogonadism in 22%, mixed PCOS/hypogonadotropic hypogonadism [defined as polycystic ovaries with low gonadotropins and low estradiol] in 5%, hyperprolactinemia in 4%, drug induced amenorrhoea in 2% and idiopathic in 1%. The mean (+SD) age at presentation was 31 (7.19) years. The mean (+SD) age for PCOS women was 27.8 (5.65) years, 30 (6.78) years for hypogonadotropic hypogonadism, 34 (7.50) years for POI and 29.2 (7.52) years in those with mixed PCOS/hypogonadotropic hypogonadism. The mean (+SD) BMI for the total number of women assessed was 26.15 (6.94). The mean (+SD) BMI for PCOS women was 28.58 (7.03), for hypogonadotropic hypogonadism 22.21 (7.80), for POI 27 (5.60) and for mixed PCOS/hypogonadotropic hypogonadism was 20 (1.38). A total of 59% of women were Caucasian, 31% Afro-Caribbean, 7% Asian and 3% Middle-Eastern. A total of 3% gave a family history of secondary amenorrhoea. The primary reason for referral was secondary amenorrhoea in 75% of women, in 5% was infertility and in 20% both- amenorrhoea and infertility. However, on questioning, a total of 38% of women had a desire for fertility. 29% of the women had previously achieved a pregnancy whereas 9% had livebirths. For women with hypogonadotropic hypogonadism, the mean (+SD) FSH, LH and estradiol levels were 5 IU/L (2.55), LH 2.8 IU/L (2.35) and 101 pmol/L (45.04), respectively. Bone density scans were performed in 14/22 women with hypogonadotropic hypogonadism and of these, 7 (50%) had osteopenia, 4 (29%) had osteoporosis and 3 had normal bone density (21%). The mean (+SD) spine T score was -1.46 (1.31) and the mean (+SD) hip T score was -0.80 (0.88). Bone density scans were performed for 20/32 women with POI and of these, 12 (60%) had normal bone density, 8 (40%) had osteopenia while none had osteoporosis. The mean (+SD) spine T score was -0.44 (1.37) and the mean (+SD) Hip T score was -0.28 (1.29).
Limitations, reason for caution: the number might be considered low. And bone density information was not available for all patients.
Wider implications of the findings: In our study, PCOS was the noted to be the commonest cause of secondary amenorrhoea (34% of cases), while hypogonadotropic hypogonadism was the cause in approximately a fifth of all women assessed. A higher prevalence of reduced bone density was noted in women with hypogonadotropic hypogonadism compared to women with POI (79% and 40%, respectively). Consideration should be given to assessment of bone density in women in the latter two groups to allow detection, treatment and to prevent further loss of bone density. The information could provide useful information for counselling patients and guiding clinicians in their practise.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Kings College Hospital NHS Foundation Trust, London.
Trial registration number: NA.
Keywords: secondary amenorrhoea, PCOS, premature ovarian insufficiency, hypogonadotropic hypogonadism, bone density

P-723 Increased prevalence of high normal thyroid stimulating hormone values in intra uterine inseminations and in vitro fertilization

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Study question: The upper reference limits for Thyroid Stimulating Hormone (TSH) are debated. We questioned the prevalence of High Normal (HN) TSH (2,5–4,5 mIU/L) values in an IUI population and a synchronous IVF population in a iodine sufficient area.
Summary answer: The prevalence of HN TSH in the IUI cohort is 21%, and in the IVF cohort 22%, compared with a population prevalence of 5%. The prevalence of HN TSH is comparable in the different groups of causes of infertility.
What is known already: In the overall euthyroid population <5% has HN TSH values. In women undergoing IVF/ICSI HN TSH is ±20%. Karmon et al. and Jatzko showed in their IUI- populations HN TSH prevalences of respectively 26,9% and 15,1% in the euthyroid women. Jatzko's HN TSH women were considered subclinical hypothyroid and therefore treated with Levothyroxine. Evidence lacks until now about beneficial effects of TSH < 2,5 mIU/L on fertility outcomes.
Study design, size, duration: In a retrospective cohort study from January 2008 till March 2012 we analyzed all women starting IUI (n = 1259) and all women starting IVF (n = 2232). We compared the prevalence of LN or HN TSH levels in the different subfertility indications.
Participants/materials, setting, methods: From the women who had (a) IUI or IVF/ICSI treatment(s), we assessed the prevalence of LN/HN TSH if measured within 2 years before treatment and not using thyrotrophic medication (IUI n = 1007, IVF n = 1451), in a single Dutch Academic University Hospital Fertility Centre in a iodine sufficient area
Main results and the role of chance: Both in the IUI- and IVF-population the prevalence of HN TSH values is about four times higher than in the overall population. This might suggest an association with their subfertility. The prevalence's of HN TSH in different indication groups are for IUI/IVF: idiopathic:20/19%, tubal 20/16%, male factor 20%/24%, PCOS 18/15%, endometriosis 20/18%, POF 18/20%.
Limitations, reason for caution: Limitations of this study are its retrospective design, the spread of analysis interval (2 years), the small groups when comparing the indications, and no prolactin nor TPO antibodies known. Finally the value of 2,5 mIU/L as separating LN from HN is debatable.
Wider implications of the findings: Higher prevalence of HN thyroid values in subfertile women than in the overall population might associate thyroid hormones and subfertility. Literature shows until now no differences in treatment or pregnancy outcomes between LN and HN TSH women nor whether this differs in the distinct indication groups. A randomized controlled trial with Levothyroxine when TSH >2,5 mIU/L is needed to know if HN TSH should be considered as subclinical hypothyroidism affecting pregnancy outcomes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, VU University Medical Center (VUmc), Amsterdam.
Trial registration number: NA.
Keywords: TSH, IVF, IUI, endocrinology, thyroid

P-724 The proliferative phase endometrium in women with PCOS presents with decreased proliferation and density of migratory CD68+ macrophages and CD8+ T-cells

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Study question: Does the endometrium in women with polycystic ovary syndrome (PCOS) present with an altered immune cell profile explaining some of the adverse reproductive outcomes and endometrial pathologies in these women?
Summary answer: PCOS endometrium showed lower proliferation (Ki67) rate with concomitant decrease in the migratory macrophage (CD68+) and T-cell (CD8+) density compared to controls, whereas no difference was observed in the density of CD56+ uterine NK-cells. The expression of estrogen receptor alpha (ERa) and progesterone receptor (PR) were similar between the groups.

What is known already: Immune cells play a crucial role both in implantation and endometrial pathogenesis as they modulate the endometrial immune environment and facilitate embryo invasion. PCOS women present with subfertility and increased risk for endometrial cancer and previous studies have shown several endometrial abnormalities in PCOS endometrium including altered cytokine secretion/response and migratory immune cell (NK-cell) density. To date, no data exists on immune cell density in proliferative phase endometrium in women with PCOS compared to controls.
Study design, size, duration: Prospective, university based, case-control study utilizing endometrium biopsies [20 PCOS (Rotterdam criteria), 18 controls with regular cycles] obtained from fertile age volunteers or patients going through benign gynaecological procedure. The study was designed to compare endometrial immune cell density, proliferation and hormone receptor expression in women with PCOS and controls.
Participants/materials, setting, methods: Study groups were matched for BMI (kg/m^2) (PCOS 27.9, controls 26.4). The proliferative phase histology was confirmed by pathologist and CD68, CD56, CD8, Ki67, ERa and PR expression were assessed by immunohistochemistry. The staining density in stroma was analysed by using computer-assisted cell counting method in ImageJ software.
Main results and the role of chance: According to ANOVA, no difference was observed in the BMI between the study groups. Interestingly, proliferative phase endometrium in women with PCOS presented with lower CD68+ macrophage ($p = 0.014$) and CD8+ lymhocyte ($p = 0.043$) density whereas no difference was found in CD56+ uterine NK cell count. Furthermore, the Ki67 staining showed lower proliferation rate in PCOS endometrium ($p = 0.01$) even though no differences were observed in ERα and PR expression.
Limitations, reason for caution: Due to relatively small sample size the patients were not divided into different BMI-groups. Also even though mean of 7–9 pictures were taken per sample to get representative staining result for ImageJ processing there still may be a chance of estimation bias for the most representative areas in the sample.
Wider implications of the findings: The present data shows low immune cell density of CD68+ macrophages and CD8+ lymphocytes in proliferative phase PCOS endometrium compared to BMI-matched controls. As immune cells play an important role during implantation, the result may relate to adverse reproductive outcomes in women with PCOS especially if the alteration persists towards window of implantation. Furthermore, as the proliferation rate was low in PCOS endometrium it does not suggest hyperplastic endometrium phenotype in these women.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s) – Sigrid Juselius Foundation, Academy of Finland, Finnish Medical Foundation, Orion-Farmos Research Foundation.
Trial registration number: NA.
Keywords: PCOS, endometrium, immune cells, proliferation

P-725 Menstrual-cycle-length: a surrogate measure of reproductive health capable of improving the accuracy of biochemical/sonographical ovarian-reserve-test in estimating the reproductive chances of women referred to ART

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Study question: Can the menstrual cycle length (MCL) be considered as a surrogate measure of reproductive health and could it be able to improve the accuracy of biochemical/sonographical ovarian-reserve-test in estimating the reproductive chances of women referred to ART?
Summary answer: In women <35 years, MCL >31 days may be associated with increased risk of OHSS and good Ovarian Sensitivity Index (OSI). In women >35 years, MCL shortening may be associated with poor-ovarian-response, low OSI, reduced fertilization-rate. MCL is a good tool to estimate biological-age and the chances before ART cycles.
What is known already: Recent evidences suggested that long MCLs are associated with a greater number of antral-follicle waves and higher ovarian-response to hormonal stimulation. On the contrary, short MCLs are associated with poor response to ovarian hyperstimulation. So, it seems intuitive that the menstrual-diary should be routinely considered as a useful tool in estimating chances and improving reproductive outcome of ART-cycles, particularly when patient report any previous ART cycle and their biological and chronological age do not match.
Study design, size, duration: Retrospective-observational study on 455 normo-ovulatory infertile women scheduled for their first fresh non-donor IVF/ICSI-treatment owning a personal menstrual-diary of the six months preceding the ART treatment (interval-time 2011–2014). According to pre-treatment ovarian reserve assessment, all patients received the most adequate stimulation protocol.
Participants/materials, setting, methods: We divided the sample according to patients age (AGE_class_1: >40 years, AGE_class_2: 35–40 years, AGE_class_3: 26–34 years, AGE_class_4: <26years) MCL (MCL_class_1: >31 days, MCL_class_2: 30–31 days, MCL_class_3: 28–29 days, MCL_class_4: 26–27 days, MCL_class_5: <26 days), AMH (AMH cohort_1: 0.1–0.4 ng/ml, AMH cohort_2: 0.5–1.1 ng/ml, AMH cohort_3: >1.1 ng/ml).
Main results and the role of chance: With an AMH <1.1 ng/ml the ovarian-response to hyper-stimulation correlates with MCL. Patients with MCL <28 days showed a reduction of ovarian-response in accordance with MCL shortening. In patients with AMH value <0.4 ng/ml (cohort 1) and 30 days MCL an average of 3.5 oocytes per-cycle were collected while only 1.2 oocytes per cycle were retrieved in those with MCL of 26 days. MCL correlates with fertilization-rate of the MII-oocytes, particularly in patients >35 years. Considering patients >40 years, we observed a fertilization-rate of almost 90% with 30–days MCL and of 50% with <26 days MCL. We found that in patients younger than 34 years MCL >31 days was associated with an OSI of about 12.5 compared to an OSI of about 5 when MCL was <30 days.
Limitations, reason for caution: Retrospective study-design, variability in ovarian-stimulation-protocols (choosing the most appropriate for each patients), use of both recombinant and purified gonadotropins, assessment of oocytes fertilization ratio using 6 oocytes as a maximum number to fertilize (according to the Italian law), may represent a reason of caution for data interpretation
Wider implications of the findings: We strongly suggest considering the introduction in routine clinical practice of MCL evaluation, because this parameter may be considered an indicator of ovarian age (better than FSH and chronological age). The large scale applicability and the good accuracy of MCL in estimating ovarian-response, OSI and fertilization-rate allows Clinicians to consider it as an inexpensive good tool capable of improving the accuracy of biochemical/sonographical ovarian-reserve-test and to better estimate the ART success rate.
Study funding/competing interest(s): Funding by University(ies) – Authors declare no funding. Authors declare no competing of interest.
Trial registration number: NA.
Keywords: menstrual cycle length, estimation of reproductive chances, ovarian sensitivity index, ovarian reserve, ovarian response

P-726 Deleterious effect of early progesterone elevation (EPE) on IVF implantation and pregnancy rates depends on different prognostic scenarios

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Study question: We analyze the impact of early progesterone elevation (EPE) during IVF ovarian stimulation in different prognostic scenarios, related to patient's age, ovarian reserve, number and quality of available and transferred embryos.

Summary answer: EPE reduces significantly clinical pregnancy and live birth rates, but the effect size depends on prognostic factors of IVF outcomes.
What is known already: Lack of opportunity for embryo implantation due to EPE could be a consequence of progesterone increase grade. It has been hypothesized that classical prognostic factors –woman's age, ovarian reserve, number and quality of obtained embryos– could modulate the dysregulatory effect of EPE; quantification of these potential modifying effects has not been yet accurately estimated. Estimation of negative effect of EPE by prognostic subgroups could help in decisions making about immediate or delayed embryo transfer.
Study design, size, duration: Retrospective cohort study of an opportunity sample of 583 IVF cycles initiated between 2011 and 2014 in a tertiary university ART centre. Exposure was defined as progesterone elevation over an specific cut-off value established by statistical criteria. Clinical pregnancy and birth rate have been considered as main outcomes.
Participants/materials, setting, methods: We analyze 583 IVF cycles with fresh embryo transfer from a tertiary ART program with routinely serum progesterone determination. Frequency of EPP exposure was 4% (23/586). Relative risk for clinical pregnancy and live birth has been estimated globally and for each prognostic stratum.
Main results and the role of chance: EPE was defined for clinical pregnancy as final serum progesterone level >1,7 ng/mL. In EPP exposed patients, clinical pregnancy and live birth rates were significantly lower (3/23 vs 241/560; p = 0,003; RR: 0,3 95% CI: 0,10–0,87) (4/33 vs 122/357; p = 0,006; RR: 0,35; 95% CI: 0,14–0,89). EPE size effect on clinical pregnancy was related to AFC (RR for <5: 0,75 95% CI: 0,11–4,75; RR for ≥5: 0,25; 95% CI: 0,06–0,93) and number of retrieved oocytes (RR for <5: 0,85 95% CI: 0,25–2,80; RR for ≥5: 0,13; 95% CI: 0,02–0,88). Effect of EPE for live birth rate estimation (>1,5 ng/mL) was significantly modified only by number of available oocytes (RR for <5: 1,14 95% CI: 0,3–4,1; RR for ≥5: 0,21; 95% CI: 0,6–0,79).
Limitations, reason for caution: Accuracy of stratified relative risk estimation is probably affected by low frequency of critical EPP, that has been defined towards IVF outcome probabilities distributions. Bivariate analysis of potential confusion or interaction effects of prognostic covariables could be underpowered.
Wider implications of the findings: Our preliminary data support a jeopardizing effect of high levels of EPE on clinical pregnancy and live birth rates. This could be a non-linear effect, probably modulated by main prognostic factors (age, AFC, retrieved oocytes and number and quality of transferred embryos.
Study funding/competing interest(s): Funding by hospital/clinic(s), Hospital General Universitario Gregorio Marañon. Madrid (Spain).
Trial registration number: Not applicable (retrospective cohort study).
Keywords: progesterone, IVF, embryo implantation, clinical pregnancy, live birth

P-727 Higher levels of chemerin were detected in the follicular fluids of PCOS than non-PCOS: possibility of involvement of chemerin in the pathogenesis of PCOS

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Study question: By checking the levels of chemerin in follicular fluids (FF) and the levels of chemerin in serum in PCOS and non-PCOS, we investigated the presence of chemerin and its possible involvement of chemerin in pathogenesis of PCOS.
Summary answer: The levels of chemerin in the FF of PCOS were significantly higher than those of non-PCOS, but no differences in the serum between PCOS and non-PCOS. And levels of FF were higher than those of serum both in PCOS and non-PCOS. Chemerin may play a role in ovary per se of PCOS.
What is known already: Chemerin, a kind of adipokine presenting in many organs and tissues, modulates immune system functions and glucose metabolism via its own receptor. It also exerts interactions with many inflammtory factors which are related to the reproductive functions and primary incentives of insulin resistance.
Study design, size, duration: Follicular fluids, serum specimens and granulosa cells were collected from 64 PCOS and 58 non-PCOS patients undergoing IVF-ET.
Participants/materials, setting, methods: Follicular fluids and serum specimens were collected from 64 patients undergoing IVF-ET with PCOS and 58 patients without PCOS. ELISA were performed to check the chemerin levels in follicular fluid and serum. Granulosa cells were insolated from follicular fluids. RT-PCR was performed to detect the expression of chemerin in ovary.
Main results and the role of chance: The levels of chemerin in the FF of PCOS were significantly higher than those of non-PCOS, but no differences in the serum between PCOS and non-PCOS. The levels of chemerin in FF were significantly higher than those of serum both in PCOS and non-PCOS. The results of RT-PCR identified the expression of chemerin in granulosa cells.
Limitations, reason for caution: immunohistochemistry or immunocytochemistry is necessary for detecting the location of chemerin in the ovary.
Wider implications of the findings: Based on these results, we proposed that chemerin may involve in the pathogenesis of ovary in PCOS. In further study we aim to investigate the role and effects of chemerin in pathogenesis of insulin resistance in ovary per se.
Study funding/competing interest(s): Funding by national/international organization(s) – Natural science foundation of China.
Trial registration number: NA.
Keywords: chemerin, insulin resistance, PCOS, granulosa cell, inflammtory factor

P-728 Do AMH levels predict outcomes for IVF patients doing PGS?

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Study question: Is there a significant difference in cycle and live birth outcomes and numbers of euploid embryos in women undergoing IVF/PGS with their own eggs depending on pre-cycle serum anti-Mullerian hormone (AMH) levels?
Summary answer: There is a significant difference in the numbers of euploid embryos and clinical pregnancy rates (CPR) and live birth rates (LBR) per cycle start for patients undergoing PGS based on AMH levels.
What is known already: Lower AMH levels result in fewer embryos to biopsy as well as lower implantation rates (Gleicher et al, Reprod Biol Endocrinol 2012:10: 48). No association between AMH levels and live births after IVF/PGS has been previously reported.
Study design, size, duration: Retrospective review of 288 IVF/PGS cycles between June 2010 and July 2013 for which AMH levels were available. 122 cycles were done in women with an AMH <1.0 ng/mL (low AMH group) and 166 in women with an AMH >1.0 ng/mL (high AMH group).
Participants/materials, setting, methods: Private U.S. IVF clinic database review. Embryos underwent trophectoderm biopsy and vitrification awaiting SNP microarray results. Data analyzed in 2 groups: AMH <1.0 and >1.0 ng/mL. Data on CPR and LBR are provided for those cycles/patients that have undergone a subsequent frozen ET. Two-tailed Fisher's exact test for statistical analysis.
Main results and the role of chance: Cycle cancellation rates were significantly higher in the low AMH group vs. high AMH group (18% vs. 5.4%, p = 0.004). The number of euploid embryos was significantly lower in the low AMH group (37.9%) vs. the high AMH group (52.6%), p = 0.036. CPR/ET was similar in the low AMH group vs. the high AMH group (55.8% vs. 66.3%, p = 0.74). CPR/retrieval was 19% and 35% in the low and high AMH groups respectively (p = 0.04). CPR/cycle start was also lower (15.6% vs. 30.1%, p = 0.011). LBR/ET were similar in the low AMH group (52.9%) vs. the high AMH group (60.2%), p = 0.74. LBR/retrieval trended lower: 18% in the low group and 32% in the high group, p = 0.066. LBR/Cycle start was significantly lower in the low vs. high AMH groups (14.8% vs. 30.1%, p = 0.019).
Limitations, reason for caution: Retrospective review so AMH levels could have influenced cancellation rates. Not all patients have undergone subsequent FET so some of the data is incomplete. This includes 6 patients in the low AMH group and 9 patients in the high AMH group that have at least one euploid embryo. AMH assays were done in different laboratories.
Wider implications of the findings: As an independent variable, AMH levels lower than 1.0 ng/mL predict higher cycle cancellation rates, more euploid embryos to transfer, and higher CPRs and LBRs and low rates of pregnancy loss. This data confirms earlier data about higher implantation rates but reports on a substantial number of live births as well. AMH levels may be useful for patient counseling.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Pacific Fertility Center, San Francisco, CA.
Trial registration number: Not a registered randomized trial. IRB Expedited review approved by Schulman IRB.
Keywords: AMH, PGS, IVF

P-729 GnRH agonist trigger in PCOS undergoing IVF with GnRH antagonist cycles: single dose vs. double dose – a randomized pilot study

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Study question: Is a single dose of Gonadotropin-releasing hormone agonist (GnRHa) trigger to induce final oocye maturation in Polycystic Ovarian syndrome (PCOS) undergoing IVF cycles with GnRH antagonist protocol sufficient to provide an optimal outcome in terms of mature oocytes (MIIs)? Is the cycle outcome better with single dose or double dose?
Summary answer: Double dose of GnRHa trigger in PCOS with GnRH antagonist cycles provides a better cycle outcome than a single dose in terms of maturity of oocytes, good quality embryos available on day 3 and day 5 with no OHSS.
What is known already: GnRHa induced LH surge consists of a short ascending phase (~4 h) and a long descending phase (~20 h), total of 24–36 h, different from the physiological LH surge (~48 h). In PCOS with subtle pituitary dysfunction, at times a single dose of GnRHa might not be able to induce LH surge above a threshold level and for a threshold duration (mimicking physiological surge) required for adequate oocyte maturation. It is therefore important to optimize the dosage and frequency of GnRHa for an optimum yield of mature oocytes in PCOS.
Study design, size, duration: This prospective, randomized, double blinded, proof of concept study was from June 2012 to December 2014 on 100 PCOS defined as per the ESHRE/ASRM Rotterdam criteria (2003) undergoing IVF in antagonist protocol. Patients were randomized by computer generated sequence into two groups of 50 each. All participants before allocation signed an informed consent form.
Participants/materials, setting, methods: Group A: single dose, 0.2 mg, 35 h prior to oocyte retrieval. Group B: 0.2 mg, 35 h before oocyte retrieval +0.1 mg 12 h following first dose. GnRHa: Triptorelin acetate (Decapeptyl), administered at least 12 h after the last dose of GnRH antagonist. Post trigger LH and progesterone levels were measured 12 h following first dose. All Embryos were vitrified. Data analysis: SPSS version16.
Main results and the role of chance: The mean age (years) and BMI (kg/m^2) in groups A and B were (29.80 ± 3.71; 28.30 ± 3.24) and (24.64 ± 3.83; 26.05 ± 4.66), respectively. Other variables like cause, duration of infertility and seminal parameters were similar in both groups. Primary Outcome: There was statistically significant odds favoring group B for higher number of MII oocytes (Odds ratio of 0.47; CI: 0.38–0.57; $p < 0.01$). Significantly higher number of MI and GVs were obtained in group A (4.78 vs. 2.76; $p < 0.01$) and (1.84 vs. 0.64; $p = 0.012$), respectively. Secondary Outcomes: Post trigger serum LH (IU/L) and progesterone (ng/ml) levels in group A and B were (42.79 ± 14.17; 41.27 ± 13.42) and (12.36 ± 3.50; 11.68 ± 2.65), respectively. Significantly higher numbers of blastocysts were obtained in group B than group A (4.00 vs. 3.04; $p = 0.023$). Both groups had no OHSS.
Limitations, reason for caution: Sample size was not statistically defined as this was a pilot study. A significant age difference ($p = 0.034$) between the two groups was obtained as the randomization was not stratified. As an extension of the study, we plan to evaluate the clinical pregnancy and live birth rates in frozen thawed embryo transfer cycles.
Wider implications of the findings: GnRH agonist trigger is a good strategy in PCOS and hyper-responders but an appropriate dosage for an optimal cycle outcome needs to be defined. This study clearly shows that a second dose of GnRHa 12hrs following the first dose probably by maintaining a sustained LH level yielded better maturity of oocytes and higher blastocysts than a single dose. Increase in the study population will allow us to confirm the present data.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Bangalore Assisted Conception Center.
Trial registration number: NA.
Keywords: GnRHa trigger, dose, GnRH antagonist, PCOS, mature oocytes (MIIs)

P-730 Effect of recombinant-LH and hCG on in vitro maturation (IVM), fertilization, and early embryonic development of mouse germinal vesicle (GV)–stage oocytes

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Study question: Do recombinant-LH and hCG affect the *in vitro* maturation (IVM), fertilization, and early embryonic development of mouse germinal vesicle (GV)–stage oocytes in the absence of FSH?
Summary answer: Our study showed that hCG acts on Lhcgr (LH/hCG receptor) in oocytes of different stages of maturation increasing considerably the nuclear maturation rate. Furthermore the percentages of fertilization and early embryonic development increased when LH was added in hCG cultures of in vitro matured GV-stage oocytes.
What is known already: During IVM, intrinsic and extrinsic f-actors must cooperate properly in order to ensure cytoplasmic and nuclear maturation. LH and hCG are integral components of the HPA axis, which controls sexual maturation and functionality. Comparison of recombinant LH (key regulator of gonadal steroidogenesis and ovulation) and hCG (active in pregnancy and fetal development) for oocyte maturation in clinical IVM offers the possibility of investigating the effects of these gonadotrophins in a well-designed in vitro system.
Study design, size, duration: This prospective observational study was performed on a study population consisted of 100 female mice and 30 male mice (C57BL/6 × CBA) F1 hybrids, which were allocated in 15 sequential experiments of 5–8 female and 2 male mice each. The experiments were performed through an 8-month period.
Participants/materials, setting, methods: Nuclear maturation of GV oocytes was evaluated in the presence of r-LH or hCG. The oocytes were then fertilized to study the role of r-LH and hCG in the fertilization and early embryonic development. The expression of Lhcgr was examined at oocytes of different stages of maturation and early embryos.
Main results and the role of chance: The in vitro maturation percentages of mouse germinal vesicle (GV)–stage oocytes of the group supplemented with hCG were significantly higher compared to the control group. The rate of early embryonic development increased in the hCG and LH cultures of GV oocytes when LH was further added while this wasn't observed when hCG was further added. The LH/hCG receptor was expressed in all different stages of in vitro matured mouse oocytes (prophase I, metaphase I and II) as well as from zygotes and embryos of different stages of development (2-cells, 4-cells, morulae) using reverse transcriptase – polymerase chain reaction (RT-PCR).
Limitations, reason for caution: We used GV oocytes from F1 mouse only. Hormones were added in the culture medium but only an indirect late hormone effect could this study reveal. Recombinant FSH is added constantly to the culture medium in clinical IVM programs, thus a condition with FSH was not included in the study.
Wider implications of the findings: The advantages of oocyte IVM in assisted reproduction as an alternative to hormone stimulation for patients with polycystic ovary syndrome are clear. Cancer patients and patients with hormonally sensitive tumors would also benefit from IVM. Developing a widespread, safe, and effective method to extract oocytes from patients followed by IVM would allow them to produce embryos with their partners that can be frozen for later use and help mitigate their possible loss of fertility
Study funding/competing interest(s): Funding by University(ies) – The study was funded by and conducted at the IVF unit, 1st Department of Obstetrics and Gynecology, Alexandra Hospital, University of Athens Medical School, Athens, Greece.
Trial registration number: No registration number required.
Keywords: IVM, GV, hCG, LH

P-731 Low-dose aspirin plus dexamethasone can improve the pregnancy rates of patients undergoing IVF-ET: a prospective clinical study

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Study question: To determine the effects of aspirin and dexamethasone on pregnancy rates in patients undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in agonist cycle regimen.
Summary answer: The use of low-dose aspirin and dexamethasone can improve the pregnancy rates in patients undergoing IVF or ICSI in agonist cycle regimen.
What is known already: The effect of aspirin and dexamethasone as adjunvant treatments in the IVF treatment is still controversial.
Study design, size, duration: Prospective clinical study. A total of 1,600 patients underwent the IVF/ICSI treatment in agonist regimen were recruited in this study from December 2012 to June 2013.

Participants/materials, setting, methods: A total of 1600 patients using agonist regimen were recruited. In the treatment group, 1041 patients received a daily dose of 100 mg of Aspirin and 0.75 mg of Dexamethasone from the beginning of the ovarian stimulation. In the control group 559 patients received no adjuvant treatment.
Main results and the role of chance: The mean age, basal FSH levels, antral follicle count, infertility etiology, endometrial thickness, and mean number of embryos replaced were similar between the two groups. However, a significantly higher clinical pregnancy rate (57.61% versus 51.60%, $P = 0.033$) and blastocyst formation rate (33.40% versus 30.82%, $P = 0.019$) were observed in the study group compared with the control group; Furthermore, the number of gonadotropin ampules used was decreased in the study group compared to the control group (32.35% versus 34.40%, $P < 0.001$).
Limitations, reason for caution: This study is a prospective clinical study without strict randomiztion. The sample size of the study group is much larger than the control group. Although the basic clinical characteristics is no different, the results of this study should be cautionly analysed.
Wider implications of the findings: The previous researches were mainly about the efficacy of use of Aspirin or Dexamethasone. The study of the combined use of low-dose Aspirin and Dexamethasone is quite limited. This is the largest study using Aspirin and Dexamethasone as co-treatment in IVF treatment. In this study, it was observed Aspirin and Dexamethasone can improve the clinical pregnancy rate and may augment the ovarian response of the patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Shenzhen Zhongshan Urological Hospital.
Trial registration number: NA.
Keywords: IVF, aspirin, dexamethasone, clinical pregnancy rate

P-732 Increased antral follicle count (AFC) does not attenuate the expected age-related decline in IVF-ET outcome and improves live birth rate only in young patients

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Study question: To clarify whether AFC differentially influences IVF-ET outcome in elderly and young patients
Summary answer: Increased AFC does not compensate for the expected decline in clinical pregnancy and live birth rates in elderly IVF-ET candidates but it is associated with a noticeable improvement of IVF-ET outcome in youngsters.
What is known already: Whereas, during aging, ovarian follicles become progressively scarce and reproductively incompetent, inter-individual variations exist in the pace of follicle loss. Moreover, follicle distribution over the various stages of growth changes, which leads to an increased antral/non-growing follicles ratio in elderly premenopausal women. Together, these physiologic features concur to explain, at least in part, why some normo-ovulating, no-PCOS women have above-average AFC despite their increasing age. Whether these quantitative variations reflect oocyte competence remains unclear.
Study design, size, duration: We studied selected youngster (26–32 years) and elderly (37–42 years) IVF-ET candidates, regularly-ovulating, devoid of PCOS, and undergoing 863 COH cycles. Prior to COH, all patients had either documented increased (20–24 follicles) or reduced (5–13 follicles) AFC (3–10 mm in diameter in both ovaries) at transvaginal ultrasound scans.
Participants/materials, setting, methods: According to age and AFC, patients were sorted into 4 groups as follows: Youngster-Increased AFC ($n = 167$), Youngster-Reduced AFC ($n = 208$), Elderly-Increased AFC ($n = 122$), and Elderly-Reduced AFC ($n = 366$). Clinical pregnancy and live birth rates were the outcome measures. In addition, serum AMH levels were assessed in all patients. Multivariate analysis was performed.
Main results and the role of chance: Clinical pregnancy and live birth rates were similar in Elderly-Increased (36.1% and 24.6%, respectively) or Elderly-Reduced (34.4% and 23.2%, respectively) AFC groups. Yet, in the Youngster-Increased AFC group, patients showed an improved IVF-ET outcome (55.1% and 46.7%) as compared to the Youngster-Reduced AFC (37.7% and 30.8%) group ($P < 0.001$ and $P < 0.002$, respectively). Similar results were obtained when using AMH instead of AFC to discriminating ovaries with good or defective follicle endowment.
Limitations, reason for caution: Pregnancy and live birth rates are often influenced by a wealth of factors. The present investigation attempted to controlling for most of them.
Wider implications of the findings: The present results indicated that, in IVF-ET candidates, the quantity of antral follicles recorded at ultrasound scans reflect the overall follicle health only in young patients (27–32 years). In elderly patients (37–42 years), even marked variations in AFC failed to influence the likelihood of live birth. These data are contributive for orientating clinical practice and raise unanswered questions on the mechanisms regulating the association between follicle quantity and quality during ovarian aging.
Study funding/competing interest(s): None
Trial registration number: NA.
Keywords: AFC, ovarian reserve, ovarian aging, AMH

P-733 The effects of thyroid autoimmunity presence on ovarian reserve in 117 women with euthyroid state

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Study question: The aim of this study was to assess the effects of antithyroid, antibodies (ATA) presence on ovarian reserve
Summary answer: The presence of thyroid antibodies is associated with decreased ovarian reserve (DOR)
What is known already: Thyroid autoimmunity is the most common autoimmune state that affects up to 5–10% of women during the reproductive age. The relationship between thyroid autoimmunity and ovarian reserve has been investigated in some studies. Several studies have denied such an association whereas others have confirmed the presence of a negative relationship between these two parameters.
Study design, size, duration: This is a retrospective cohort study including 117 women positive for (ATA) at 18–38 years of age from November2014 to January2015 at our hospital.
Participants/materials, setting, methods: For ovarian reserve measurement we used AMH levels and antral follicle count. Thyroid function was tested by serum TSH and FT4 levels. Antithyroid peroxidase and antithyroglobulin antibodies (anti-TPO and anti-TG, respectively) were detected as markers for thyroid autoimmunity. Women were classified according to AMH levels. In group A, AMH ≤0.5 ng/ml and in group B AMH >0.5 ng/ml
Main results and the role of chance: No differences were observed between two groups regarding mean female age, mean BMI. There were significant differences observed between two groups regarding AMH levels (respectively; $P < 0.001$), Women with DOR had higher serum levels of anti-TPO in comparison to controls (40.9 ± 59.7 and 17.6 ± 10.7 IU/mL, respectively; $P < 0.05$) and no significant difference was found in serum levels of TSH, or FT4 between the two groups. Patients with DOR had a higher prevalence of positive results for anti-TG and/or anti-TPO in comparison to controls (25.6% and 5.3%, respectively; $P < 0.05$), anti-TPO alone (18.6% and 4.3%, respectively; $P < 0.05$) and anti-TG alone (22.4% and 4.5%, respectively; $P < 0.05$).
Limitations, reason for caution: Our results demonstrate a negative correlation between DOR and thyroid autoimmunity. However, our study does not consent to draw definitive conclusions and further evidence is required prior to show the effects of thyroid autoimmunity on ovarian reserve
Wider implications of the findings: There is a strong association between the presence of thyroid autoantibodies and DOR. High prevalence of thyroid antibodies in euthyroid patients with DOR refers to the importance of investigation for thyroid autoimmunity in those patients. Screening for thyroid disorders in women with DOR is controversial but might be important to detect thyroid autoimmunity to follow-up these parameters in these patients during pregnancy.
Study funding/competing interest(s): Funding by hospital/clinic(s) –There is no conflict of interest. No funding source.

Trial registration number: NA.
Keywords: thyroid autoimmunity, AMH, decreased ovarian reserve

P-734 Randomised control trial of low dose gonadotropin in polycystic ovarian syndrome for controlled ovarian stimulation with GnRH agonist long protocol

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Study question: Low dose (100 IU/day-112.5 IU/day) gonadotropin with Gonadotropin Releasing Hormone (GnRH) agonist long protocol in patients with polycystic ovarian syndrome (PCOS) results in multiple follicle development with minimal iatrogenic side effects.
Summary answer: Gonadotropins with GnRH agonist long protocol for controlled ovarian stimulation, leads to multiple follicle development, reducing risk of ovarian hyperstimulation syndrome (OHSS). Embryo transfer is done in the same cycle; extra embryos are cryopreserved, cost effective, providing flexibility to start ovarian induction at any point after desirable down regulation
What is known already: PCOS patients are difficult to stimulate as higher doses can lead to OHSS, a serious complication. IVM is used as an alternative for these patients, however success rates are limited. GnRH antagoinst is also a good substitute, but discrepancy in follicle size is seen and flexibility to start ovulation induction (OI) is a limiting factor.
Study design, size, duration: 400 cycles of PCOS patients 25–37 years, with duration of infertility 2–14 years, from January 2012 to December 2014 were selected and randomly distributed. PCOS patients with tubal blockage, male-factor infertility and endometriosis were included. Cycles were divided into two groups after down regulation with GnRH agonist long protocol.
Participants/materials, setting, methods: After all investigations, first group received recombinant FSH (100–112.5 IU/day) and second group 150 IU/day for 10–15 days. Oocyte retrieval was performed after 36 h of hCG injection. OI started when estradiol <50 pg/ml, endometrial thickness <5 mm, LH <5 mIU/ml and serum progesterone <1 ng/ml. Statistically analysed by Student's *t-test.*
Main results and the role of chance: Total dose of FSH, in-group one was significantly lower in comparison to group two, $p = 0.0003$. High E2 levels on day of HCG were also observed in both the groups. Symptoms of OHSS, such as abdominal pain, abdominal bloating, nausea and vomiting were noted in an increased number of patients in group two, which was statistically significant, $p < 0.05$. IVF cycles were cancelled in patients who complained about the above symptoms before the hCG injection. There were no cycles cancelled in the low dose group and statistical significance was also achieved when group one was compared to group two $p = 0.0268$.
Limitations, reason for caution: Dose of FSH could not be increased due to risk of severe hyper stimulation.
Wider implications of the findings: COH can safely be done in PCOS patients with low dose of gonadotropins with GnRH agonist long protocol for different indications. This avoids the risk of OHSS and extra embryos are available for cryo-preservation. This method can be a good alternative for *In-Vitro* maturation (IVM) and ovarian stimulation with GnRH antagonist.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Saini IVF and Fertility Research Centre, Dehradun, India.
Trial registration number: Not Applicable in India and consent from patients was taken before the treatment at the centre.
Keywords: gonadotropin releasing hormone (GnRH) agonist, polycystic ovarian syndrome (PCOS), ovarian hyperstimulation syndrome (OHSS), in-vitro maturation (IVM), human chorionic gonadotropin (hCG)

P-735 Follicle stimulating hormone and antral follicle count in combination better predicts live birth rate than anti-Mullerian hormone in women with diminished ovarian reserve

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Study question: This study aims to determine whether endocrine markers (anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH)) or ultrasound markers (antral follicle count (AFC)) are capable of predicting live birth rate after IVF among women with diminished ovarian reserve (DOR).
Summary answer: FSH in combination with AFC has a greater tendency to predict with live-birth after assisted conception. Although AMH, independently of age and assay used has an association with the same, however, its predictive accuracy in the particular cohort of women is less.
What is known already: Serum levels of FSH and AFC are commonly used as indicators, with high FSH levels and low AFC point to reduced reproductive potential. Since 2005, AMH has emerged as an established marker of ovarian reserve and a good predictor of poor or excessive ovarian response after controlled ovarian hyperstimulation; however, weakly associated with pregnancy following fertility treatment. It is debatable whether it can predict the ultimate outcome of assisted conception, live birth.
Study design, size, duration: A prospective cohort study was conducted from August 2013 to April 2014 including 512 women having irregular menstrual cycle and DOR who were referred to fertility treatment at our centre. The control group included 409 non-PCOS, poor responder infertile females undergoing fresh IVF treatment cycles using standard conventional protocol.
Participants/materials, setting, methods: FSH, LH, E_2 and AMH were measured by chemiluminescence. Serum FSH and AMH levels with AFC were assessed as predictors of clinical pregnancy. Data was compared using multivariate analysis and receiver operator characteristic (ROC) curve. Odds ratio (OR) was estimated as a measure to support ROC findings.
Main results and the role of chance: DOR cohort had lower ($p < 0.001$) AFC (4.42 ± 0.98 vs. 7.11 ± 1.23) and AMH (0.8 ± 0.2 vs. 2.1 ± 0.78) but higher basal FSH levels (9.8 ± 1.9 vs. 5.5 ± 2.1). In multivariate analysis the combination of AFC and basal FSH was superior ($p < 0.001$) to individual entity or AMH alone ($p < 0.007$). Area under curve (AUC) for each parameter according to ROC analysis revealed a combination of FSH and AFC (0.821) performed better in live birth prediction compared with AMH (0.673), basal FSH (0.534) and age (0.497). OR for women with DOR with the combination were 4.63 (95% CI: 2.75–7.81) in comparison to AMH 3.91 (95% CI: 1.85–4.78) and basal FSH alone (2.31 (95% CI: 1.56–3.11)).
Limitations, reason for caution: As conversion of AMH levels from different immunoassays are highly inaccurate, AMH Gen II assay (Beckman Coulter Ltd) was used which is currently the most reliable assay for AMH. Samples were determined by a single experienced laboratory technician and assay result precision was validated using linearity of dilution assessment.
Wider implications of the findings: The combination of AFC and basal FSH in predicting live birth is better than AMH. It recalls and re-establishes the age-old tool to envisage the occurrence of the ultimate outcome of assisted conception, live birth. Though serum AMH levels tend to be cycle independent, however, the diagnostic accuracy of the same in predicting live birth is poor. However, AMH should be considered as an alternative covariate while counseling couples before undergoing fertility treatment.
Study funding/competing interest(s): Funding by hospital/clinic(s) –The study was funded by our own private infertility centre: Institute of Reproductive Medicine.
Trial registration number: NA.
Keywords: diminished ovarian reserve, anti-Müllerian hormone, antral follicle count

P-736 Age-related normograms of antral follicle count (AFC) – prospective cohort study of 3,677 infertility patients and 3,457 oocyte donors

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Study question: What is the normal age-related decline in AFC in fertile women and does this differ in subfertile women?
Summary answer: The association of log(AFC) with advancing age is linear. The age related decline in AFC is more pronounced in the subfertile population than in women with normal fertility potential.

What is known already: AFC is widely used to assess the fertility potential of individuals and guide stimulation protocols. The lack of technical and methodological standardisation of AFC measurement has limited the development of large population reference ranges. Small single centre studies have reported a linear negative association between age and AFC, however, models have not been validated and whether the same associations apply to fertile women is unknown.
Study design, size, duration: A large prospective study of 7,134 women undergoing AFC assessment on day 2 or 3 of their cycle across 13 fertility centres by 93 doctors from January to December 2013. 3677 out of them underwent investigations for subfertility and the rest (n = 3457) attended the clinics as potential oocyte donors.
Participants/materials, setting, methods: Identical protocol and machine characteristics were used for AFC assessment across the different centres. The infertility cohort was randomly split into training (1729) and validation (1728) dataset; with similar splitting for donors (1839, 1838). Candidate regression models were fitted on the training set and validated on the validation set.
Main results and the role of chance: Oocyte donors had a mean age of 25.4 years (range 18.0–38.0) and a mean BMI of 22.0 kg × m^{-2} (range 16.4–33.8). Infertility patients were older; (mean 36.6 years, range 22–45) and had a higher BMI (mean 23.1 kg × m^{-2}, range 15.8–49.3). Obstetric characteristics differed between the groups, with more termination of pregnancies and term births in oocyte donors. The linear model provided the most adequate fit in both the training and the validation dataset compared to more complicated nonlinear models (quadratic and piecewise linear). The age related decline in AFC was summarised as log(AFC) = 3.313–0.014*(age) for donors and log(AFC) = 4.551–0.063*(age) for infertility patients.
Limitations, reason for caution: The negative association of AFC with age is based on cross-sectional data so the longitudinal application of the model in predicting the decline of AFC in individuals across their reproductive lifespan requires further investigation. Oocyte donors may have been on hormonal contraception at the time of the AFC measurement.
Wider implications of the findings: This is the first internally validated normogram describing the age related decline in AFC with 95% confidence intervals for both fertile and subfertile women. The normograms can be used in other populations with similar baseline characteristics to infer the normal range of AFC for a given age. External validation of the normograms in populations with different characteristics will grant additional validity on the predicted age specific variation in AFC.
Study funding/competing interest(s): Funding by national/international organization(s) – SI is funded by a Joint UK Research Council Grant (G1001357).
Trial registration number: NA.
Keywords: AFC, Age, normogram

P-737 MicroRNA (miRNA) signature in granulosa cells specific to polycystic ovary syndrome (PCOS)

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Study question: The aim of this study is to identify the expression profiles of miRNAs in granulosa cells which is one of the somatic cells of oocyte microenvironment, obtained from PCOS patients compared to normal samples.
Summary answer: As a result of this study 3 miRNAs that have not been previously associated with PCOS were identified.
What is known already: miRNA expression studies in the oocytes and ovaries are still at the very beginning and the roles of miRNAs expressed in oocytes or ovarian somatic cells (theca, granulosa, cumulus), have still not been revealed in reproductive infertility disorders such as PCOS.
Study design, size, duration: In this study, miRNA microarray analysis was conducted with granulosa cells obtained from 14 PCOS patients and 9 healthy controls that are subjected to in vitro fertilization (IVF) procedure.
Participants/materials, setting, methods: Microarray study was performed with Affymetrix miRNA 3.0 array system and data analysis was performed with BRB-ArrayTools to find out differentially expressed (DE) miRNAs. Identification of predicted target genes of DE miRNAs and pathway enrichment analysis was performed by databases. Target genes were validated bioinformatically by an independent microarray study.
Main results and the role of chance: Among the DE miRNAs 2 were found to be upregulated while one of them was found to be downregulated (up to 2.35 fold and -1.9 fold, respectively; $p < 0.05$). 58 of the predicted miRNA target genes like LDLR, TGFB1, PRKACA were validated with an independent microarray study (GSE34526). The validated target genes were found to be enriched significantly in pathways like MAPK, TGF-ß, ovarian steroidogenesis and insulin signaling pathway, which were previously associated with PCOS ($p < 0.019$).
Limitations, reason for caution: The study was performed with human granulosa cells which were isolated from follicular fluid. Follicular fluid was obtained from patients during IVF procedure.
Wider implications of the findings: The validation of the target genes with an independent study increases the reliability of the relationship of these miRNAs with PCOS. The data obtained in this study emphasizes the potential roles of miRNAs in the molecular regulation of oocyte microenvironment in PCOS.
Study funding/competing interest(s): Funding by national/international organization(s) – TUBITAK – The Scientific and Technological Research Council of Turkey.
Trial registration number: NA.
Keywords: microRNA (miRNA), polycystic ovary syndrome (PCOS), granulosa, microarray, in vitro fertilization (IVF)

P-738 Impact of vitamin D levels on ovarian reserve and ovarian response to ovarian controlled hyperstimulation in egg donors

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Study question: Is there any correlation between total and bioavailable serum 25-OH vitamin D, ovarian reserve and ovarian response to controlled stimulation in egg donors. Do they correlate with recipients' pregnancy outcome?
Summary answer: Total and bioavailable vitamin D did not correlate with ovarian reserve and ovarian response to OCH in egg donors. Patients receiving oocytes from vitamin D deficient donors had lower implantation rates, but similar gestational rates. Bioavailable vitamin D did not influence any investigated parameter.
What is known already: There is a global epidemic of vitamin D carency. Studies on its role in human reproduction are controversial. Some demonstrated worse outcome of assisted reproductive technologies in vitamin D non replete patients. A diffuse theory is that vitamin D currency reduces endometrial receptivity. On the contrary we failed to find any correlation of the vitamin and the reproductive outcome in oocytes recipients. Few authors investigated correlations between vitamin D and ovarian function.
Study design, size, duration: Retrospective study on a total of 269 egg donors who were submitted to 310 cycles of ovarian stimulation from June 2013 to April 2014
Participants/materials, setting, methods: 269 egg donors, treated in our University affiliated clinic, we analyzed serum total and bioavailable vitamin D and their relation with antimullerian hormone (AMH), antral follicular count (AFC), number of total and mature oocytes retrieved. We also investigated differences in gestational outcome of their recipients.
Main results and the role of chance: Among all donors, 23 (8.5%) were vitamin D replete (vitamin D >30 ng/ml), 94 (34.9%) had vitamin D insufficiency (20–30 ng/ml) and 152 (56.3%) presented deficiency (<20 ng/ml). AFC and AMH were similar in the three groups. Number of oocytes retrieved, number and rate of mature oocytes and cycle characteristics were comparable in the three groups. Implantation rate was lower in patients receiving oocytes from vitamin D deficient donors compared to replete ones with a difference of -1.843 (P 0.04), while pregnancy rates were independent from donors' total serum vitamin D. Bioavailable vitamin D did not correlate to ovarian reserve and response nor with recipients' reproductive outcome.
Limitations, reason for caution: Being a retrospective study with a limited sample size and the fact that implantation is slightly lower just comparing the group of vitamin D replete and deficient donors with a p value very near to 0.05 ($p = 0.04$), while bioavailable vitamin D has no influence on reproductive outcome
Wider implications of the findings: Donors who are not vitamin D replete nor had a decreased ovarian reserve neither a lower response to COH. Further

studies on patients are necessary to confirm the lack of influence also in a population of infertile women. Lower implantation rate of embryos coming from vitamin D deficient donors could reflect lower oocyte and embryo quality but the etiopathogenetic mechanisms by which they could be affected have still to be investigated. The effect of the supplementation with D vitamin in these patients should be validated in larger trials as well as intervention studies.
Study funding/competing interest(s): Funding by hospital/clinic(s) –IVI Fertility Clinics.
Trial registration number: 1406-MAD-035-JG.
Keywords: egg donors, D vitamin, implantation rate, deficiency, IVF

P-739 High prevalence of vitamin D deficiency in female patients from an Italian assisted reproduction center

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Study question: What is the baseline vitamin D profile of women attending an infertility center and what are its non-dietary determinants?
Summary answer: Vitamin D serum levels are highly deficient in women seeking medical help for couple's infertility and are significantly associated with body composition, seasonal modifications and specific causes of infertility.
What is known already: Vitamin D is known to be involved in many processes of the human reproductive system in both genders. Assisted Reproduction Technology (ART) represents a valuable model to draw inferences on vitamin D deficiency in specific aspects of human fertility as it allows to evaluate separately the various steps of the reproductive process. Negative effects of vitamin D deficiency on clinical pregnancy outcomes have been reported although the debate in this regard is still open.
Study design, size, duration: This was a cross-sectional analysis of a cohort of 1072 women attending a single Italian academic infertility center between January 2011 and December 2013.
Participants/materials, setting, methods: Serum levels of 25-hydroxy-vitamin D [25(OH)D] was analyzed in relation to demographic characteristics, seasons, causes of infertility and selected general health risk factors. Both unadjusted and adjusted levels of serum 25(OH)D were examined. Data for global solar radiation were also correlated with 25(OH)D levels.
Main results and the role of chance: Serum 25(OH)D circulating levels fluctuated according to a seasonal cycle. Median 25(OH)D concentration was below 30 ng/ml for 89% of the entire year. Over the whole year, 6.5% of patients had 25(OH)D levels ≤10 ng/ml, 40.1% ≤20 ng/ml, and 77.4% ≤30 ng/ml. Relative proportions of patients with serum 25(OH)D concentration below all the three cutoff levels analyzed were always significantly higher in the first year trimester compared to the third and fourth trimesters. Global solar radiation was weakly correlated with vitamin D levels. At multivariable analysis, 25(OH)D levels were inversely associated with weight ($p = 0.0003$); conversely, 25(OH)D levels were positively associated with height ($p < 0.0001$) and a history of endometriosis ($p = 0.033$).
Limitations, reason for caution: The cross-sectional design may limit casual inference on the effects of season and infertility causes on vitamin D status. Moreover, our results were derived from a single center in Northern Italy, therefore we are uncertain that our results are generalizable to other infertility centers across the country and in other countries.
Wider implications of the findings: The high prevalence of vitamin D deficiency in infertile women referring to an ART procedure is likely to be mirrored by a similar prevalence during pregnancy. More in general, the demonstration of an advantage of having adequate levels of vitamin D in ART cycles would be strengthened by the potential subsequent advantage also in terms of prevention of obstetrics complications.
Study funding/competing interest(s): Funding by hospital/clinic(s) –IRCCS San Raffaele Scientific Institute, Milan, Italy.
Trial registration number: NA.
Keywords: vitamin D, 25-hydroxyvitamin D, IVF

P-740 Altered expression of homeobox (HOX) family genes in cumulus cells of mature MII oocytes from patients with polycystic ovary syndrome

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Study question: Is there any correlation between altered expression of *Homeobox (HOX)* family genes in cumulus cells (CCs) of mature MII oocytes and polycystic ovary syndrome (PCOS)?
Summary answer: Several members of *HOX* family genes showed significantly differential expression profile in CCs of PCOS patients *vs.* fertile women with male infertility problems. Present alterations may cause the abnormal folliculogenesis and reduce oocyte competence in PCOS patients.
What is known already: Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility which associated with altered gene expression profiles in oocyte and its enclosing cells. Of essential genes for development of Müllerian tract in the embryonic period and adult function are *HOX* family genes. As the important role of CCs in successful folliculogenesis, oocyte maturation, ovulation and fertilization; evaluation of correlation between expression of *HOX* genes and PCOS can represent better insight into mentioned disorder.
Study design, size, duration: This is a case-control laboratory study involving 20 PCOS patients and 20 fertile women with male infertility problems aged 18–36 year old, referred to the Royan Institute to underwent IVF-ICSI with GnRH antagonist protocol between 15 February 2014 and 20 December 2014. Informed consents were obtained from the participants.
Participants/materials, setting, methods: Thirty six hours after hCG injection, ovaries were punctured and cumulus oocyte complexes were dissected. Cumulus cells were collected from 223 and 191 MII oocytes of PCOS and fertile women, respectively. After RNA extraction and cDNA synthesis qRT-PCR was performed using specific primers for *HOXA1-5, HOXB1-5, HOXC4-5* and *HOXD1-4* genes.
Main results and the role of chance: Expression profile of *HOX* family genes of CCs, revealed significant decrease in mRNA levels of *HOXA1*, *HOXC4* and *HOXD4* ($p \leq 0.05$) and significant increase in *HOXA2*, *HOXA3*, *HOXA5*, *HOXB1*, *HOXB2*, *HOXB4* and *HOXB5* ($p \leq 0.05$) in PCOS patients *vs.* control group. There were no significant changes in expression levels of *HOXA4*, *HOXB3*, *HOXC5*, *HOXD1* and *HOXD3* ($p \leq 0.05$) among two studied groups.
Limitations, reason for caution: Owing to the strict selection criteria (the same stimulating protocol, without chromosomal defects, having no special disease and ovarian cautery), and since total number of oocytes for each participant were between 5 and 20 with more than 93% MII, this study included small sample size. Thus, further investigations using a large cohort of participants are needed to confirm these results.
Wider implications of the findings: These findings imply significant correlation between altered expression of *HOX* family genes and PCOS disorder and provides new insights to understand the pathogenesis of PCOS.
Study funding/competing interest(s): Funding by national/international organization(s) – Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Trial registration number: NA.
Keywords: PCOS, HOX family genes, MII oocyte, cumulus cells

P-741 Exercise training reduces acute physiological severity of menopausal hot flushes

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Study question: We aimed to determine whether the acute physiological severity of HFs, measured objectively are responsive to intervention, by comparing the thermoregulatory and (cerebro)vascular changes that occur during HFs following exercise training.
Summary answer: This is the first study to demonstrate that objective physiological responses during HFs are responsive to an exercise intervention. Supervised exercise training reduces the frequency, subjective severity and the acute physiological severity of menopausal HFs in symptomatic females.

What is known already: Menopausal hot flushes (HF) occur due to a reduction in oestrogen causing thermoregulatory and vascular dysfunction. A HF consists of a feeling of intense heat, skin reddening, cutaneous vasodilation (CVC), profuse elevations in sweating, heart rate, and reduced blood pressure and brain blood flow, with a combination of these factors defining HF severity. Exercise training enhances thermoregulatory responsiveness of sweating, CVC and improves brain blood flow that may reduce menopausal HF.
Study design, size, duration: Feasibility study; Seventeen symptomatic postmenopausal females were recruited for this via the Liverpool Women's Hospital and local G.P practices and performed either 16 weeks of supervised exercise training (n = 10, 52 ± 4y, 29 ± 6 kg/m^2) or a no-exercise control intervention (n = 7, 52 ± 6y, 30 ± 7 kg/m^2). Intervention was based on patient choice and not randomly assigned.
Participants/materials, setting, methods: A heat stress (48ºC water-perfused suit) was used to induce HF. Sweat rate, CVC, blood pressure, heart rate, middle-cerebral artery velocity (MCAv) were measured during the HF. HF were objectively identified and divided into eight equal segments for analysis. Females also subjectively rated HF's over 7 days.
Main results and the role of chance: Exercise training decreased HF duration by 63s (95% CI: 14, 113) compared to of 17s (95% CI: -43, 66) following control (P = 0.08). During the HFs sweat rate decreased by 0.04 mg·cm^2·min^{-1}(95% CI: 0.02, 0.06) after exercise training compared to no change in control (P < 0.005). This was accompanied by a reduction in chest CVC of 26 AU (95% CI: 21, 30) following exercise training compared to no change in control (P = 0.01). MCAv was attenuated by 3.4 cm/s (95% CI, 0.7, 5.1) during a HF following exercise training compared to control [0.6 cm/s (95% CI, -0.7, 1.8) P = 0.04]. Exercise training reduced HF frequency [39 (47, 31) vs 5 (16, 6) HF·wk] and severity [101 (121, 80) vs 9 (37, 20) AU] compared to control (P < 0.005).
Limitations, reason for caution: Preliminary evidence, to further establish these positive effects, a RCT that assesses the effects of exercise training compared to a no-exercise control is warranted. Importantly, a RCT would allow for a mediation analysis on the data to highlight the causal pathway(s) for the benefits of exercise training on HF.
Wider implications of the findings: This is the first study to show that the physiological changes that occur during HF are responsive to an intervention. Supervised exercise training reduces the frequency, subjective severity and the acute physiological severity of menopausal HFs in symptomatic females. Exercise training could be implemented at low cost by sympomatic females. Once recommendations about exercise prescription are established, this information can be communicated via clinicians and other health professionals.
Study funding/competing interest(s): Funding by University(ies) –Funding by national/international organization(s).
NHS Liverpool
Liverpool John Moores University
Trial registration number: NA.
Keywords: hot flush, menopause, exercise training

P-742 The contribution of FSH receptor polymorphism on ovarian response and treatment efficiency following stimulation with FE 999049, a recombinant FSH from a human cell-line

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Study question: What is the relation between different FSH receptor (FSH-R) single-nucleotide polymorphisms (SNPs) and ovarian response and gonadotropin use in patients undergoing controlled ovarian stimulation with FE 999049?
Summary answer: The pharmacodynamic parameters of gonadotropin stimulation, including number of oocytes retrieved, were significantly influenced by the daily FE 999049 dose and the patient's anti-Müllerian hormone (AMH). In the AMH range studied, the SNP FSH-R profile did not contribute with additional significant predictive value of ovarian response beyond that already explained by daily FE 999049 dose and AMH. Concerning treatment efficiency, daily FE 999049 dose predicted total dose and duration of stimulation, while neither AMH nor SNP FSH-R profile added significant predictive value.
What is known already: Threonine (Thr) rather than alanine (Ala) at position 307, serine (Ser) rather than asparagine (Asp) at position 680, and an AA genotype in the -29 position in the promotor region have been associated with reduced ovarian response and/or increased exogenous gonadotropin consumption.
Study design, size, duration: Planned analysis of a RCT (Arce et al, Fertil Steril 2014) where 222 IVF/ICSI patients underwent stimulation with FE 999049 at fixed doses of 5.2, 6.9, 8.6, 10.3 or 12.1 mg/day. Randomisation was stratified by serum AMH (low stratum: 5.0–14.9 pmol/L, high stratum: 15.0–44.9 pmol/L; Beckman Coulter Gen2 ELISA).
Participants/materials, setting, methods: Genomic DNA was analysed for SNP at positions -29, 307 and 680 of the FSH-R at the University of Modena and Reggio Emilia, Italy. ANCOVA models were used to determine the predictive value (as expressed by R^2, the proportion of variation explained) on estradiol, inhibin A, inhibin B and follicles at end of stimulation and number of oocytes retrieved as well as total gonadotropin consumption and duration of stimulation. The ANCOVA models for the endocrine parameters included the respective log-transformed baseline values obtained at stimulation day 1. The ANCOVA model for oocytes retrieved included sites. Log(dose) was included as covariate while AMH strata and SNP at positions -29, 307 and 680 were included as factors in these models.
Main results and the role of chance: Distribution of SNP FSH-R combinations: AA 7%, AG 35% and GG 58% for position -29; Thr/Thr 29%, Ala/Thr 54% and Ala/Ala 17% for position 307; and Asn/Asn 30%, Asn/Ser 53% and Ser/Ser 17% for position 680. The distribution for each position and for the overall combinations were not significantly different between the low and high AMH strata.

Frequency of SNP FSH-R (-29,307,680)

A-Thr-Asn/A-Thr-Asn	3%
A-Thr-Asn/A-Ala-Ser	3%
A-Ala-Ser/A-Ala-Ser	1%
A-Thr-Asn/G-Thr-Asn	10%
A-Thr-Asn/G-Ala-Ser	20%
A-Ala-Ser/G-Ala-Ser	5%
G-Thr-Asn/G-Thr-Asn	16%
G-Thr-Asn/G-Ala-Ser	30%
G-Ala-Ser/G-Ala-Ser	11%

Regarding ovarian response, daily FE 999049 dose and AMH were both significant (p < 0.001) predictors of estradiol, inhibin A, inhibin B and follicles at end of stimulation as well as of oocytes retrieved. SNP had no additional significant predictive value on any of these parameters.

ANCOVA for number of oocytes retrieved

	R^2	p
FE 999049 dose	0.208	< 0.001
FE 999049 dose + AMH	0.328	< 0.001
FE 999049 dose + SNP-29	0.217	0.325
FE 999049 dose + SNP307	0.220	0.218
FE 999049 dose + SNP680	0.218	0.286
FE 999049 dose + SNP-29 + SNP307 + SNP680	0.247	0.324

Regarding treatment efficiency, daily FE 999049 dose was a significant predictor of total dose (R^2 = 0.564, p < 0.001) and duration of stimulation (R^2 = 0.215, p < 0.001) with no additional significant predictive value of AMH or SNP on these parameters.
Limitations, reason for caution: The present investigation is applicable for FE 999049, a recombinant FSH derived from a human cell-line. The trial included mainly Caucasians and was limited to patients within a specified AMH range. The analysis was restricted to three positions on the FSH-R.
Wider implications of the findings: In this investigation, FSH-R SNP had no relevance beyond daily gonadotropin dose and AMH in predicting ovarian response and gonadotropin efficiency in patients undergoing controlled ovarian stimulation with FE 999049. As FSH-R SNP as a stand-alone parameter has previously been associated with ovarian response and treatment efficiency, it should be considered to include daily gonadotropin dose and AMH in future analyses as well as to investigate different gonadotropin preparations to provide further clarity on the role of FSH-R SNP.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Ferring Pharmaceuticals.
Trial registration number: NCT01426386.
Keywords: single-nucleotide polymorphism, FSH receptor gene, AMH, FE 999049, ovarian response

P-743 To evaluate which factors determine the incidence of extremely elevated progesterone on the day of HCG administration

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Study question: To investigate which factors caused the extreme progesterone (P4) elevation on the day of human chorionic gonadotropin (hCG) administration.
Summary answer: The most strong factor related with the extremely elevated P4 concentration on the day of human chorionic gonadotropin (hCG) administration is the choice of the GnRH agonist protocol(odd ratio:2.786).
What is known already: Extremely high P4 concentrations on the day of hCG administration has an obvious negative impact on pregnancy outcome and the live birth rate. The variable factors which would increase the incidence of extreme progesterone elevation on the day of HCG administration are still not clear.
Study design, size, duration: This is a retrospective observational, single-center cohort study. The data was collected from infertile couples who underwent 2,000 fresh IVF and/or intracytoplasmic sperm injection (ICSI) embryo transfer cycles from January 2000 to December 2014 in our institution
Participants/materials, setting, methods: This retrospective observational, single-center cohort study was based on the medical records of infertile couples undergoing IVF treatment. To further analyze the association between variable factors involved in extreme progesterone elevation on the day of HCG administration, multivariate logistic regression was performed.
Main results and the role of chance: When all cycles were divided into those with P4 < 1.94 ng/ml(*n*:1791) and 1.94 ng/mL(*n*:209) on the day of hCG administration, we found no statistically significant difference between the groups in female age, BMI, body height, primary or secondary infertility, etiology of infertility, LH used or not, and the number of oocytes retrieved. Only five factors: protocol choice (GnRH agonist or GnRH antagonist), the number of dominant follicle, LH values on the day of hCG administration, total FSH dosage, and E2 on day of hCG administration were positively associated with extremely elevated P4 concentration(odd ratio:2.786,1.098,1.085,1.023,1.001, $p < 0.001$ for all). After omitting the antagonist cycles, no factors were statistically significantly associated with extremely elevated P4 concentration in GnRH agonist group.
Limitations, reason for caution: This present study is retrospective cohort study
Wider implications of the findings: The total FSH dosage, luteinizing hormone values on the day of hCG administration, estradiol values on the day of hCG administration, and the number of dominant follicles (>1.6 cm) could only slightly increase the incidence of extremely elevated progesterone on the day of HCG administration in this present study. Clinicians should consider shifting the GnRH agonist protocol to GnRH antagonist protocol in women who had previous cycle associated with extremely elevated P4 concentrations.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital.
Trial registration number: This is not an RCT.
Keywords: extremely elevated progesterone, GnRH agonist

P-744 The effect of LH-activity components contained in HMG on the clinical outcomes during progestin-primed ovarian stimulation in normalovulatory women undergoing IVF/ICSI treatments compared with u-FSH

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Study question: To evaluate the effect of LH-activity components(LH and HCG) contained in HMG preparations on the clinical outcomes of normalovulatory women undergoing IVF/ICSI treatments in the progestin-primed ovarian stimulation(PPOS)protocol in terms of the hormone profile, embryo results and pregnant outcomes after frozen embryo transfer(FET)compared with urinary FSH
Summary answer: There were no significant correlations between the concentration of serum LH, serum b-hCG, and urinary b-hCG on the trigger day and embryological characteristics which indicated LH-activity components contained in HMG preparations had no influence on the clinical outcomes during PPOS
What is known already: Premature LH surges can be inhibited by natural progesterone secreted by corpus luteum or medroxyprogesterone acetate(MPA)during ovarian stimulation without down-regulation in normalovulatory women, which were concluded both in the follicular-phase and luteal-phase ovarian stimulation in combination with' freeze-all' strategy. It was still a controversy whether LH-activity components contained in HMG preparations affected the clinical results in down-regulation protocol, while no relevant investigations were reported in PPOS
Study design, size, duration: 180 patients were recruited from September to November in 2014 in this prospective controlled cohort study and allocated into three groups according to the gonadtropin used: group A, u-hMG-A(containing 16.77IU HCG per 75 IU HMG); group B, u-HMG-B(containing 7.86IU HCG per 75 IU HMG);group C, u-FSH(containing only FSH)
Participants/materials, setting, methods: Normalovulatory patients with 25–40 years old were eligible to participate. MPA 10mg/d and Gn 225IU were administered from cycle day 3. When the dominant follicles reached mature, GnRH-a 0.1mg and HCG 1000IU were used for trigger. The clinical results were compared in terms of the types of Gn, levels of β-hCG and LH.
Main results and the role of chance: The number of oocytes retrieved in group A, B, C was 10.72 ± 5.78, 11.33 ± 5.19 and 13.38 ± 8.97, respectively, with no statistic significance ($p > 0.05$). Other indicators such as the number of mature oocyte, fertilization, cleavage and viable embryos were similar ($p > 0.05$). The LH level on the trigger day ranged from 0.1 mIU/ml to 6.44 mIU/ml, with no premature LH surges detected, have no relation with embryo results ($p > 0.05$). The concentration of serum and urinary β-hCG on the trigger day were higher in group A than group B (3.12 ± 1.77 mIU/ml vs 1.9 ± 0.73 mIU/ml, 6.76 ± 5.05 mIU/ml vs 4.43 ± 3.01 mIU/ml, $p < 0.05$), which were not associated with embryo results ($p > 0.05$). There were no significant difference in the clinical pregnancy rate after FET among the three groups (41.46% vs 51.43% vs 41.46%, $p > 0.05$)
Limitations, reason for caution: The sample size is limited as a preliminary trial, therefore, a well designed, adequately powered, randomized, controlled trial should be undertaken to verify these findings
Wider implications of the findings: Our study concluded the clinical characteristics were not affected by the LH-activity components contained in HMG in normalovulatory women undergoing PPOS. The hormone indicators in urine were introduced for the first time in this study to reflect the state of Gn metabolism in different individuals. Further studies will be performed to optimize and individualize the selection of Gn undergoing PPOS in different populations with advanced age, poor responders or polycystic ovarian syndrome (PCOS).
Study funding/competing interest(s): Funding by national/international organization(s) – This study was funded by the Natural Science Foundation of Shanghai (grant number: 14411964300).
Trial registration number: The trial was registered with the Chinese Clinical Trial Registry (ChiCTR-OPN-14005276).
Keywords: urinary HMG, human chorionic gonadotropin, luteinizing hormone, progestin-primed ovarian stimulation, frozen embryo transfer

REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-745 A prospective randomized controlled study (RCT) depicting favourable IVF-ICSI outcomes following anti-tubercular treatment on the sole basis of abnormal hysteroscopic findings

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Study question: Does anti-tubercular treatment (ATT) on the sole basis of abnormal hysteroscopic findings yield better IVF-ICSI outcome in developing countries like India with a very high prevalence of Tuberculosis disease?

Summary answer: Yes, infertile women given anti-tubercular treatment (ATT) for abnormal hysteroscopic findings suggestive of tubercular mycobacterial infection had better IVF-ICSI outcome.
What is known already: Genital Tuberculosis is a major cause of infertility in developing countries like India. Prompt anti-tubercular treatment (ATT) can dramatically improve female reproductive function. *The prevalence of the* disease is steadily increasing and has emerged in previously unrecognized populations. The diagnosis is often difficult or unconvincing.
Study design, size, duration: 682 infertile patients below 42 years were evaluated by laparoscopy and hysteroscopy between July 2006 and December 2014. Out of these, 138 patients having abnormal hysteroscopic findings were prospectively randomised into Anti-Tuberculosis treatment and non-treatment groups by a computer generated list, before undergoing IVF-ICSI cycles.
Participants/materials, setting, methods: This was an intervention study done on 138 positive abnormal hysteroscopic women who were randomized into two groups, of 58 patients each, one receiving standard anti-tubercular treatment and the other receiving no treatment before IVF/ICSI cycles. Out of 138 patients, 22 were excluded from the study either because of failure to comply to drug therapy, or other reasons. The primary outcome measured was the implantation rate and cumulative pregnancy rate following IVF-ICSI, and the secondary outcome was the miscarriage rate.
Main results and the role of chance: There was a statistically significant difference between the two groups, the cumulative pregnancy rate being higher following IVF-ICSI in the group who took prior anti tubercular treatment (39% (23/58) versus 21% (12/58)). Although the miscarriage rate was lower in the treatment group, it was not statically significant (15% (3/20) versus 17% (2/12)).
Limitations, reason for caution: The presence of mycobacterial DNA (TB-PCR) is considered as more sensitive for TB than a positive TB smear for diagnosis and treatment TB (Menzies et al., 2011). Further, there are some limitations of the PCR test itself such as the contamination leading to false positive and the presence of inhibitory substance to false-negative tests (Laifer et al., 2004). We also could not include the study of other markers of infertility in the endometrium which would suggest the TB involvement. The group of women in this study had a high clinical probability of TB in our settings after exclusion of other causes of infertility.
Wider implications of the findings: Indication for the treatment of tuberculosis mycobacterial infections is not easy to define due to difficulty in obtaining appropriate tissue samples for mycobacterial smear and culture positivity. In resource poor developing countries where IVF-ICSI cycles are self-funded and difficult to afford, prior treatment of abnormal hysteroscopic findings of tuberculosis mycobacterial infection cases with no other demonstrable cause improves the IVF-ICSI results.
Study funding/competing interest(s): Funding by hospital/clinic(s) –BTTB CENTRE.
Trial registration number: BTTBC/2006/07.
Keywords: genital-tuberculosis, abnormal-positive-hysteroscoy, antitubercular-treatment(ATT), IVF-ICSI, pregnancy-rate

P-746 Frozen-thawed embryo transfer (FET) after elective single embryo transfer is cost-effective for up to two FET cycles

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Study question: What is the impact of consecutive frozen-thawed embryo transfer (FET) cycles on cumulative live birth rate (cLBR) after elective single embryo transfer (eSET) in the fresh cycle?
Summary answer: FET is cost-effective and cLBR increases significantly until the second FET, but if the treatment has resulted in a live birth, all remaining embryos should be transferred.
What is known already: eSET in the fresh IVF or ICSI cycle minimizes the incidence of multiple pregnancies. Usually, one or more supernumerary good quality embryos are frozen and transferred in FET cycles, but the impact of the number of FET following the fresh cycle on cLBR and costs has not been investigated.
Study design, size, duration: This cost-effectiveness analysis examined consecutive treatment cycles performed during January 2000–June 2013 in three infertility clinics. Total number of collected cycles was 23826, from which we analyzed 2771 first eSET cycles (January 2000–June 2011) followed by 4230 FET cycles (January 2000–June 2013).
Participants/materials, setting, methods: In each FET, the best available embryo container was thawed. cLBR/stimulation was calculated after one, two and ≥3 FET. Cycles were analyzed until the second live birth. Incremental cost-effectiveness ratios (ICERs) for change in cost/change in live births used our previously published data on fresh and FET cycle costs.
Main results and the role of chance: cLBR (first birth) increased from 46.8% (1296/2771) after the first FET to 51.0% (1413/2771, $P < 0.0001$) after the second FET and to 53.4% (1480/2771, $P > 0.2$) after ≥3 FETs. After the first birth, 683 couples (46.2%) continued treatment and 30.2% (206/683) had a second birth. 22% of these (46/206) occurred after the third consecutive FET. A live birth cost 8954 in the fresh cycles and 3393 in FET. ICER of a first birth was lowest after the first FET vs. fresh cycles (2646 /birth), gradually increasing to 3217 /birth after ≥3 FETs. The second birth vs. first had higher FET costs (1975 € /birth). However, total costs of the second birth were much lower than those of the first birth with ICER 6975 € /birth.
Limitations, reason for caution: About 8% of women would have needed more time to complete all possible FET cycles. Total duration of the study was long, but there was no trend in yearly fluctuations of cLBR during the study period (42.3–58.2%, linear-by-linear association $P = 0.1$).
Wider implications of the findings: Results are important for health policy makers. Clinicians and patients should be aware that most live births (93.9%) occurred in the fresh and up to two FET cycles. However, 22% of second births occurred after the second FET. Since second births are associated with low costs, the use of FET should not be limited after the first birth. If no live birth has occurred after the second FET, a new stimulation might be considered.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s), Funding by national/international organization(s) – University of Helsinki, Helsinki University Hospital, the Sigrid Juselius Foundation, Academy of Finland, University of Oulu, Oulu University Hospital.
Trial registration number: NA.
Keywords: elective single embryo transfer, frozen-thawed embryo transfer, live birth, cost-effectiveness

P-747 Natural fertility and outcomes of assisted reproductive technologies in different ethnicities

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Study question: Are natural fertility and in vitro fertilization (IVF) outcomes related to patient's ethnicity?
Summary answer: There is a correlation between reproductive potential and ethnicity, both in spontaneous fertility than in assisted reproductive treatments, in terms of spontaneous pregnancy rate, sterility duration, causes of sterility and IVF outcomes.
What is known already: Ethnicity widely influences reproduction because it is associated with different economic, cultural and genetic characteristics that correlate with reproductive performance and onset of reproductive pathologies. Puberal onset, menopausal age, hormonal levels, prevalence of polycystic ovary syndrome, endometriosis and myomas are related to ethnicity. Some studies underline better in vitro fertilization outcomes in Caucasian women. Unfortunately, in literature there are only a few studies and most of the them include small cohorts of subjects.
Study design, size, duration: This is a cohort study. 4798 patients (86.8% Italian; 1.9% North European, 5.1% East European; 0.1% North American, 1.3% South American, 2.5% African; 2.3% Asian) who underwent in vitro fertilization between 2004 and 2014 were included in the study.
Participants/materials, setting, methods: The different ethnicity groups have been compared in terms of hormonal values, duration and reasons for sterility, in vitro fertilization outcomes (number of oocytes retrieved, ovarian hyperstimulation rate, embryos obtained, implantation rate, pregnancy rate, miscarriages rate).

Main results and the role of chance: The main differences among the ethnicities concern sterility duration, sterility causes, rate of oocyte cryopreservation, embryo quality and pregnancy rate. Endometriosis and polycystic ovarian syndrome are more frequent in Asian women, tubal factor is the main cause of infertility in East European women. Rate of cycles with cryopreservation of supernumerary oocytes is significantly higher in Asian women than in African women (37% vs 16% of all retrievals). Embryo quality resulted better in North European women (68.5% of grade 1–2 embryos), whereas the rate of good quality embryos was low in African women (42.6% of grade 1–2 embryos transferred).
Limitations, reason for caution: The population of the study is not equally divided among the different ethnicities, because the Italian group is much more numerous than the others. Moreover, the groups are very heterogeneous because each one includes several subgroups (for example Chinese, Japanese, Indian in the Asian group).
Wider implications of the findings: The evaluation of fertility in different ethnicities is particularly significant due to the surge in worldwide immigration. The differences in fertility's characteristics and IVF results, observed among the ethnicities, permit to personalize the medical approach in terms of prevention of fertility related diseases, diagnostic path and treatment. Furthermore, these data are helpful in improving personalized patients' counselling about the expected results.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) –University of Bologna. Sant'Orsola Malpighi University Hospital.
Trial registration number: NA.
Keywords: ethnicity, IVF, fertility counselling

P-748 Knowledge and attitudes regarding fertility and assisted reproductive technology in Portuguese childbearing population

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Study question: How good is the fertility knowledge and what are the attitudes regarding Assisted Reproductive Technology (ART) in a Portuguese sample of men and women in reproductive age and without children?
Summary answer: The Portuguese childbearing population had a poor knowledge about fertility and ART. They would like to have two children in the future. The marital and financial stability and the family support are perceived as the most important factors influencing the decision to have children.
What is known already: Human fertility is threatened by several factors, such as the postponing of the first pregnancy. Young adults should be aware of risk factors so they can make high quality reproductive decisions. However, research reveals that young individuals have little knowledge about their fertility and about what to do in face of reproductive problems. There is no study about the knowledge and attitudes regarding fertility and ART in a Portuguese sample.
Study design, size, duration: It was developed a cross-sectional epidemiological study over a 5-month period, between August and December of 2014. It was performed a face-to-face, home-based random-route questionnaire in the 5 regions of Continental Portugal. This was a national representative sample.
Participants/materials, setting, methods: Participants were 2403 individuals (1595 women); median age 33 years old. 28% of the participants were higher educated and 68% were involved in an intimate relationship. The inclusion criteria were the age between 18 and 45 years old and the absence of children. Fertility and ART knowledge and attitudes were assessed.
Main results and the role of chance: Participants revealed poor knowledge about (in)fertility. Artificial insemination was the best-known treatment by this sample and 19% of the women have already heard about fertility preservation techniques. Female participants referred that the ideal age to start having children is 28 years old, to a maximum age of 40 years old. In terms of having children in the future, participants perceived the marital stability, the family support and the financial stability as the most important factors influencing this decision. Most of the individuals (42%) plan to have two children in the future and 27% of the individuals revealed that do not pretend to have any child.
Limitations, reason for caution: It is important to take into account the possible presence of a social desirability bias in this study. This bias means that the sample could have answered in a manner favorably perceived by the researchers.
Wider implications of the findings: Educational interventions to general population should be implemented to improve knowledge about fertility and ART. Moreover, primary care healthcare professionals should discuss fertility and reproductive plans with their young patients. Future studies should try to understand how knowledge and attitudes about ART affect reproductive decisions.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) –Merck Portugal S.A.
Trial registration number: NA.
Keywords: childbearing intentions, attitudes towards assisted reproduction, Portugal

P-749 Effect of ethnicity on live birth rates after IVF/ICSI treatment: analysis of 60,955 treatment cycles from a national database

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Study question: To evaluate the effect of ethnicity of women on the clinical outcome of In-Vitro Fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment.
Summary answer: Live birth rates following IVF or ICSI treatment were significantly lower in some of the ethnic minority groups (White Irish, Indian, Bangladeshi, Pakistani and Black African) compared with white British women, which suggests that ethnicity is a major determinant of live birth following IVF or ICSI treatment.
What is known already: Despite the rapid advancement in the IVF field, ethnic disparities has attracted limited attention unlike other areas in medicine where ethnicity is a primary prognostic consideration. In the literature, a scarce number of reports were published on the relationship between ethnic background and IVF outcome.
Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of IVF and ICSI treatment in the UK. Data from 2000 to 2010 involving 60,955 treatment cycles from 38,709 women were analysed.
Participants/materials, setting, methods: Data on all women undergoing their first stimulated fresh IVF treatment cycle during the period from 2000 to 2010 were analysed for live birth rate per cycle and cumulative live birth rate. Data analysed after adjusting for age, cause and type of infertility and treatment type (IVF or ICSI) to express results as odds ratio and 95% confidence intervals.
Main results and the role of chance: While white Irish (0.73; 0.60–0.90), Indian (0.85; 0.75–0.97), Bangladeshi (0.53: 0.33–0.85), Pakistani (0.68; 0.58–0.80), Black African (0.60; 0.51–0.72), and other non-Caucasian Asian (0.86; 0.73–0.99) had a significantly lower odds of liver birth rates per fresh IVF/ICSI cycle than White British women, ethnic groups of White European (1.04; 0.96–1.13), Chinese (1.12; 0.77–1.64), Black Caribbean (0.76; 0.51–1.13), Middle Eastern (0.73; 0.51–1.04), Mediterranean European (1.18; 0.83–1.70) and Mixed race population (0.94; 0.73–1.19) had equivalent live birth rates. The cumulative live birth rates were also showed similar pattern across different ethnic groups.
Limitations, reason for caution: Controlling for confounders like women's BMI, smoking status, treatment protocol and gonadotrophin dose could not be done because these data were not available.
Wider implications of the findings: Ethnicity should be considered while counseling women and couples about their realistic chances of IVF/ICSI success. Further research is needed to understand the reasons behind the variation in treatment outcome between ethnic groups and towards tailoring the protocol to maximize their IVF/ICSI success.
Study funding/competing interest(s): Funding by University(ies), Funding by hospital/clinic(s) –Nottingham University Hospitals.
Trial registration number: 12/EM/0202.
Keywords: ethnicity, IVF, ICSI, live birth rate, assisted conception

P-750 Relationship between pollutants concentration in ambiance and lunar phases with pregnancy rate during IVF cycles

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Study question: Increasing concentrations in pollutants as nitrogen dioxide (NO_2) and ozone (O_3) and changes in percent of the moon illuminated (PMI) from oocyte retrieval (OR) to embryo transfer (ET) day could affect pregnancy rate (PR) in women undergoing an IVF cycle.
Summary answer: Increasing concentrations in NO_2 is associated with a lower chance of pregnancy from oocyte retrieval to embryo transfer. Changes in O_3 concentration and in lunar phases did not affect the outcome of the IVF cycle.
What is known already: Most papers link chemical contaminants and spontaneous pregnancy but there are few bibliography that relate pollutants with IVF outcome; some observed a relationship between a higher level of NO_2 with lower pregnancy and birth rates in all phases of an IVF cycle. Others studies showed that an increase in fine particle matter ($PM_{2.5}$) and particular matters (PM_{10}) were associated with a drop in pregnancy rate.
Study design, size, duration: Retrospective study. *Size:* 363 women undergoing an IVF cycle with ET in day2. *Period:* January 2013–July 2014. Climatological and pollutants factors were collected on a daily basis by the Air Quality Network of Castilla-LaMancha. We compared data relating to lunar phases and contaminants in order to ascertain how they affect pregnancy rate.
Participants/materials, setting, methods: Mean concentration of pollutants(MCP)was calculated during the culture period (NO_2 = 12.16mg/m^3 and O_3 = 78.46mg/m^3). Full moon was considered as PMI >75%. Chance of pregnancy was analyzed depending on MCP and PMI since OR to ET. Chi square Fisher's test and multivariable binary logistic regression analysis was performed to determinate the variables related to pregnancy rate
Main results and the role of chance: We found a statistically significant increase of pregnancy rate when NO_2 concentration in the environment during the embryo culture period was lower than the mean concentration (n = 90) (OR: 2.02, CI 95%: 1.21–3.38. p = 0.048). No significant differences were found in pregnancy rate when O_3 was below the mean concentration (n = 100) (OR: 0.75 CI 95%: 0.46–1.24 p = 0.265). On the other hand, if PMI was above 75% (n = 99) a marginally significant effect on pregnancy rate was observed (OR: 0.65, CI 95%: 0.41–1.03 p = 0.068). Consequently, we could say that NO_2concentration has a deleterious effect on pregnancy rates in women undergoing IVF. These results also suggest a possible link between lunar phases and pregnancy rate.
Limitations, reason for caution: We are measuring the only period in which embryos and mother are separated, so we could hypothesize that the toxic effect would be lower. However, not all laboratories are equipped with good air filtration system. Other limitation is the limited period of time. We could extend the study to stimulation and implantation period
Wider implications of the findings: The impact of exposure to environmental contaminants on human fertility remains controversial. Some studies show a relationship between $PM_{10}$and a drop in spontaneous fertility. In agreement with Legro et al. (2010), we found a persistent negative association between pregnancy and NO_2 concentration. The most prominent sources of NO_2 are internal combustion engines like those used in motor vehicles. Further studies are necessary but these results highlight the fact that pollution is in someway affecting human fertility.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Universitary General Hospital from Albacete.
Trial registration number: NA.
Keywords: pollution, IVF, contamination, lunar phases, pregnancy rate

P-751 Publication bias in the field of subfertility

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Study question: Is the statistical significance of the results from subfertility randomised controlled trials (RCTs) that have been presented at conferences associated with the probability of publication of these RCTs as full-text articles? A preliminary report.
Summary answer: This study found no evidence to suggest that subfertility RCTs of conference abstracts that reported statistically significant (SS) results were more or less likely to be published than studies that did not report SS results.
What is known already: Publication bias due to the failure to publish study results based on the direction or strength of study findings can lead to a misleading interpretation about the effectiveness of an intervention and ultimately impact patient care. Publication bias has been detected in many clinical areas including subfertility. This study aimed to investigate

- whether publication bias exists in a recent cohort of conference abstracts, and
- associations of other factors with the probability of full-text publication.

Study design, size, duration: Eligible abstracts were subfertility RCTs reporting ≥1 reproductive outcome. The Cochrane Menstrual Disorders and Subfertility Group specialised register (MDSGSR) was searched for abstracts presented between 01/01/2007 and 31/12/2010. A search was performed to identify full-text publications in MDSGSR, Pubmed, Medline, Embase, and Cinahl December 2014 to February 2015 (ongoing).
Participants/materials, setting, methods: Two authors independently screened abstracts for eligibility and extracted data. One author searched for full-text publications not found by the other author. Odds ratios (OR) and 95% confidence intervals (CI) will be calculated with the use of multiple regression to identify additional factors independently associated with probability of publication.
Main results and the role of chance: As at January 2015, 229 articles were eligible from a total of 337 retrieved. Preliminary analysis indicates that: 37% of abstracts were oral presentations, <1% were stated as registered, 3% were stated as interim or preliminary analyses, 11% acknowledged industry funding, while the source of funding was not reported in 68% of studies. Overall, 50% of the abstracts were found to be published as full-text articles. There was no SS difference between likelihood of publication of abstracts reporting SS results compared to abstracts reporting non-SS results (OR = 1.16 95% CI 0.66–2.02). Of studies not reporting SS results, 13% made a positive statement about the findings, for example describing a trend towards improved outcomes.
Limitations, reason for caution: The included RCTs were only those captured by the MDSGSR and which investigated interventions for subfertility. To assess the true extent of publication bias a more comprehensive survey might be conducted using a cohort of RCTs from trial registers. Further, these results are interim analyses only.
Wider implications of the findings: Publication bias is a problem for evidence based health care, and the presence of this bias has been detected across many clinical areas. The absence of significant publication bias in subfertility, if confirmed, will enhance our confidence in the results of published systematic reviews of evidence in this field.
Study funding/competing interest(s): Funding by University(ies) – University of Auckland.
Trial registration number: NA.
Keywords: publication bias, publication deficit, subfertility, abstract publication

P-752 Retrospective six year analysis of ART directory of India

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Study question: How have ART practices and outcomes changed in India over the last six years.
Summary answer: Over the last six years there has been a progressive increase in the number of ART clinics offering ART services including surrogacy. Most common indication for ICSI being male factor infertility. Long protocol still remains most preferred but increase in antagonist cycles is noted.
What is known already: World data on the availability, safety and effectiveness of ART have been published since 1989. The number of embryos transferred is a major determinant of the increase in multiple pregnancies.

Study design, size, duration: : Retrospective analysis of data over last 6 years (2007–12) from all IVF centres in India registered with ISAR (Indian Society of Assisted Reproduction)
Participants/materials, setting, methods: Data from total of 139 registered centres regarding number of ART clinics, types of cycles and procedures, pregnancy, delivery and multiple birth rates.
Main results and the role of chance: The number of reporting centres increased from 113 in 2007 to 139 in 2012. Total 30,270 IVF and ICSI cycles were performed in 2012. Maximum number of centres 69% performed <50 cycles in a year with 3.5% performing >500 cycles a year. Long luteal was the preferred protocol in all years with increase in the number of antagonist cycles. Number of single embryo transfer cycles remains constant around 11–13% but still around 14% cycles had 4 embryos transferred. The overall pregnancy rate was 38.5% and multiple pregnancy rate 21% for 2010 and 41.6% with 22 % multiples in 2012. The overall pregnancy rate for FET cycles egg donation and embryo donation cycles was around 35.6%, 43.5%, and 47.6%, respectively.
Limitations, reason for caution: Still many centres in India are not registered with any body and not reporting their results. There is need for a central regulatory body to monitor the working and practices of the various IVF centres.
Wider implications of the findings: To compare the ART practice and outcomes with international Data. To assess need for guidelines regarding embryo transfer policy and encourage more transparency amongst centres.
Study funding/competing interest(s): Funding by national/international organization(s) – Indian society of assisted reproduction
Trial registration number: NA.
Keywords: ART directory India, NARI

P-753 Pre-treatment serum vitamin D levels and fertilization rates among women undergoing assisted reproduction

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Study question: Is there an association between levels of 25-hydroxyvitamin D (25OH-D) and fertilization rate among women undergoing assisted reproduction (ART)?
Summary answer: Serum 25OH-D levels were positively related to fertilization rate and this relation was stronger in conventional insemination cycles than in intracytoplasmic sperm injection (ICSI) cycles.
What is known already: In female rats, vitamin D deficiency results in decreased litter size and impaired neonatal growth. Human data is inconclusive; some studies have found that women undergoing ART with higher serum levels of vitamin D significantly more likely to achieve implantation and clinical pregnancy while other studies have failed to document a benefit.
Study design, size, duration: 100 women (contributing 158 ART cycles) randomly sampled from participants of an ongoing prospective cohort study (EARTH study) of couples recruited from a Fertility Center in a university hospital between 2007 and 2013.
Participants/materials, setting, methods: Pre ART treatment serum levels of 25OH-D were measured by immunoassay. Generalized linear mixed models with random intercepts were used to evaluate the association of serum 25OH-D with fertilization rate accounting for repeated treatment cycles and adjusting for age, BMI, infertility diagnosis, protocol, race and dietary factors.
Main results and the role of chance: The median serum 25OH-D levels were 87.5[58.3–119.3] ng/mL. Vitamin D levels were positively related to fertilization rate. The adjusted fertilization rates (95% CI) for women with increased quartiles of serum 25OH-D were 0.68 (0.58–0.76), 0.60 (0.50–0.69), 0.71 (0.62–0.79) and 0.78 (0.70–0.84) (p, linear trend = 0.01). This relation was stronger in conventional insemination cycles than in ICSI cycles. Specifically, the adjusted fertilization rates (95% CI) in increasing quartiles of 25OH-D were 0.65 (0.52–0.78), 0.51 (0.37–0.64), 0.74 (0.66–0.81) and 0.83 (0.72–0.90) among women undergoing conventional insemination cycles (p, linear trend = 0.005), and 0.74 (0.61–0.83), 0.64 (0.47–0.78), 0.62 (0.42–0.79) and 0.79 (0.66–0.88) among women undergoing ICSI cycles (p, linear trend = 0.22).
Limitations, reason for caution: None of the women included in this analysis were vitamin D deficient. In addition, residual confounding cannot be ruled out.
Wider implications of the findings: If these findings are replicated in randomized control trials, vitamin D supplementation could be offered as an inexpensive adjuvant to infertility treatment.
Study funding/competing interest(s): Funding by national/international organization(s) – National Institutes of Health (NIH) grants and the Early Life Nutrition Fund from Danone Nutricia US.
Trial registration number: NA.
Keywords: vitamin D, reproductive techniques, assisted

P-754 Patients experience of viewing time-lapse sequences, a prospective survey study

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Study question: How do patients value the chance to view time-lapse sequences of their own embryos during treatment?
Summary answer: Patients found viewing the sequences to be a relevant and important aspect of their treatments. There was a clear interest in obtaining a copy of the time-lapse sequence but only if the treatment resulted in a pregnancy.
What is known already: Studies are lacking on patients experience of using time-lapse monitoring in ART. We consider it to be highly important to study the patient-oriented aspects of this new technology which currently is spreading rapidly and is already considered an important aspect of treatment in many centers internationally.
Study design, size, duration: This prospective, observational questionnaire study was carried out at three of IVF Sweden`s centers in Sweden. We report the results from the first 163 self evaluated scores on patient´s experience reported anonymously.
Participants/materials, setting, methods: Participants in the study are patients who are offered to view time-lapse sequences of embryos selected for transfer at privately funded ART clinics. After the embryo transfer, patients were asked to fill in a questionnaire where a 5-grade Likert-scale instrument was used for assessing patient's experience.
Main results and the role of chance: Patients found the offer to view the time-lapse sequences to give important and relevant information regarding their treatment (average score 4.5) and felt that this gave them increased participation in the treatment (4.7). Extremely few patients preferred not to know the details of the development of their own embryos (1.1). A modestly positive attitude towards extra payment for time-lapse monitoring was expressed in the study (3.2) but viewing the sequences increased satisfaction with the treatment (3.6). Patients wish to obtain a copy of the time-lapse sequence, but only if a pregnancy is achieved (2.3). The results from the study are collected from a large number of patients and in three different private ART clinics and do not vary between clinics.
Limitations, reason for caution: This may be considered a pilot study as it is the first to assess patients experience of viewing time lapse sequences. Further studies are required to analyze for example how closely patients identify with the embryos before implantation.
Wider implications of the findings: Embryologists and clinicians may consider whether the positive expectations of an ART treatment may lead to feelings of regret and sorrow if the treatment is unsuccessful. The results do not indicate that viewing time-lapse-sequences specifically adds extra stress in this aspect. Although time-lapse technology is a useful innovation in the field of ART, it is important not to forget the individuals behind the gametes and embryos being cultured in the ART laboratories.
Study funding/competing interest(s): Funding by hospital/clinic(s) – IVF Sweden – The authors have no financial interests to declare.
Trial registration number: The regional ethics committee at Lund University considered the study to lie outside the boundaries of ethical vetting because of the non-invasive and anonymous nature of the study.
Keywords: time-lapse, patient experience

P-755 Sequential awareness surveys on clinical application of uterine transplantation among Japanese general public

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Study question: Uterine transplantation (UTx) has been applied clinically in some countries recently, and the first childbirth was obtained in Sweden in 2014. However, evaluation of social consensus is not enough yet. To address this point, sequential awareness surveys were performed among Japanese citizen by gathering public comments for the new technology.
Summary answer: Although UTx seems to be acceptable for Japanese general peoples, the awareness for the reproductive technology has not markedly changed even after successful childbirths with UTx has been reported. These results may indicate that more information concerning UTx procedure should be opened and spread to public.
What is known already: It has been proven that even the patients with absolute uterine factor infertility are able to be pregnant and give birth by themselves, and many studies related to experimental and clinical UTx technologies have been reported recently. However, no analyses of public awareness on clinical application of UTx have been available. Moreover, no sequential surveys were performed though the social intensions for third party reproduction are always changing.
Study design, size, duration: Cross-sectional surveys were conducted on Japanese general population aged from 20 to 39 years. The infertile persons were excluded. The first and second surveys were performed on June 2012 and December 2014, respectively. Answers were obtained from 300 females and 300 males at each survey.
Participants/materials, setting, methods: The age of the participants (mean ± SD) was 30.1 ± 5.6 years old. In them, 33.5% in the females and 22.0% in the males had children. Self-reported questionnaire consisting of ethical, social and clinical aspects on UTx and gestational surrogacy was used through the Internet.
Main results and the role of chance: More than 3/4 (79.3% of the females and 76.3% of the males) of the peoples permitted the clinical UTx application morally, and about 40% of peoples (44.7% in females and 36.0% in males) showed interest in the UTx. Both 22.7% of the females and 19.9% of the males hoped for UT treatment if she or his partner has lost uterus. These rates were greater than those of surrogacy, however, the answer 'unknown' was occupied more than 50%. As uterine donor candidates, physical woman with gender identity disorder who would receive sex reassignment surgery was selected equally to cadaver and her or his partner's mother/sister. No significant differences in these ratios were observed between the first and second surveys.
Limitations, reason for caution: Because these surveys were carried out through the Internet, the participants were restricted to the Internet users who could access the website and answer the questionnaire. Opinions were collected only from reproductive aged people, which mean that the data may not reflect overall social mood.
Wider implications of the findings: To progress UTx clinically, not only agreement between patients and medical staffs but also social consensus should be established. Because ethical, legal, ethnic, religious situations for third party reproduction including both UTx and gestational surrogacy are different in each country, an independent national direction has to be assessed. For this purpose, continuous awareness survey for clinical application of UTx has to be performed among general public in each regions of the world.
Study funding/competing interest(s): Funding by University(ies) – Kyoto University.
Trial registration number: NA.
Keywords: uterine transplantation, clinical application, awareness survey, ethics

P-756 Pregnancy planning and among women attending antenatal care in Sweden and Denmark

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Study question: To what level have pregnant women entering antenatal care in Sweden and Denmark planned their pregnancies and is pregnancy planning associated with folic acid supplement intake?
Summary answer: In both Sweden and Denmark three out of four women considered their pregnancy to be highly or quite planned. Women with planned pregnancies were more likely to have taken folic acid, but in total only 29% of Swedish and 48% of Danish women took folic acid prior to the pregnancy.
What is known already: Unplanned pregnancy is associated with unfavorable outcomes for mother and infant. Pregnancy planning is often measured in an imprecise and dichotomous manner and consequently the prevalence of planned and unplanned pregnancies varies between countries. Since the most critical period for organ development occurs before many women even know they are pregnant; the first contact with antenatal care is often too late for advice about health promoting changes such as intake of folic acid supplement.
Study design, size, duration: Cross sectional studies among women attending antenatal care in Sweden and Denmark in 2013–2014. In Sweden 5494 women were asked to participate at the first visit to the antenatal clinic. In Denmark 4616 women received a link to a clinical questionnaire before the first visit to the antenatal clinic.
Participants/materials, setting, methods: In Sweden the participation rate was 68% and in Denmark 87%. Women answered a multiple-choice questionnaire and pregnancy planning was measured with one single question with five alternatives; highly planned/quite planned/neither planned nor unplanned/quite unplanned/highly unplanned. A dichotomous question measured intake of folic acid supplements.
Main results and the role of chance: Of the Swedish women (n = 3343) answering the question about pregnancy planning 47% (n = 1557) had a highly planned pregnancy, 27% (n = 915) had a quite planned pregnancy, 14% (n = 468) had a pregnancy that was neither planned nor unplanned pregnancy, 4% (n = 145) had a quite unplanned pregnancy and 8% (n = 458) had a highly unplanned pregnancy. Of the Danish women (n = 3805), 46% (n = 1750) had a highly planned pregnancy, 29% (n = 1087) had a quite planned pregnancy, 17% (n = 646) had a pregnancy that was neither planned nor unplanned pregnancy, 4% (n = 154) had a quite unplanned pregnancy and also 4.4% (n = 168) had a highly unplanned pregnancy. The level of pregnancy planning was associated to intake of folic acid ($p < 0.001$) in both countries.
Limitations, reason for caution: Conclusions should only be drawn on women who aim to pursue their pregnancies. Women with immigrant background were underrepresented.
Wider implications of the findings: The level of pregnancy planning is not but should be inquired routinely at registration to antenatal clinics to enable individualized counselling and support, and improved data on outcomes of planned and unplanned pregnancies.
Study funding/competing interest(s): Funding by University(ies) – Funding by national/international organization(s) – The Family Planning Foundation in Uppsala, The Faculty of Medicine, Uppsala University, Sweden, The Uppsala-Örebro Regional Research Council, Sweden.
Trial registration number: NA.
Keywords: pregnancy planning, folic acid supplement, pregnancy outcome

P-757 Knowledge, attitudes, and intentions toward fertility awareness and oocyte cryopreservation among United States obstetrics and gynecology (OB/GYN) residents

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Study question: What knowledge, attitudes, and intentions do US OB/GYN residents have toward discussing age-related fertility decline and oocyte cryopreservation with their patients, and at what ages and with what types of patients would they have such discussions? Do opinions differ toward medically indicated versus elective oocyte banking?
Summary answer: US OB/GYN residents are likely to initiate discussions regarding age-related fertility decline and oocyte cryopreservation in patients with cancer, but are less likely to initiate discussions of elective oocyte banking. Most residents believe that age-related fertility decline should be discussed during well-woman annual exams, but not oocyte cryopreservation.
What is known already: Currently, no studies of US OB/GYN residents exist that question their knowledge, attitudes, and intentions toward discussing age-related fertility decline and oocyte cryopreservation with patients. Oocyte cryopreservation is no longer an experimental technique, as current literature suggests that fertilization and pregnancy rates using vitrified oocytes are similar to fresh in vitro fertilization or intracytoplasmic sperm injection cycles. However, not all practitioners are familiar with this technique or are comfortable counseling patients about these options.
Study design, size, duration: A cross-sectional online survey was conducted during the fall of 2014 to evaluate residents in American Council for Graduate (ACOG) Medical Education-approved OB/GYN resident programs. Program directors were emailed via the ACOG Council on Resident Education in Obstetrics and Gynecology server listing and asked to solicit resident participation.
Participants/materials, setting, methods: Participants included 238 residents evenly distributed between post-graduate years 1–4 with varied post-residency plans. 90% of residents were women, and 75% were 26–30 years old. The survey was divided into three sections: demographics, fertility awareness, and attitudes toward discussing fertility options with patients. Descriptive and inferential statistics were conducted.
Main results and the role of chance: Residents (83%) believed an OB/GYN should initiate discussions about age-related fertility decline with patients (mean age 31.8), and 73% believed these discussions should be part of an annual exam. However, 93% of residents overestimated the age at which female fertility slightly declines and 47% overestimated the age it markedly declines. Residents were likely to support oocyte cryopreservation in cancer patients no matter the age, but much less likely to support elective oocyte banking. For elective oocyte cryopreservation, 40% believed OB/GYNs should initiate discussions with patients (mean age 31.1), while only 20% believed this topic should be part of an annual exam. Interestingly, only 25% of residents were 'familiar' or 'very familiar' with oocyte cryopreservation, although 63% worked in training settings that offered it.
Limitations, reason for caution: Because the study invitation was sent through US OB/GYN resident program directors rather than directly to residents, it is possible that some residents did not receive the invitation to participate. This limits the generalizability of the findings.
Wider implications of the findings: This study highlights a critical need for improved education and curricular offerings among OB/GYN residencies related to issues of age-related fertility decline and oocyte cryopreservation. To promote informed reproductive decision-making among patients, efforts should be made to help OB/GYNs provide comprehensive fertility education, while also respecting patient choices. Differences in attitudes towards egg banking with and without medical indication warrant further study.
Study funding/competing interest(s): Funding by University(ies) – Yale University.
Trial registration number: NA.
Keywords: oocyte cryopreservation, oocyte banking, age-related fertility decline, fertility preservation, OB/GYN residents

P-758 IVF – follow up study; cumualtive pregnancy rate in a new series of IVF attempts following 3 or more failed attempts

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Study question: What is the cumulative pregnancy rate in a new series of IVF-treatment cycles following three or more previous IVF-treatment cycles without live birth?
Summary answer: 46.3% of subjects starting treatment achieved an ongoing pregnancy within one year, and of those that went through the intended three cycles, almost 70% achieved an ongoing pregnancy.
What is known already: It is known that the accumulated pregnancy rate in a series of IVF-treatments is high; however, there is insufficient information about couples that change clinics and start a new series of IVF treatments following three failed cycles. Some studies suggest that success rates are still high even after a series of negative attempts.
Study design, size, duration: This was a prospective, observational cohort trial. 149 subjects were included in the trial and were followed for three cycles in one year from their first treatment, between June 2009 and May 2014. Approximately 33% of the subjects did not complete the intended three cycles within 1 year.
Participants/materials, setting, methods: Subjects having had three or more previous IVF-treatment cycles without live birth were recruited to this study. The treatment cycle commenced with a routine agonist or antagonist treatment with GONAL-f®. Subjects were assessed and treated according to routine clinical practice at the study site.
Main results and the role of chance: Of the 149 subjects included in the study, 69 achieved an ongoing pregnancy within one year (46.3%). 99 subjects completed the intended three cycles, with an accumulated ongoing pregnancy rate of 69.7%. Some of the remaining subjects had planned new attempts but did not complete them within the study period of one year. A longer study period might have given a more accurate result on a per subject basis.
Limitations, reason for caution: Approximately one third of the subjects did not complete the intended three cycles within the allotted time-frame. This could reduce the results since some subject might become pregnant on a second or third attempt occurring after the study period.
Wider implications of the findings: Most subjects benefit from undertaking a new series of IVF-treatments even if they have had three or more failed treatments. While most subjects get pregnant within their first three treatments, hope is not lost for the remaining population. The study suggests that completing a new series of three IVF attempts can give an accumulated ongoing pregnancy rate approaching 70%.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Merck Serono.
Trial registration number: 2008-002840-41.
Keywords: IVF, previous failed attempts, cumultative pregnancy rate

P-759 Comparison of reproductive parameters in European and middle eastern minority patients

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Study question: Is there a difference in reproductive parameters and outcome between middle eastern minorities and Europeans?
Summary answer: Couples of middle eastern origin did not show any difference in pregnancy and implantation rates but some reproductive parameters differed significantly.
What is known already: US studies revealed that racial minorities show differences in reproductive outcome parameters. Little is known about European populations.
Study design, size, duration: Observational cohort study of patients being treated between 2000 and 2011.
Participants/materials, setting, methods: 2993 European- and 288 middle eastern women undergoing their first IVF treatment in a private IVF institute.
Main results and the role of chance: Middle eastern patients were significantly younger at treatment (30.84 vs. 34.35 years, $P < 0.001$ for women and 34.98 vs. 37.82 years, $P < 0.001$ for their male partners) and first wish for a child (23.75 vs. 28.99 years, $P < 0.001$). Still, duration of infertility was significantly longer in women of middle eastern origin (6.39 vs. 4.63 years, $P < 0.001$). Middle eastern women showed significantly less tubal and more male and PCO indications for fertility treatment ($P = 0.005$). When corrected for age, middle eastern women had a significantly lower number of oocytes ($P = 0.003$, OR 0.96). There was no difference in pregnancy outcome, even when corrected for

age (34.9% vs. 41.2%, in the middle eastern vs. the European group, $P = 0.689$, OR 1.07) and implantation rates ($P = 0.834$).
Limitations, reason for caution: retrospective study; these results do not point out solely racial differences. Differences between the two groups can be explained by multifactorial cultural and ethnical differences as well as life-style factors.
Wider implications of the findings: Clinicians should be aware of the medical impact of patient's origin to support ideal medical care
Study funding/competing interest(s): Funding by hospital/clinic(s) – None.
Trial registration number: NA.
Keywords: IVF, ethnicity, Middle East, European

P-760 Seasons in the sun: the impact on IVF results

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Study question: We evaluated if weather conditions determined by temperature, rain and sunshine at the start of ovarian stimulation or one month before had an effect on the outcome of IVF in terms of number of mature and fertilized oocytes, pregnancy and live birth rates.
Summary answer: The live birth rate per cycle was statistically different between cohorts of patients that were stratified into quartiles of sunshine hours during the month before the start of ovarian stimulation.
What is known already: Several retrospective studies have evaluated seasonal variations in the outcome of IVF treatment. Some also included weather conditions, mostly temperature and hours of daylight. The results were conflicting. We focused on individual variables provided as monthly results by our national meteorological institute. We shifted the results in IVF outcome to the weather results of one month earlier, as we supposed that the selection of good quality oocytes may start in the weeks before.
Study design, size, duration: Retrospective cohort study. Between January 2007 and December 2013, the IVF outcome of all Belgian patients treated in our university center was compared to the quarter of the year and monthly mean values of temperature, rain fall, rainy days and sunshine hours during the month when gonadotropins were started or the month before.
Participants/materials, setting, methods: 11494 patients started an IVF cycle and were included. Firstly bivariate correlation was performed by linear modelling between monthly weather conditions and IVF results. Secondly the same IVF outcome variables were plotted against the weather results stratified per quartile for each individual meteorological variable (Kruskal-Wallis Test).
Main results and the role of chance: There was no relationship between IVF outcome and the quarter of the year. When looking for a linear correlation between IVF results and the mean monthly values for the weather, the results were inconsistent. However, when the same analysis was repeated with the weather results of 1 month earlier, there was a clear trend towards better IVF outcome with higher temperature, less rain and more sunshine hours. This was most striking, although not statistically significant, for live birth rate and sunshine hours (Pearson Correlation Coefficient 0.230, p 0.052). The live birth rate per cycle was significantly different (p 0.019) between different groups (Q = quartile) of mean number of sunshine hours (Q1 = 60.75, Q2 = 136.00, Q3 = 174.50).
Limitations, reason for caution: Because of the retrospective design of the study, further adjusting for possible confounding factors such as age of the woman, type of infertility and indication for IVF is mandatory. The weather conditions seem to have their strongest impact on live birth rates.
Wider implications of the findings: The impact of sunlight on the early selection of good quality oocytes is an important finding for future research. Maybe we should focus further on environmental factors during the early phases of oocyte recruitment during the period just before ovarian stimulation for IVF. A strong impact of light, as illustrated by the importance of sunny weather, brings melatonin again in the picture for further research and treatment options.
Study funding/competing interest(s): Funding by hospital/clinic(s) – UZ Gent.
Trial registration number: NA.
Keywords: seasonal variations, fertilization rate, live birth rate

REPRODUCTIVE SURGERY

P-761 Antral follicle count: a new marker predicting ovarian response in women with ovarian endometriomas

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Study question: Ovarian responsiveness to hyper-stimulation is actually considered the best non-invasive surrogate measurement of ovarian reserve. The aim of this study is to evaluate the accuracy of Antral Follicular Count (AFC) in estimating ovarian reserve and responsiveness in ovaries with endometriomas or with a past history of surgical excision of endometriomas.
Summary answer: AFC performs similarly in unaffected ovaries, in ovaries with endometriomas and in ovaries with a history of surgery for endometriomas. AFC is a reliable mean to estimate ovarian reserve in gonads with endometriomas or with a past history of surgical excision of endometriomas.
What is known already: The evaluation of the ovarian reserve in women with current or past ovarian endometriomas is challenging. In most cases, these cysts are unilateral, thus hampering the validity of hormonal assessments. As a matter of fact, sonographic assessment of AFC is the unique mean to obtain independent data on the ovarian reserve of an ovary. However, to the best of our knowledge, AFC in ovaries with current or past endometriomas has never been validated.
Study design, size, duration: Retrospective review of 84 women with present or operated endometriomas and/or with a history of surgery for endometriomas who underwent IVF.
Participants/materials, setting, methods: The outcome was the total number of developing follicles. A linear regression model that included age, BMI and the mean daily dose of gonadotropin was used to calculate the adjusted B coefficients. The capacity of AFC to predict low (≤2 follicles) or hyper-response (³7 follicles) was evaluated using ROC curves.
Main results and the role of chance: The adjusted B coefficients in non-operated gonads without endometriomas ($n = 45$), in gonads with endometriomas ($n = 68$) and in operated gonads ($n = 76$) were 0.55 (95% CI: 0.10–1.10, $p = 0.013$), 0.76 (95% CI: 0.54–0.98, $p < 0.001$) and 0.50 (95% CI: 0.25–0.75, $p < 0.001$), respectively. The Areas Under the Curve-AUC for prediction of low response were 0.84 (95% CI: 0.69–0.99, $p = 0.001$), 0.82 (95% CI: 0.72–0.92, $p < 0.001$) and 0.73 (95% CI: 0.62–0.85, $p = 0.001$), respectively. The AUCs for prediction of hyper response were 0.85 (95% CI: 0.72–0.98, $p < 0.001$), 0.78 (95% CI: 0.67–0.90, $p < 0.001$) and 0.77 (0.60–0.94, $p = 0.003$), respectively. Data were insufficient to draw a reliable estimate for the prediction of hyper-response in the group of previously operated gonads with recurrences. Statistically significant AUCs emerged for all groups for the prediction of poor response.
Limitations, reason for caution: The study is retrospective and we used the number of developing follicles as a marker of ovarian reserve. However, we deemed more appropriate to refer to the number of follicles rather than the number of oocytes retrieved because this outcome is not influence by technical difficulties in oocytes retrieval.
Wider implications of the findings: AFC is a reliable mean to estimate ovarian reserve in gonads with endometriomas or with a past history of surgical excision of endometriomas. In particular, the presence of an endometrioma does not affect the diagnostic performance of AFC.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Not funding. Retrospective study in hospital.
Trial registration number: NA.
Keywords: endometrioma, antral follicular count, IVF

P-762 Comparison of vaginal misoprostol and dinoprostone for cervical ripening before diagnostic hysteroscopy in nulliparous women

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Study question: To compare the effectiveness of vaginal misoprostol and dinoprostone for cervical ripening before diagnostic hysteroscopy in nulliparous women.
Summary answer: In a prospective, randomized study, vaginally administered dinoprostone before diagnostic hysteroscopy is more effective than misoprostol for inducing cervical ripening.
What is known already: One of the most significant problems during diagnostic hysteroscopy is the difficulty of the instrument to go along the cervical canal and to pass the internal os and the related complications such as cervical injury or tear, bleeding, a false track or uterine perforation. In order for this passing to be easier there needs to be cervical ripening and widening of the cervical canal at a specific diameter.
Study design, size, duration: A total of 90 women with suspected intrauterine lesions (such as uterin polyps, filling defects in the uterine cavity) on the basis of abnormal findings from hysterosalpingo-graphy, ultrasonography or saline-infusion-graphy were admitted to the study between July 2012 and December 2013.
Participants/materials, setting, methods: Ninety women in reproductive age eligible for diagnostic hysteroscopy were recruited. Patients were randomly assigned to receive 400 mcg misoprostol (n = 30), 10 mg dinoprostone (n = 30) vaginally before diagnostic hysteroscopy; the control group (n = 30) did not receive any cervical priming agent.
Main results and the role of chance: Primary outcome; the number of women requiring cervical dilatation. In the control group, 23 (76.7%) patients needed cervical dilatation before hysteroscopy; in the misoprostol group, 17 (56.7%) patients needed it, whereas only 9 (30%) patients in the dinoprostone group did. There was significant difference between misoprostol and dinoprostone group (p = .037) and placebo and dinoprostone group (p < .001), but there was not difference between placebo and misoprostol group (p = .100).
Limitations, reason for caution: A potential weakness of the present study is the subjective measurement of cervical width. To overcome this situation, it is recommended to use a tensinometer but most of the previous studies the cervical width was achieved by Hegar's bougies.
Wider implications of the findings: To date, only one study has evaluated the effects of vaginal misoprostol and dinoprostone for cervical ripening in nulliparous women before operative hysteroscopy. Preutthiron and colleagues found that vaginal misoprostol was more effective than dinoprostone for cervical priming in nulliparous women before hysteroscopic surgery. The discripency of results between our study and Preutthipan's study might be that we have employed different procedures, we performed diagnostic hysteroscopy.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Konya Education and Research Hospital.
Trial registration number: NCT01620814.
Keywords: misoprostol, dinoprostone, cervical ripening, hysteroscopy

P-763 Congenital adrenal hyperplasia: outcomes surgical correction of ambiguous genitalia

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Study question: Outcomes of surgical treatment of congenital adrenal hyperplasia (CAH).
Summary answer: Considering the results of surgical treatment of adolescent girls with CAH simple virilizing form female plastic of external genitals must be conducted at the age of 2–3 years in one-stage operation. For the patients with CAH salt-wasting form two-stage operation of external genitals female plastic is recommended.
What is known already: -
Study design, size, duration: 103 subjects 15–26 year old (middle age 19,4) participated in this study. All subjects were divided into 2 treatment groups: 67 subjects with CAH simple virilizing form entered into the first treatment group, 36 subjects with CAH salt-wasting form entered into the second treatment group. Feminizing genitoplasty was conducted for all subjects. For the patients of the 1 group it was performed when they were 3–6 years old including clitoris plastic and section of urogenital sinus in one-stage operation. For the patients of the second group two-stage operation was conducted: female plastic of external genitals was performed at the age of 2–6 years and introit plastic was conducted at the age of 14–16 years. Of these 35 subjects 8 subjects underwent operation with subsequent vaginal dilatation in the postoperative period.
Participants/materials, setting, methods: Several postoperative complications were registered: vaginal vestibule cicatricial deformity, formation of vaginal stricture, coarse fibrosis, stenosis. Most of the complications were registered in the second group. All first group girls with CAH simple form were sexually adapted. 4 second group girls could not be sexually active due to postoperative complications
Main results and the role of chance: -
Limitations, reason for caution: -
Wider implications of the findings: -
Study funding/competing interest(s): Funding by national/international organization(s) – National Scientific Center of Endocrinology.
Trial registration number: L28.
Keywords: feminizing genitoplasty, congenital adrenal hyperplasia, ambiguous genitalia

P-764 Immunohystochemical features of IL-6 in pelvic peritoneal adhesions of various etiologies at reproductive age women

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Study question: To examine the morphological structure and expression of IL-6 in the tissues of the pelvic peritoneal adhesions at reproductive age women and forecast scientifically the future behavior of formed adhesions after adhesiolysis.
Summary answer: Regardless of the genesis of morphological features and adhesions in women of the reproductive age, the immunohistochemical study of IL-6 observed tissue staining of varying intensity, the highest level of IL-6 marker's expression was detected in adhesions at patients with external genital endometriosis.
What is known already: Increased production of IL-1 and TNF-a in traumatic inflammation or infection correlates with an increase in the number and severity of adhesions. Unlike IL-1 and TNF-a, the role of IL-6 in the adhesion process is characterized by conflicting data reports. Some authors have suggested that IL-6 is involved in the formation of adhesions, possessing the properties of proinflammatory cytokines. According to other researchers, its concentration doesn't correlate with the presence and severity of peritoneal adhesions.
Study design, size, duration: The materials of this study were fragments of the surgical specimens (adhesions and their parts) n = 100, obtained from reproductive age women during operative laparoscopy. The morphological and immunohistochemical study of adhesions were carried out by standard procedures using paraffin blocks of Dako reagents and monoclonal antibodies to IL-6.
Participants/materials, setting, methods: The adhesions of 38 patients with a history of chronic inflammatory diseases, pelvic adhesions of 32 patients with endometrial disease (12 patients with ovarian endometriosis and 20 patients with peritoneal endometriosis outside) and 30 patients who previously underwent surgeries of pelvic and abdominal cavity.
Main results and the role of chance: During the immunohistochemical study of adhesions obtained from women undergoing surgery of pelvic organs, the expression of IL-6 was extremely low and was equal to 33 ± 0,2 points, only a few positively stained cells were found in the walls of arteries. Tissue adhesions in women with a history of inflammatory diseases of the pelvic organs, characterized by moderate expression of IL-6, was 65 ± 0,3 points. Positive staining was detected mainly in the mesothelial cells covering the walls of the spike and the arteries. The material of adhesions in case of external genital endometriosis, was characterized by a pronounced expression of IL-6. The most commonly observed diffuse was the one of the moderate severity staining of fibroblasts and arterial walls and mesothelial cells. It reached 154 ± 0,6 points.
Limitations, reason for caution: Age limitation, only women aged 19–49 years took part in this study. Exclusion criteria the following for the groups: acute gynecological diseases, malignant diseases of female genitalia and ovarian tumors.
Wider implications of the findings: Regardless of the genesis of morphological features and adhesions in women of the reproductive age, the immunohistochemical study of IL-6 observed tissue staining of varying intensity. The highest

level of IL-6 marker's expression was detected in adhesions at patients with external genital endometriosis. Such characteristics of IL-6 as a system device indicate the need for more active management of patients with external genital endometriosis, using anti-inflammatory and immunomodulatory therapy.
Study funding/competing interest(s): Funding by University(ies) – Crimean State Medical University named after S. I. Georgievsky.
Trial registration number: This study isn't an RCT.
Keywords: pelvic peritoneal adhesion, morphology, interleukin 6, reproductive age women

P-765 Unidirectional knotless barbed suture for laparoscopic myomectomy (bsLM): fertility, pregnancy and delivery outcomes

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Study question: Which are the fertility, pregnancy and delivery outcomes in women undergoing bsLM?
Summary answer: Fertility and pregnancy outcomes are similar to those reported in the literature for laparoscopic myomectomy with standard suture.
What is known already: During laparoscopic myomectomy, the use of barbed suture aids surgeons by reducing operative time, difficulty of suturing, suturing time, and blood loss. No information is available on fertility and pregnancy outcomes in patients treated with bsLM.
Study design, size, duration: This prospective single-centre cohort study included 61 women who tried to conceive after bsLM that was performed between January 2010 and August 2013.
Participants/materials, setting, methods: The study included patients with at least one myoma with diameter ≥4 cm and at least one FIGO type 3, 4 or 5 myoma who tried to conceive for at least 12 months during follow-up. Patients with infertility factors (i.e. endometriosis, hydrosalpinx) were excluded from the study.
Main results and the role of chance: The mean (± SD) age at bsLM was 35.9 (±3.8) years. The median number of myomas removed was 2 (range, 1–5) with a mean diameter of the larger myoma of 6.7 (±1.9) cm. The mean length of follow-up was 2.9 (±1.0) years. 34 patients (55.7%) spontaneously conceived; the mean time to conception was 5.7 (±3.9) months. 11 patients (18.0%) conceived by assisted reproductive technologies. There were 17 cesarean sections (13 because of previous myomectomy); labor was successful in 12 women (73.3% of all vaginal delivery trials). There were 5 miscarriages and 1 voluntary termination of pregnancy (because of Down Syndrome); 10 pregnancies are on-going. There was no uterine rupture or placental complication.
Limitations, reason for caution: The small sample size is the major limitation of the study which is unpowered to draw definitive conclusions on the risk of uterine rupture during vaginal delivery in patients treated by bsLM.
Wider implications of the findings: The use of barbed suture during laparoscopic myomectomy does not decrease spontaneous fertility and does not affect pregnancy outcome. Further investigations are required before bsLM can be routinely performed in women wishing to undergo vaginal delivery.
Study funding/competing interest(s): Funding by University(ies) – University of Genoa.
Trial registration number: NA.
Keywords: laparoscopic myomectomy, barbed suture, fertility, pregnancy outcome, vaginal delivery

P-766 Salpingostomy in the treatment of hydrosalpinx: a systematic review and meta-analysis

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Study question: What is the chance of natural conception when salpingostomy is used to treat hydrosalpinx?
Summary answer: The rate of natural clinical pregnancy after salpingostomy is performed for hydrosalpinx is 26% (95% CI 24–29%).
What is known already: Tubal surgery is now not commonly offered for women with hydrosalpinges. Surgical correction of hydrosalpinges has declined simulateously with the increasing utilisation of in vitro fertilisation. This is the first systematic review to investigate natural conception rates following salpingostomy in the treatment of hydrosalpinx.
Study design, size, duration: We conducted a systematic review of 21 observational studies. These studies encompassed 2376 patients that underwent salpingostomy and then attempted natural conception. Follow up was over a variety of time periods.
Participants/materials, setting, methods: Literature searches were conducted to retrieve observational studies which reported salpingostomy for hydrosalpinx. Databases searched included MEDLINE, EMBASE, Cochrane Register and CINAHL. Only studies that focused on salpingostomy (rather than other tubal conserving surgeries) for the treatment of hydrosalpinx were included. 21 studies matched the inclusion criteria.
Main results and the role of chance: The pooled natural clinical pregnancy rate from the 21 observational studies (including 2376 patients) was 26% (95% CI 24–29%) after salpingostomy was performed for hydrosalpinx. The cumulative clinical pregnancy rates were 6.4% (95% CI 3.2–12.8%) at 6 months, 21.9% (95% CI 19.0–26.0%) at 12 months, 22.1% (95% CI 18.8–26.0%) at 18 months and 28.2% (95% CI 22.9–34.7%) at 24 months. The pooled live birth rate (eight studies, 954 patients) was 24% (95% CI 21–28%) after salpingostomy was performed for hydrosalpinx. The pooled ectopic pregnancy rate (18 studies, 2228 patients) was 10% (95% CI 8–11%). The pooled miscarriage rate (seven studies, 924 patients) was 7% (95% CI 6–9%). The included studies scored well on the Newcastle Ottawa quality assessment scale.
Limitations, reason for caution: Strict inclusion criteria were used in the conduct of the systematic review. However, the studies included are clinically heterogeneous in many aspects including patient characteristics, surgical technique and duration of follow up after salpingostomy.
Wider implications of the findings: The findings of this systematic review suggest that there may be an alternative treatment strategy in patients presenting to fertility services with hydrosalpinx. Further prospective, large and high quality studies are needed to identify the sub-population that would most benefit from tube conserving surgery.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Birmingham Womens NHS Foundation Trust.
Trial registration number: No trial registration number is available as this is a systematic review.

P-767 Uterine allotransplantation in a rabbit model using aorto-caval anastomosis: a long-term viability study

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Study question: Can a large vessel aortic-caval vascular patch technique, whereby the recipient and donor aortae and vena cavae are anastomosed together, bring about long-term graft functionability and animal survival following an allogeneic uterine transplant in a rabbit model.
Summary answer: Our method used a macrovascular patch technique to ensure adequate blood supply to the donor uterine graft. We have demonstrated the feasibility of uterine allotransplantation using this technique in the rabbit, demonsatrted by a resulting pregnancy, but were unable to demonstrate a higher long-term survival percentage.
What is known already: Women with absolute uterine factor infertility are considered as being 'unconditionally infertile'. Currently, the only two fertility management options are surrogacy or adoption. These women may also benefit from a possible third option: uterine transplantation. Allogeneic uterine transplantation has been attempted in a number of animal models, but achieving an adequate blood supply for the transplanted uterus still presents the biggest challenge. Microvascular re-anastomosis was unsuccessful in a number of animal models.
Study design, size, duration: This was a longitudinal study involving a small-animal model. The study was performed at the Royal Veterinary College and Imperial College London, United Kingdom between May 2011 and December 2012. Nine donor and nine recipient allogeneic New Zealand White rabbits of proven fertility were chosen for the cross-transplantations.

Participants/materials, setting, methods: An aortic-caval macrovascular patch was harvested as part of the uterine allograft. Tacrolimus was administered for immunosuppression. If long-term survival was ensured, embryos were transferred into each cornua via mini-midline laparotomy. The pregnancy was monitored with regular reproductive profiles and trans-abdominal ultrasound. The recipients were monitored until death or euthanasia.
Main results and the role of chance: In this case series, long-term rabbit survival was 11% ($n = 1$). Surgical survival was 56% ($n = 5$). Three rabbits (UTx #3, #4, #8) died intra-operatively as a result of blood aspiration, ventricular hematoma, and massive hemorrhage. Three does (#1, #2, #7 and #9) died within the first 24h as a result of the veno-vena and anastomosis breakdown. Does #6 and #9 died secondary to pre-operative pneumonia and a pulmonary embolus, respectively. Only one rabbit survived longer than a month. In this doe, a gestation sac was visualised on ultrasound from day 9 post-embryo transfer but by D18 the gestation sac had reduced in size, suggesting fetal resorption had occurred. Scheduled necropsy on D27 and histopathology confirmed evidence of a gravid uterus and presence of a gestational sac.
Limitations, reason for caution: The biggest limitation of the study is the paucity of data and therefore we recognize that it is difficult to make any conclusions regarding our immunosuppression regimen. The combination of a CNI and a steroid can clearly slow down the rejection process, if not completely inhibit it. If UTx were available in humans, one can envisage the addition of a monoclonal antibody for the induction the end. Disappointingly our sole pregnancy did not result in the birth of healthy term offspring. Also, tissue typing between the donor and the recipient does was not performed.
Wider implications of the findings: Despite the end-result i.e. failed pregnancy, the study represents only the third example of conception and pregnancy following an allogeneic animal uterine transplant. The surgical anatomical macrovascular model was successful. The cause of fetal demise is most likely secondary to inadequate prevention of the immunological rejection response.
Study funding/competing interest(s): Funding by national/international organization(s) – Funded by a registered UK charity – Womb Transplantation UK.
Trial registration number: NA.
Keywords: uterus, transplantation, fertility, rabbit, Allogeneic

P-768 Uterine auto-transplantation in a sheep model using iliac vessel anastomosis

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Study question: To investigate, develop and evaluate anatomical, surgical and anastomotic aspects necessary for a successful uterine transplant in a large-animal (sheep) model, whereby uterine autotransplantation will be carried out in an orthotopic position in the pelvis and with vascular anastomosis to the external iliac vessels.
Summary answer: Internal to external iliac vessel anastomosis, whereby the external iliac vessles may be used as recipients of the graft vessels in order to allow for a secure blood flow, is an acceptable surgical technique that should be applied in a human uterine transplantation model to ensure adequate subsequent uterine perfusion.
What is known already: Women with absolute uterine factor infertility are considered as being 'unconditionally infertile'. Currently, the only two fertility management options are surrogacy or adoption. These women may also benefit from a possible third option: uterine transplantation. There is a need to further develop the large animal uterine transplantation model in order to study anastomotic and other surgical techniques. The large animal model resembles the human pelvis much more closely than small animals.
Study design, size, duration: This was a longitudinal study involving a large-animal model. The model itself was a sheep model. The study was performed at the Royal Veterinary College and Imperial College London, United Kingdom between January and June 2013. Five ewes of proven fertility were chosen for the auto-transplants.
Participants/materials, setting, methods: The uterine allograft along with the internal iliacs, and uterine arterial and venous tree all intact were harvested en bloc. An end-to-side anastomosis was performed between the external iliac and the internal iliac vessels. Successful reperfusion of the graft was judged by the color shift of the uterus during reperfusion.
Main results and the role of chance: The main outcome measures were as follows: operative details (retrieval, ischaemic, clamping, reperfusion and recipient hysterectomy duration); physiological profiles; gross morphology and histopathology. Five autotransplants were performed in the sheep model. One procedure was abandoned because of the inappropriate size of sheep model. Another procedure was halted because the animal suffered from respiratory failure in the immediate intra-operative period. Three transplants were completed. In those, at least two of four possible anastomoses were finished and the grafted uteri demonstrated immediate perfusion and appropriate viability 45 min post-operatively.
Limitations, reason for caution: Five cases were attempted, with only three completed. The reasons for the non-completion of autotransplants #1 and #4 were non-surgical. The next study should assess long-term uterine function by attempting conception, with analysis of fetal weight/well-being. Fluid balance must be recorded to prevent future occurrences of renal failure.
Wider implications of the findings: The present study was the first time that the UK team attempted a macrovascular uterine transplant large-animal model. The surgical technique was very similar to what would be used in a human model. The external iliac vessels may be used as recipients of the graft vessels in order to allow for a secure blood flow. A future experiment should attempt to achieve normal pregnancies after removal and subsequent auto-/allo-transplantation of the sheep uterus.
Study funding/competing interest(s): Funding by national/international organization(s) – Funded by a registered UK charity – Womb Transplantation UK.
Trial registration number: NA.
Keywords: uterus, transplantation, fertility, sheep, anastomosis

P-769 IVF treatment should not be postponed for patients after exeresis of ovarian endometrioma with normal basal FSH concentration: a retrospective study

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Study question: Whether IVF treatment should be delayed in patients after exeresis of ovarian endometrioma with normal basal FSH concentrations.
Summary answer: IVF treatment should not be delayed in patients after exeresis of ovarian endometrioma with normal basal FSH concentrations.
What is known already: Infertile patients with endometriosis have a similar concentration of Follicular-fluid anti-Müllerian hormone (AMH) as compared with non-endometriotic patients, and basal concentrations of serum FSH are routinely used as a marker for predicting ovarian reserve and as a screening test for patients undergoing IVF. However, there are few studies to investigate basal FSH fluctuations in women after exeresis of ovarian endometrioma.
Study design, size, duration: A total of 146 controlled ovarian hyperstimulation (COH) cycles were registered; 3 of these cycles had ovarian hyperstimulation syndrome, which had been excluded. So we retrospectively evaluated 76 cycles after previous exeresis of ovarian endometrioma and 67 cycles with male factor infertility from January 2008 to January 2013.
Participants/materials, setting, methods: (1) All patients' basal FSH level was lower than 10 IU/l, infertility associated with ovarian endometrioma diagnosed by laparoscopy, (2) age ≤40 years old, (3) body mass index (BMI) <30 kg/m, (4) absence of polycystic ovarian syndrome and other endocrine disease. The treatment protocols were long agonist protocol.
Main results and the role of chance: The patients' characteristics and mostly the IVF treatment outcome parameters between the two groups had no significant differences. However, the number of retrieved oocytes was significantly higher in group II than that in group I ($P = 0.02$) and the number of retrieved oocytes significantly decreased in patients less than 1 year and 2–3 year after exeresis of ovarian endometrioma ($P = 0.028$, $P = 0.005$, $P = 0.04$). The post-operative period independently contributed to the FSH units used in a COH cycle ($P = 0.018$) and the FSH units used significantly increased in more than 4 years after exeresis of ovarian endometrioma ($P = 0.000$, $P = 0.001$, $P = 0.012$, $P = 0.001$, $P = 0.004$).
Limitations, reason for caution: our study did not take into account the impact of frozen–thawed cycles on the cumulative pregnant rates (due to data currently

not being available as some patients still have some frozen embryos which are not transferred in).
Wider implications of the findings: This study shows that basal FSH concentration is not useful for the clinician deciding on whether to delay treatment in a patient after exeresis of ovarian endometrioma with a normal FSH concentration. It would therefore be appropriate to treat infertile patients if they are normally cycling with the full knowledge of the smaller number of retrieved oocytes.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Reproductive Medicine Center of the First Affiliated Hospital of Wenzhou Medical University.
Trial registration number: NA.
Keywords: endometrioma, in vitro fertilization, ovulation induction, FSH

P-770 The effect of laparoscopic myomectomy on fertility of infertility patients

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Study question: What is the effect of laparoscopic myomectomy for infertility patients?
Summary answer: This study confirmed that laparoscopic myomectomy may be effective for infertility patients with intramural uterine myoma.
What is known already: Submucous myomas led to decreased fertility. Hysteroscopic myomectomy for submucous myomas is likely to improve pregnancy rate.
Study design, size, duration: A retrospective study was performed from January 2008 through December 2012 on 96 infertility patients with intramural myomas. They had undergone laparoscopic myomectomy.
Participants/materials, setting, methods: Out of 96 patients analyzed, 40 were laparoscopic myomectomy cases and 56 were laparoscopic assisted myomectomy cases. Sixty-seven cases were analyzed for their fertility improvement. During the same period, patients who had submucous myoma and undergone hysteroscopic myomectomy were studied for the pregnancy after the surgery for comparison.
Main results and the role of chance: Forty out of 67 (61.2%) patients become pregnant within one year with or without infertility treatment. Twenty-seven pregnancies followed an IVF/ICSI procedure. There were 6 IUI cases and 8 natural conception cases. Twenty cases were conceived within 1–3 months, 11 cases were within 4–6 months, 8 cases were within 7–9 months and 2 cases were within 10–12 months Conception tends to occur in early months after surgery. The cumulative pregnancy rate of group which had distortion of uterine cavity were 59.3% (32/54) and that of group which had no distortion of uterine cavity were 64.3% (9/14). The cumulative pregnancy rate of the patients who had undergone hysteroscopic myomectomy were 55.6%. Laparoscopic myomectomy for infertility patients was not inferior to hysteroscopic myomectomy for submucous myomas.
Limitations, reason for caution: Possible biases related to retrospective studies and limited number of cases.
Wider implications of the findings: A prospective randomized clinical trial is needed to confirm our findings.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Teikyo University School of medicine University Hospital, Mizonokuchi.
Trial registration number: NA.
Keywords: uterine myoma, laparoscopic myomectomy, fertility

P-771 Almost half of unilateral hydrosalpinx need additional contralateral fallopian tubal surgery

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Study question: When we perform unilateral salpingectomy due to unilateral hydrosalpinx on HSG, is really contralateral fallopian tube normal?
Summary answer: With a unilateral hydrosalpinx on HSG, abnormalities in contralateral fallopian tube were found in 45.6% and performed additional surgeries salpingectomy or fimbrioplasty.
What is known already: Tubal factor is about one third causes of infertility. For tubal factor evaluation, the most commonly used tests are hysterosalpingography (HSG) and laparoscopy. HSG has high specificity for diagnosis of tubal occlusion and low sensitivity in cases with peritubal adhesions. Tubal patency on HSG does not necessarily indicate normal tubal function. Among women with patent tubes on HSG, 18% were found to have tubal obstruction or peritubal adhesions on laparoscopy.
Study design, size, duration: This retrospective study included 178 salpingectomy cases due to hydrosalpinx on HSG between January 2010 and December 2014.
Participants/materials, setting, methods: Total 178 cases salpingectomy were performed. 99 cases were excluded (69 cases - bilateral salpingectomy due to bilateral hydrosalpinx, 30 cases – unilateral salpingectomy due to unilateral hydrosalpinx with contralateral tubal obstruction on HSG/previous salpingectomy).
Main results and the role of chance: We analyzed 79 cases with unilateral hydrosalpinx and contralateral normal patency on HSG. Among 79 cases, 43 cases were performed only unilateral salpingectomy (US). 14 cases were performed US and contralateral salpingectomy due to unexpected hydrosalpinx. 22 cases were performed US and contralateral fimbrioplasty or adhesiolysis due to peritubal adhesion or abnormal result on dye test. Therefore in case of unilateral hydrosalpinx on HSG, 45.6% (36/79) were found abnormality of contralateral tube and needed additional surgery.
Limitations, reason for caution: The subjectivity of radiologists may be involved in interpretation of HSG. Also during operation, the subjectivity of surgeon may be involved in decision making of contralateral tubal surgery.
Wider implications of the findings: An abnormal tubal condition affects negatively natural pregnancy or IVF outcomes, therefore proper surgery is needed. Among women with normal tubes on HSG, 18% were found abnormalities on laparoscopy. But with unilateral hydrosalpinx, proportion of requiring opposite fallopian tubal surgery is increased by 45.6%. Therefore, in case of elective unilateral salpingectomy due to unilateral hydrosalpinx on HSG, operators must examine opposite tube carefully and make full explanation about incidental bilateral salpingectomy and possibility of IVF.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Mamapapa & baby OB&GY clinic.
Trial registration number: NA.
Keywords: hydrosalpinx, salpingectomy, hysterosalpingography

P-772 The outcome of "conservative debulking surgery" in infertile women with uterine adenomyosis

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Study question: Does conservative debulking surgery of adenomyosis have clinical efficacy in infertile women?
Summary answer: Conservative debulking surgery could be an effective method for increasing pregnancy rate and conservation of fertility potential in infertile women with adenomyosis.
What is known already: Adenomyosis 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, hysterectomy has been a standard surgical treatment for eradication. Until now, there is no agreement on the most appropriate therapeutic methods. There have been several reports that surgical procedures, including endometrial resection, myometrial reduction and excision by electrocautery, reduced the need for hysterectomy in patients with adenomyosis
Study design, size, duration: Prospective clinical trial was conducted. The subjects consisted of 39 infertile patients with adenomyosis undergoing In Vitro Fertilization-Embryo Transfer (IVF-ET) and were enrolled from December 2007 to June 2014.
Participants/materials, setting, methods: 39 patients having unexplained infertility, adenomyosis were enrolled after the failure of IVF. This newly designed operation included transverse H-incision on the adenomyotic wall, excision of adenomyosis tissue using argon laser under the monitoring of intraoperative ultrasonography. After debulking surgery, patients underwent follow up for checking symptom relief and pregnancy.

Main results and the role of chance: The mean age of the patients was 35.15 ± 3.05 years and the volume of excised specimens of adenomyosis was 88.00 ± 71.45g. The relief of dysmenorrhea was observed clearly in all patients at 6 months after operation (NRS; 7.88 ± 2.50 vs. 1.76 ± 1.86, $p < 0.001$). The CA 125 level was significantly decreased at the time of 6 months after operation (187.55 ± 221.13 vs. 26.78 ± 19.29, $p < 0.016$). Post-operational complication occurred in three patients (ureter fistula, uterus necrosis and POF). Of 31 patients who attempted pregnancy, 10 patients conceived by IVF-ET or Thawing-ET after the operation (10 of 31; 32.3%). Miscarriage occurred in three patients, ectopic pregnancy in one patient and six patients delivered by cesarean section at term.
Limitations, reason for caution: The sample size was 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 conservative debulking surgery was related to the symptom relief of dysmenorrhea and increasing pregnancy rate, implying that this surgical approach could be considered as a useful method for infertile women with adenomyosis who need conservation of uterus. This is the first report on the pregnancy status of surgical approach in infertile women with uterine adenomyosis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Dongguk University Ilsan Hospital.
Trial registration number: NA.
Keywords: adenomyosis, debulking surgery, infertility, dysmenorrhea

P-773 Does laparoscopy still have a role in patients with unexplained infertility?

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Study question: Do patients with unexplained infertility benefit from undergoing laparoscopy?
Summary answer: Laparoscopy still has an important role in diagnosis and management of cases of unexplained infertility.
What is known already: Unexplained infertility remains a challenge in diagnosis and management even after the introduction of IVF technology. It is well known that patency of the fallopian tubes by hyterosalpingogram does not rule out pelvic adhesions that can only be diagnosed by laparoscopy. Laparoscopy can diagnose cases with mild endometriosis that are not diagnose clinically of with ultrasound. It is debatable whether laparoscopy should be a mandatory step in sub-fertility workup.
Study design, size, duration: Case control study included 229 patients with unexplained infertility that underwent laparoscopy during the period from January 2012 to December 2012 (study group). The control group included 300 patients with unexplained infertility treated expectantly or with ovulation induction during the same period.
Participants/materials, setting, methods: We reviewed the files and operative reports of 229 patients with unexplained infertility that underwent laparoscopy at the endoscopy unit in Minia University Hospital, Minia, Egypt within one year. We traced these patients to know whether pregnancy was achieved or not within one year after laparoscopy. We traced patients in the control group during the same duration of time.
Main results and the role of chance: Among the 229 patients that underwent laparoscopy, pelvic adhesions were found in 20 patients, tubal problems not revealed by hysteroslpingram were found in four patients and mild endometriosis was found in 9 patients. Laparoscopic adhesolysis, tuboplasty and diathermy of endometriotic spots were done respectively. Eighty four patients achieved pregnancy within one year after undergoing laparoscopy. In the control group ($n = 300$), fifty one patients achieved pregnancy during the same period either spontaneously or on top of ovulation induction. Odds ratio for achieving pregnancy after laparoscopy in patients with unexplained infertility was 2.83 which was statistically significant ($P < 0.01$).
Limitations, reason for caution: Laparoscopic procedures in this study were done in a tertiary hospital by experts in laparoscopic surgery. That allowed proper managements of cases with pelvic adhesions and endometriosis with subsequent high pregnancy rate after laparoscopy. Laparoscopic surgeons with equal expertise are not available for all laparoscopic procedures done for patients with unexplained infertility.
Wider implications of the findings: Patients with unexplained infertility constitute a large proportion of infertile patients. Laparoscopy can diagnose and treat problems like pelvic adhesions and mild endometriosis in these patients. Laparoscopy is recommeneded to be done as a part of sub-fertility workup in patients with unexplained infertility.
Study funding/competing interest(s): Funding by University(ies) – Minia University, Minia, Egypt.
Trial registration number: The study is not a clinical trial.
Keywords: laparoscopy, unexplained infertility

P-774 Effectiveness of bilateral uterine artery chemoembolization with methotrexate and gelatine foam in the treatment of caesarean scar pregnancy

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Study question: The aim of this study was to investigate the efficacy and safety of uterine artery embolization (UAE) with intra-arterial methotrexate (MTX) and gelatine foam combined with dilation and curettage (D&C) for the treatment of caesarean scar pregnancy (CSP).
Summary answer: Bilateral Uterine Artery Chemoembolization with the use of absorbable embolizing material combined with D&C seems to be an effective and safe method, and may be beneficial for women wishing to preserve fertility.
What is known already: Caesarean scar pregnancy (CSP) is the rarest type of ectopic pregnancy. It is a life-threatening abnormal form of implantation of a gestational sac in the myometrium at the site of a previous Caesarean scar. The incidence of this pathology ranges from 1/1800 to 1/2200 pregnancies and its rate is 0.15% in women with previous Caesarean sections. CSP can lead to massive haemorrhages, uterine rupture, and disseminated intravascular coagulation or death. Early diagnosis is essential for effective treatment to avoid life-threatening complications. Despite increasing incidence rates of Caesarean scar pregnancy, no universal guidelines for its management and treatment have been suggested.
Study design, size, duration: In this retrospective observational study the studied group consisted of 10 patients between 6 and 9 weeks of pregnancy.
Participants/materials, setting, methods: Caesarean scar pregnancy was confirmed by transvaginal ultrasound, using the following criteria: an empty uterine cavity, empty cervical canal, development of the gestational sac in the anterior part of the uterine isthmus and blood flow around the gestational sac. The therapeutic procedures were performed under local anaesthesia by puncturing the right femoral artery using the Seldinger technique. The 5F vascular sheath was inserted; the Pigtail catheter was placed in the abdominal aorta. Digital subtraction angiography of both internal iliac arteries was performed. Using the cross-over method, the 5Fr Robert catheter was moved to the opposite side. The uterine artery was selectively cannulated using the Progreate microcatheter; 25 mg of methotrexate was infused bilaterally (total 50 mg) administered in a slow bolus and the vessel was embolized using the gelatine foam (technical pearls: two screw syringes, three way stopcock, gelatin sponge cut into small pieces, add contrast, mix it between the syringes through a stopcock) until stasis was achieved. After 48 h, all patients were treated with D&C.
Main results and the role of chance: A total of 10 women diagnosed with caesarean scar pregnancy were successfully treated with Bilateral Uterine Artery Chemoembolization (UTACE) with local MTX and Gelatine Foam combined with uterine curettage. None of the treated patients required a hysterectomy. The mean blood loss was estimated at 125 ml.The mean duration of hospital stay was 5 days. The average time for beta-hCG to decline to normal values was 24 days. The mean time to recovery of normal menstruation was 32 days.
Limitations, reason for caution: Limitation of the study is a small group of patients studied.
Wider implications of the findings: UTACE with the use of absorbable embolizing material combined with D & C is a new, safe and effective treatment method of caesarean scar pregnancy allows to preserve the uterus.
Study funding/competing interest(s): Funding by University(ies) – Medical University of Lublin.

Trial registration number: KE-0254/120/2012.
Keywords: caesarean scar pregnancy, ectopic pregnancy, embolization

P-775 How often can FloSeal®, a gelantine-thrombin matrix sealant, effectively achieve haemostasis after laparoscopic stripping of ovarian endometriomas?

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Study question: To assess how effective FloSeal®, a gelantine-thrombin matrix sealant, is in achieving haemostasis after laparoscopic stripping of ovarian endometriomas.
Summary answer: FloSeal® is an effective method in achieving haemostasis after laparoscopic stripping of ovarian endometriomas and should be considered as an alternative to traditional electrocoagulation for haemostasis, especially in those women with fertility wish or low ovarian reserve.
What is known already: Laparoscopic ovarian cystectomy by the stripping method is the most common operative technique to treat endometriomas. Traditional methods for controlling haemostasis are by electrocoagulation and by laparoscopic suturing. FloSeal® consists of a bovine-derived Gelatin Matrix and a human derived Thrombin component and has been an effective alternative method of haemostasis in various operations. There is limited data on its use in gynaecological reproductive surgery.
Study design, size, duration: This is an observational study which is a part of a randomized controlled study in assessing ovarian function after laparoscopic stripping of ovarian endometriomas conducted at the Department of Obstetrics and Gynaecology of Prince of Wales University Hospital in Hong Kong between July 2013 and December 2014.
Participants/materials, setting, methods: All women aged 18–40 years old with clinical and ultrasound diagnosis of unilateral or bilateral endometriomas was enrolled. Patients were consented to use FloSeal® for haemostasis after laparoscopic stripping of ovarian endometriomas.
Main results and the role of chance: A total of 20 patients aged 34 ± +4.44 years old underwent laparoscopic ovarian cystectomy for ovarian endometriomas during the study period. Forty-eight percent of the participants have unilateral ovarian endometrioma over the left side, 36% over the right side and 16% of them had bilateral endometriomas. The average diameter of the endometrioma examination was 4.82 ± +1.93 cm. The average AFS score for endometriosis was 46.5. The average duration for the operation was 69.2 minutes and postoperative haemoglobin drop was 0.7 ± +1.1g/dL. All but one case (95%) was able to achieve haemostasis with FloSeal® within 5 minutes with no additional haemostatic procedure required. No case required intraoperative blood transfusion and there were no intraoperative or postoperative complications noted.
Limitations, reason for caution: Our study was limited by the small sample size.
Wider implications of the findings: Controlling haemostasis with FloSeal® after laparoscopic stripping of ovarian endometriomas is an effective alternative haemostatic method and it avoids further ovarian tissue damage. It should be considered in those who want to preserve the ovarian reproductive function especially in women with fertility wish.
Study funding/competing interest(s): Funding by national/international organization(s) – The study was supported by the Hong Kong Obstetrical and Gynaecological Trust fund.
Trial registration number: Joint CUHK- NTEC Clinical Research Ethics Committee of the Chinese University of Hong Kong (CRE-2011.296).
Keywords: floseal, haemostasis, endometrioma, laparoscopic, stripping

P-776 Laparoscopic resection of deeply infiltrating endometriosis of the lower urinary tract

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Study question: Our study aimed to assess the outcome of different laparoscopic surgical approaches in the treatment of deeply infiltrating endometriosis of the lower urinary tract.
Summary answer: There was no difference regarding the intra-and postoperative complications in patients who underwent mucosal skinning when compared to partial cystectomy.
What is known already: Involvement of the urinary tract by deeply infiltrating endometriosis (DIE) is estimated in 1–2% of all cases, most frequently affecting the bladder. Extrinsic ureteral DIE is more common than the intrinsic form [1]. The treatment's goal is the alleviation of the patient's symptoms and prevention of renal insufficiency.
Study design, size, duration: Between 10/07/2009 and 31/12/2014 at the 1st Dept. of OB/GYN, Semmelweis University, Budapest a series of 21 laparoscopic partial (Group A) CO2-laser bladder resection and 26 extramucosal resection (Group B) was performed for bladder DIE. Ureterolysis was performed in 190, segmental ureteral resection in 3 cases.
Participants/materials, setting, methods: A prospective database was established for all elective patients undergoing laparoscopic bladder/ureteral surgery by the same surgical team. The main outcome measures assessed were operative duration, conversion rate, incidence of early complications, length of hospital stay, morbidity and mortality.
Main results and the role of chance: There was no difference regarding the intra-and postoperative complications in patients who underwent mucosal skinning when compared to the partial cystectomy. In our series of 190 laparoscopic ureterolysis ureteral injury occured in one case (0.5%). In case of segmental ureteral resections we observed no intra-and/or postoperative complication.
Limitations, reason for caution: The major limitation of our cohort study was the low number of patients intrinsic ureteral DIE. For this reason we were not able to make a meaningful comparison between the surgical outcome of laparoscopic ureterolysis and segmental ureteral resection.
Wider implications of the findings: According to our results both mucosal skinning and partial cystectomy are safe and reproducible surgical methods for the treatment of bladder DIE.
Study funding/competing interest(s): Funding by University(ies) – Semmelweis University 1st Department of OB/GYN.
Trial registration number: NA.
Keywords: laparoscopic surgery, bladder/ureteral endometriosis, DIE

P-777 Reliability of the ESHRE/ESGE and ASRM classification systems of uterine congenital malformations

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Study question: What is the reliability of diagnosis of congenital uterine anomalies, especially in differentiation between septate uterus/others and between anomaly/norm using the European Society of Human Reproduction and Embryology-European Society for Gynaecological Endoscopy (ESHRE-ESGE) classification in comparison to the American Society for Reproductive Medicine (ASRM) classification supplemented with absolute morphometric criteria?
Summary answer: Standardized interpretation indicate that the ESHRE-ESGE system has substantial/good or almost perfect/very good reliability ($\kappa > 0.60$, and >0.80, respectively), but other interpretation way showed insufficient reliability for clinical use ($\kappa < 0.90$), especially in diagnoses of septate uterus, and poorer reliability than ASRM, which was sufficient ($\kappa > 0.95$).
What is known already: The ESHRE-ESGE classification, its criteria and results are different than ASRM, however both are based on anatomy and use the same terms as 'congenital anomaly' and 'septate uterus'. Surgical treatment of septate uterus by ASRM has improved reproductive outcomes, unlike most of the other malformations and normal uterus. One of the conditions to ensure the appropriate management of uterine malformations is the reliability in diagnoses using the same classification system. Three-dimensional ultrasound is the recommended method to diagnose uterine anomalies by ESHRE-ESGE, which was an accurate and reproducible method of diagnosis using ASRM classification supplemented by morphometric criteria.
Study design, size, duration: A reliability study based on 112 prospective acquired 3D ultrasound single volumes of uterus, selected from women in

reproductive age, who were enrolled between June and September 2013, and these stored volumes re-evaluated in June 2014 during the three-day rounds.
Participants/materials, setting, methods: The same 3D volumes of uterine malformations (n = 50; consecutively included) and normal uterus (n = 62; randomly selected) by ESHRE-ESGE were independently offline evaluated by two experienced blinded raters (A and B), using the same ultrasound machine, both classifications criteria and methodology of evaluation and measurements, in a private medical centre. Rater A once again evaluated these volumes after two weeks. Inter- and intrarater repeatability of measurments of main benchmarks for malformation diagnosis (ASRM; internal fundal identation, external cleft, ESHRE-ESGE; added myometrial thickness) was estimated. The reliability/agreement for both systems in diagnosis of specific uterine congenital anomalies, recognition of septate uterus and differentiation between anomaly/norm was calculated and compared.
Main results and the role of chance: Intraclass correlation coefficient for measurments of internal fundal identation was >0.99 (interpreted as very good included lower limit of CIs), and for external cleft and uterine wall thickness was 0.95–0.99 (interpreted as good). Reliability / agreement of the ESHRE-ESGE classification in diagnoses of uterine malformations was lower (interrater p_o = 0.92, κ = 0.86, 95% CI 0.71–1.00; intrarater p_o = 0.88, κ = 0.80, 95% CI 0.65–0.95) than using ASRM system (interrater p_o = 0.99, κ = 0.98, 95% CI 0.87–1.00; interrater p_o = 0.98, k = 0.96, 95% CI 0.85–1.00). The greatest difference between these systems concerned the interrater reliability/agreement in diagnosis of septate uterus (ESHRE-ESGE, p_o = 0.88, k = 0.76, 95% CI 0.57–0.94; ASRM, p_o = 0.99, κ = 0.96, 95% CI 0.78–1.0). The relative risk of disagreement in recognition of septate uterus in different time of evaluation by the same rater and between two raters using ESHRE-ESGE classification relative to ASRM classification was significantly higher (RR intrarater disagreement; 9.0; 95% confidence interval 1.2–70; P = 0.04, RR interrater disagreement; 13; 95% CI 1.8–98; P = 0.04).
Limitations, reason for caution: Estimates of reliability/agreement may be greater, than those to be expected in clinical practice, where are additional sources of variation in comparison to offline analysis of the same stored 3D volumes.
Wider implications of the findings: Consequence of lower reliability of diagnosis by ESHRE-ESGE may be lack of agreement in uterine malformations management and biased research interpretation. Improvement of ESHRE-ESGE system is needed. The use of the simple morphometric criteria supplemented ASRM classification may be temporary better.
Study funding/competing interest(s): Funding by University(ies) – This work was supported by Jagiellonian University.
Trial registration number: NA.
Keywords: congenital uterine anomalies, septate uterus, reliability, interobserver intraobserver agreement, three-dimensional ultrasonography

P-778 Reproductive outcome and recurrence rate of surgically treated endometrioma patients and its relation to endometriosis fertility index

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Study question: What is the cumulative pregnancy and recurrence rate in advanced stage endometriosis patients treated laparoscopically for endometrioma with the stripping technique and its relation with the novel endometriosis fertility index (EFI).
Summary answer: Age of the patient and EFI score are two independent factors affecting pregnancy rate after laparoscopic surgery. Endometriosis patients with very low EFI scores (<4) should be directed to assisted reproduction without expected management since the have very poor pregnancy outcomes after surgery.
What is known already: Surgery for endometriosis improves spontaneous conception rates in infertile patients, however pregnancy rates after endometrioma excision for advanced stage endometriosis is limited. EFI is a newly formulated staging system and it is validated to be clinically useful for surgically confirmed endometriosis patients who desire pregnancy.
Study design, size, duration: This was a retrospective study in 71 patients managed for ovarian endometriomas with the laparoscopic stripping technique who were desiring pregnancy. The same surgeon performed all the operations. EFI score was calculated for each patient (range 1–10). Patients were fallowed expectantly with a mean follow up of 46,7 ± 20,2 months.
Participants/materials, setting, methods: Patients had standard conservative laparoscopic surgery for endometriosis at a university hospital. At operation all the visible endometriotic foci were destructed or excised, tubo ovarian anatomy was restored and ovarian endometriomas were excised. Bilateral tubal occlusion at the end of the surgery or severe male factor infertility patients were excluded
Main results and the role of chance: The mean age of the patients was 31.9 ± 5.0 and the mean duration of infertility was 36.9 ± 30.9 (12–204) months. Forty-six (64.8%) patients had primary infertility, while the remaining 25 (35.2%) had secondary infertility. Forty-two (59.2%) patients had monolateral and 29 (40.8%) had bilateral endometrioma. The cumulative pregnancy and delivery rates were 54.9% and 47.9%, respectively. The mean time to pregnancy was 16.8 ± 13.2 months. The mean EFI score was 6.49 ± 1.90. EFI score was significantly higher in pregnant patients compared to non-pregnant patients (7.33 ± 1.43 vs 5.47 ± 1.91, $p < 0.001$). Pregnancy rate was significantly higher in high EFI score patients (8–10) compared to moderate (4–7) and low (0–3) EFI score patients (78.3%, 48.8%, and 0%, respectively p < 0,001). Twelve patients had endometrioma recurrence (16.9%). Mean recurrence time was 27.5 ± 16.3 months.
Limitations, reason for caution: The retrospective nature of the study may limit to make firm conclusions. Preoperative and postoperative ovarian reserve of the patients was not available which may have impact on the pregnancy results.
Wider implications of the findings: Conservative laparoscopic surgery for advanced stage endometriosis patients has a cumulative pregnancy rate of over 50%. Endometriosis fertility index which includes both historical and surgical factors of the patient has a strong association with the probability of pregnancy after surgery. Therefore, it may guide the clinicians in deciding the treatment option for endometriosis patients after surgery
Study funding/competing interest(s): Funding by University(ies) – No funding.
Trial registration number: NA.
Keywords: endometrioma, laparoscopy, reproductive outcome

P-779 The septate uterus: a systematic review on the features of the septum

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Study question: What is known about the histology and pathophysiology of the septate uterus?
Summary answer: The precise histological aspects of the intrauterine septum remain unclear since different studies report contradictory findings, but it seems to be more comparable to normal uterine tissue then currently assumed. The pathophysiology of the risk for subfertility, miscarriages or preterm delivery of women with a septate uterus remains unknown.
What is known already: Women with a septate uterus are at risk for subfertility, recurrent miscarriages and preterm birth. Aiming to improve the fecundity of these women, resection of the septum is standard procedure in many countries, although evidence regarding its effectiveness is lacking. The exact pathophysiological processes underlying the poor reproductive outcome remain unclear. Hypotheses include differences in vascularization, contractility or vascular endothelial growth factor receptors, but a thorough overview is missing.
Study design, size, duration: Systematic review of the literature.
Participants/materials, setting, methods: Relevant studies were identified by searching Medline, Pubmed, EMBASE and the Cochrane Controlled Trials Register from 1900 until December 2014.
Main results and the role of chance: We found 7 observational and prospective studies, who report contradictory findings on the histology of a intrauterine septum. No reports were found about vascularization. Six studies who compared biopsies of the septum with normal uterine tissue, all found a difference in one of the following histological aspects: more myometrial tissue, more

muscular fibers, less connective tissue, a decrease in VEGF receptor expression or a decrease in sensitivity to preovulatory hormonal changes. One prospective study revealed no statistically significant differences in septate and myometrial tissue and concluded that the histology of the septum corresponds almost completely to normal uterine tissue.
Limitations, reason for caution: Evidence is limited, studies are small and mostly published before 2000.
Wider implications of the findings: This review, although based on a limited number of studies, questions the assumption that a intrauterine septum exists of mainly abnormal uterine tissue. This finding may imply that the higher risk to subfertility, miscarriages and preterm birth in women with a septate uterus could be due to other, to be investigated, factors.
Study funding/competing interest(s): Funding by University(ies) – Academic Medical Center Amsterdam.
Trial registration number: NA.
Keywords: septate uterus, histology

P-780 Diagnosis, management, and outcome of obstructed hemivagina and ipsilateral renal agenesis (OHVIRA syndrome) in 16 cases

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Study question: To discuss the management options, need for laparotomy, and outcome in patients with OHVIRA syndrome.
Summary answer: Most patients with OHVIRA syndrome, with indentation of the vaginal wall with hematocolpos and short distance between the hematocolpos and the vagina may be operated vaginally with vaginal septum resection with no complications and no recurrences.
What is known already: Early diagnosis of the syndrome is essential to preserve fertility. Conservative treatment is effective and has a good prognosis for the patients' reproductive function.
Study design, size, duration: Retrospective study.
Participants/materials, setting, methods: Retrospective evaluation of patients operated between 2001 and 2014.
Main results and the role of chance: The number of patients diagnosed with OHVIRA syndrome and operated in our clinic between 2001 and 2014 was 16. The mean age of the patients was 16.9 ± 6.0 (12–33). The presenting complaint was pain after menarche in 11 of the patients. One patient presented with acute abdomen. Three patients presented over 20 years of age (25, 27, 33), two of them with the complaint of infertility, and one presented with a pelvic mass. Two of the patients over 20 years of age had not undergone any diagnostic method or operations before. One patient presenting with the complaint of infertility had a history of diagnostic laparoscopy, but vaginal septum resection was not performed. Among the patients presenting before 20 years of age, one presented with pyocolpos, the rest presented with cryptomenorrhea. One had undergone diagnostic laparotomy and hysteroscopy, but vaginal septum resection was not performed. All of the 16 patients were diagnosed with OHVIRA syndrome before the operation by ultrasonography and MRI findings. 12 patients underwent vaginal septum resection only with no complications. In four patients laparotomy had to be performed in order to complete the procedure. One of the patients was the one who had previously undergone diagnostic laparotomy and hysteroscopy. Access to the hematocolpos was impossible in this patient due to lack of indentation in the normal vagina and access to the pelvic cavity was needed for safe resection of the vaginal septum. In the other 3 patients, it was impossible to perform vaginal septum resection without laparotomy because there was over 8 cm distance between the hematometrocolpos and the normal vagina in the MRI. In one of these, anastomosis was impossible and hysterectomy was performed. No complications developed in any of the procedures. And none of the patients suffered from vaginal septum reclosure after the procedure.
Limitations, reason for caution: Main limitation of this study is the retrospective nature of the study.
Wider implications of the findings: OHVIRA syndrome should be considered among the differential diagnoses in young females with renal anomalies presenting with pelvic mass, dysmenorrhea, symptoms of acute abdomen, and infertility. It may be impossible to perform vaginal septum resection in some cases where there is no indentation in the vagina and the distance between the hematocolpos and normal vagina is long. Hysterectomy may be needed in these cases.
Study funding/competing interest(s): Funding by University(ies) – Istanbul University Istanbul Medical Faculty.
Trial registration number: NA.
Keywords: OHVIRA syndrome, mullerian anomaly, uterus didelphys, obstructed hemivagina, infertility

P-781 Diagnostic accuracy of ultrasonography for diagnosing endometrial pathologies in postmenopausal women with bleeding or asymptomatic thickened endometrium

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Study question: How accurate is the transvaginal ultrasound measurement of endometrial thickness for predicting endometrial carcinoma in postmenopausal women with vaginal bleeding or asymptomatic thickened endometrium?
Summary answer: Sonographic endometrial thickness measurement have a high diagnostic accuracy for detection of endometrial carcinoma in postmenopausal women with bleeding, but it does not have sufficient accuracy in women with asymptomatic thickened endometrium.
What is known already: Endometrial assessment is indicated in all postmenopausal women with vaginal bleeding. Ultrasonography is preferred as the initial test for the investigation of postmenopausal bleeding and has a high negative predictive value for ruling out malignancy. Different guidelines use different cut-off levels of endometrial thickness that should warrant endometrial sampling, varying from 3 to 5 mm. The significance of asymptomatic postmenopausal thickened endometrium remains unclear. Routine endometrial sampling for these women is not recommended.
Study design, size, duration: Study design: Cross sectional study. Study size: Six hundred and two women. Study duration: Between June 2012 and December 2014.
Participants/materials, setting, methods: Participants/materials: A total of 602 postmenopausal women. Setting: Tertiary education and research hospital. Methods: Two hundred and seventy-four women with vaginal bleeding and 328 asymptomatic women with increased endometrial thickness (³5 mm) were underwent endometrial sampling by the Pipelle® instrument. The histopathological findings were investigated.
Main results and the role of chance: In women with vaginal bleeding, 8 (2.9%) were found to have endometrial carcinoma. Three (0.9%) cases of endometrial carcinoma were diagnosed in asymptomatic women. The best cut-off point for endometrial thickness in predicting endometrial carcinoma established by the Receiver Operating Characteristics (ROC) was 8.2 mm with diagnostic accuracy of 88.3%, a sensitivity of 75% and a specifity of 74% in women with bleeding (p = 0.0001). In asymptomatic women, endometrial thickness of 7.2 mm was an optimal cut-off point, with diagnostic accuracy of 76.5%, a sensitivity of 66.7% and a specifity of 65.8%, but the area under ROC was not statistically significant (p = 0.114). The diagnostic accuracy of endometrial thickness for prediction of polyp was 77.5% for symptomatic women and 61.0% for asmptomatic women.
Limitations, reason for caution: The detailed evaluation of the effects of clinical variables that can improve the diagnostic performance of ultrasonography such as age, parity, body mass index, hormone replacement therapy usage, presence of hipertension and diabetes is needed. Larger prospective studies including patient characteristics could provide better results.
Wider implications of the findings: Endometrial thickness measurement has a high diagnostic accuracy for detection of endometrial carcinoma in postmenopausal women with bleeding, but it does not have sufficient accuracy in women with asymptomatic thickened endometrium. Endometrial thickness has a moderate diagnostic accuracy for polyp detection in symptomatic women, but its predictive accuracy is low in asymptomatic women. In clinical practice, endometrial thickness assessment along with clinical variables could provide better results for predicting endometrial pathologies in asymptomatic patients.
Study funding/competing interest(s): Funding by hospital/clinic(s) – Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey.

Trial registration number: This study is not an RCT.
Keywords: endometrial thickness, endometrial biopsy, postmenopause, ultrasound, vaginal bleeding

P-782 Why myomas grow during ulipristal acetate treatment?

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Study question: Contrary to previously reports, uterine fibroids increase their volume during the initial phases of ulipristal acetate (UPA) treatment. Additionally, some myomas may increase their volume at the end of preoperative treatment with UPA. Which pathophysiologic mechanism is behind this phenomenon?
Summary answer: Using preoperative 3D-ultrasound and Doppler technology and its correlation to postoperative pathological examination, we describe the adaptive mechanism that generates considerable hydropic and cystic degenerative changes in leiomyomas under UPA treatment leading to a transitory increase in their volume.
What is known already: Uterine leiomyoma is the most common benign uterine tumor in women of reproductive age. Symptomatic women usually experience pelvic pain, anemia, and infertility. Contemporary therapeutic approaches principally comprise surgical interventions. Recently, UPA, a selective progesterone receptor modulator (SPRM), has become an effective and well-tolerated option for the preoperative treatment of leiomyomas. According to clinical data, UPA improves haemoglobin levels in anaemic patients, and it grants a significant reduction in the size of fibroids.
Study design, size, duration: A prospective, longitudinal study conducted at our Department of Obstetrics, Gynaecology and Reproductive Medicine from September 2013 to January 2015, comparing the effect of UPA and triptorelin on leiomyoma volume. The study was approved by the institutional review Board and all participants gave informed consent for the trial.
Participants/materials, setting, methods: 50 premenopausal women with symptomatic myomas were treated with UPA (5 mg/d) during 12 weeks preoperatively. VOCAL software for 3D ultrasound was used to measure leiomyoma volume and blood flow indices before treatment and weekly until surgery. As a control group 25 women received triptorelin (3.75 mg/mo) before surgery.
Main results and the role of chance: No differences were observed in either volume or vascularization between both groups at baseline. Moreover, reduction in myoma volume and vascularization indices at the end of the treatment protocol was similar in both groups ($P > 0.05$). However, myomas treated with UPA experience an increase in its volume during the initial 6 weeks of treatment, followed by a rapid decrease in the next 6 weeks. Nevertheless, up to 15% of the myomas in the UPA group presented an increased volume at the end of the preoperative treatment. Pathological examination revealed a significant increase in hydropic and cystic degenerations among myomas treated with UPA. On the other hand, myomas treated with triptorelin presented increased areas with necrosis and thrombotic vessels.
Limitations, reason for caution: We cannot exclude potential selection bias due to pretreatment histopathological differences between our groups.
Wider implications of the findings: An overall decrease in the volume of uterine leiomyomas treated with UPA is generated by an apoptotic phenomenon. However, the intercellular compartment seems to undergo more often degenerative hydropic degenerations, which may contribute to a transitory increase in the volume of myomas or even a complete cystic degeneration with a considerable increase in its size. Therefore, clinicians have to be aware of this circumstance in order not to misdiagnose it with malignant pathology.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – Universidad de Valencia, Hospital Clínico Universitario.
Trial registration number: NA.
Keywords: ulipristal acetate, leiomyoma, degeneration, 3D-ultrasound, pathology

P-783 Safeguarding fertility with whole ovary cryopreservation in ewe: slow freezing compared to vitrification

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Study question: Should slow freezing or vitrification be used on whole ovaries, as cryopreservation method, before micro-vascular transplantation, for efficiently safeguarding fertility?
Summary answer: In a ewe model, both methods led to poor fertility outcomes. A gestation was obtained in the slow freezing group, but fertility outcomes weren't significantly different between groups.
What is known already: Numerous live births have been obtained after non vascular grafting of cryopreserved fragments of ovarian cortex. However, fragments suffer ischemia during revascularization, which could limit the lifespan of these grafts. Micro-vascular transplantation of cryopreserved whole ovaries could allow immediate revascularization and improve fertility outcomes. To date, this method allowed gestations only in a ewe model, and remains in the research field. The most appropriate method for whole ovary cryopreservation -slow freezing or vitrification- is unknown.
Study design, size, duration: In this animal study, 12 ewes were randomized for micro-vascular auto-transplantation, with either slow-frozen or vitrified ovary. The remaining ovary was removed. Fertility and ovarian function were assessed for the 3 subsequent years. Ovarian follicular count of native ovaries (control) and transplants was performed at the end of the study.
Participants/materials, setting, methods: Ovaries from 6 ewes were slow-frozen using DMSO as cryoprotector. The ovary of 6 other ewes were exposed to increasing concentration of the vitrificant solution "VM3", while gradually lowering the temperature to reduce toxicity, and vitrified. After thawing/warming, cryoprotector(s) was(were) gradually removed, and vessels were anastomosed to the systemic circulation.
Main results and the role of chance: Warming phase crystallization occurred in each ovary vitrified with "VM3". All anastomoses from the slow frozen group were permeable, whereas 2 primary failures of blood flow reestablishment occurred in the vitrification group. Two ewes from the slow frozen and vitrification group died 4 week and 7 months after transplantation, respectively, death cause being independent from the present protocol. Hormonal resumption occurred in 4/5 ewes from the slow frozen group and 6/6 ewes from the vitrified group. A ewe from the slow frozen group delivered healthy twines, 1 year 9 months and 12 days after transplantation. Estimated whole follicle survival was dramatically low in each group, although significantly higher in the vitrification group ($0.3\% \pm 0.5\%$), than in the slow-frozen group ($0.017\% \pm 0.019\%$, $p < 0.05$).
Limitations, reason for caution: The limited number of animals used, didn't allow drawing any evidence-based conclusion for fertility outcomes. The extrapolation from ewe model to human is not established.
Wider implications of the findings: Micro-vascular transplantation of slow frozen ovaries was confirmed to allow fertility in a ewe model. However, poor follicular survival and fertility outcomes observed after transplantation of cryopreserved whole ovary call in question the ability of this technique to efficiently safeguard fertility, whatever the cryopreservation method used.
Study funding/competing interest(s): Funding by national/international organization(s) – Research grants from "Agence de la Biomédecine", and "Fondation de l'Avenir."
Trial registration number: This study was approved by the Ethics Committee of the Veterinary School of Lyon (ENVL no: 0804).
Keywords: fertility preservation, vitrification, slow freezing, whole ovary, ewe model

P-784 The role of diagnostic and therapeutic mini invasive surgery in couples with unexplained infertility: a restrospective observational study

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Study question: In the era of ART (assisted reproductive techniques), does the mini invasive surgery still play a role in the diagnostic work-up and therapeutic management of the infertile couple?
Summary answer: Combined laparoscopy and hysteroscopy, associated with chromopertubation with methyleneblue, allow to identify and contextually treat

pelvic and uterine abnormalities that may impair fertility, with a resulting reliable rate of spontaneous pregnancies.
What is known already: Laparoscopy, with the direct visualization of the pelvis, is the only method able to identify specific peritoneal factors, such as minimal endometriosis and pelvic adhesions, which may impair fertility. ESHRE-ASRM guidelines indicate surgery for women with symptoms, or risk factors, or abnormal hysterosonography/ultrasonography, with no other reasons to undergo ART. However, the role of laparoscopy in asymptomatic women with normal imaging is debated, as few studies previously evaluated the pregnancy rate following the procedure.
Study design, size, duration: The operative findings, interventions and the subsequent pregnancy rate were retrospectively analyzed in 70 women undergoing surgery for unexplained infertility from January 2010 to December 2012 in our tertiary care academic medical center.
Participants/materials, setting, methods: 358 infertile women underwent combined hysteroscopy-laparoscopy during the observation period. We excluded from the analysis subjects presenting at least one of the following: age >38 years, infertility duration <3 years, proven or suspected pelvic organs abnormalities before surgery, anovulation, secondary infertility, male factors. 70 women resulted eligible for the evaluation.
Main results and the role of chance: On hysteroscopy, intrauterine lesions were detected and contextually treated in 12 women (17%). On laparoscopy, endometriosis was found in 16 women (23%): 13 minimal or mild, 2 ovarian and 1 deep endometriosis. Pelvic adhesions were observed in 23 patients (33%). All cases of pelvic abnormality were surgically treated. The bilateral tubal patency, documented prior to surgery in all selected patients by imaging, was confirmed on the chromopertubation in 60 women. 9 subjects exhibited unilateral tubal patency, and one case of bilateral obstruction, without macroscopic alterations, was observed. The cultural examination of the peritoneal fluid resulted negative in all cases. A spontaneous pregnancies occurred in 20 women (28%), independently of age, in the following 18 months. This percentage achieved 50% in the subgroup treated for endometriosis.
Limitations, reason for caution: It is a retrospective study done in a single unit. Even if we found an high percentage of spontaneous pregnancy, it cannot be ruled out that factors other than surgery may play a role. In this context, the lack of a control group does not allow to draw definitive conclusions.
Wider implications of the findings: The considerable rate of success in terms of spontaneous pregnancies makes the surgical management of unexplained infertility an intriguing option, in particular for its diagnostic potential. Even if it implies general anesthesia and hospital stay, the cumulative economical cost, psychological burden and complications rate is comparable to those of II-III level ART.
Study funding/competing interest(s): Funding by University(ies) – Catholic University, Rome.
Trial registration number: NA.
Keywords: laparoscopy, infertility

STEM CELLS

P-785 B:Role of HECTD1 in cell movement during embryogenesis

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Study question: HECTD1 was first identified as E3 ubiquitin ligase for inhibin B receptor which mutant phenotype was characterized by impaired placental development, severe neural tube defects (spina bifida) and growth retardation. We are interested in the HECTD1 functional molecular networks in cell spreading and migration during embryogenesis.
Summary answer: Together, our findings indicate that IQGAP1 and Hax1 serve as important downstream factors controlled by ubquitination of HECTD1, impacting on formation of focal adhesions and cytoskeleton, and further regulating cell spreading and migration in embryogenesis.
What is known already: Three models about HECTD1 molecular signaling were proposed, one was about increased secretion of Hsp90 in the cranial mesenchyme of HECTD1 mutants may be responsible for the altered behavior of these cells. Another model suggested that HECTD1 promotes the adenomatous polyposis coli (APC) protein-Axin interaction to negatively regulate Wnt signaling. The latest theory proposed that ubiquitination of PIPKIγ90 by HECTD1 and consequent degradation of PIP2 controls focal adhesion dynamics and cell migration.
Study design, size, duration: Using the secretory-trap approach, we generated HECTD1 mutant mice by insertion of β-geo expression cassette between intron 26 and 27. The wild type mouse embryos are taken as control. Whenever we do DNA/siRNA transfection, there are always non-targeting control groups.
Participants/materials, setting, methods: Mouse embryonic fibroblasts were cultured from E12.5 embryos. General approach: cell culture/transfection, gene knockout, mass spectrometry, yeast two hybrid, immunohistochemistry, immunoprecipitation, Western blots, FACS, ubiquitination assay, half-life assay, protein phosphatase activity assay.
Main results and the role of chance: Loss of HECTD1 exerts shorter spreading time and irregular cell shapes in cell spreading, enhanced velocity with decreased directionality in directional cell migration. The formation of focal adhesions, adherens junctions and cytoskeleton are also impaired in HECTD1 knockout MEFs. We confirmed IQGAP1 and Hax1 are two binding partners of HECTD1. Over-expression of IQGAP1 and Hax1 together with high activity of GTPases in HECTD1 knockout MEFs reminiscent of IQGAP1 and Hax1 may act as substrates of HECTD1. Comparing with wild type cells, ubiquitination of IQGAP1 and Hax1 in HECTD1 knockout cells was decreased but the half-life of the two proteins were increased. siRNA knockdown of IQGAP1 and Hax1 in HECTD1 knockout cells significantly rescued the expression of focal adhesions, cell spreading time and migration velocity.
Limitations, reason for caution: As having more generations, it is getting harder for Heterozygotes to be pregnant, which makes it difficult for us to compare the role of HECTD1 in different stages of embryos.
Wider implications of the findings: Our results provide new mechanisms of HECTD1 in regulating cell spreading and migration during embryogenesis. IQGAP1 and Hax1 serve as essential downstream factors of HECTD1 which might be the potential therapeutic targets for growth retardation and neural tube defects.
Study funding/competing interest(s): Funding by national/international organization(s) – The Repronatal Fundation, Basel, Switzerland.
Trial registration number: NA.
Keywords: HECTD1, cell movement, embryogenesis

P-786 The effect of BMP-4 and RA on histone modifications in human amniotic epithelial cells expressing DAZL and VASA

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Study question: The effects of introducing human amniotic epithelial cells (hAECs) to a microenvironment consistent of necessary cues for primordial germ cells (PGCs) differentiation was tested to induce histone modification in hAECs expressing germ cells markers (DAZL and VASA).
Summary answer: The extrinsic inducers bone morphogenic protein (BMP)4 and retinoic acid (RA) may regulate intrinsic factors such as DAZL and VASA, thus affecting cell fate. BMP4 and RA were also found to affect epigenetic state in hAECs already committed to the germline differentiation, via specific histone modification which characterizes pre-colonized PGCs.
What is known already: Previous reports describing germ like cells differentiation from various cell types have shown that exogenous addition of BMP4 and RA improves recruitment of PGCs and enhances their maintenance in vitro. The crucial role of epigenetic patterning via histone modifications, which establish a unique PGCs pluripotent state, was also found in these studies. hAECs model was recently found to be an in vitro culture system that allows us to study germline differentiation pathway.
Study design, size, duration: hAECs isolated from term placentas and passaged every 8 days. Three differentiation protocols used: spontaneous protocol; hAECs cultured in SSM. BMP4 protocol; 50 ng/ml BMP4 added to freshly

isolated hAECs cultured in SSM for 10 days. RA protocol; BMP4 protocol followed by addition of 1 μM RA for 30 hours.
Participants/materials, setting, methods: Characterization of freshly isolated-, passage 3 and 5 hAECs was conducted by the examination of DAZL and VASA germ cell markers, Dnmt3a expression and H3K9me2, H3K27me3, H3K9ac, H3K4me3 and H3S10phosph. expression in OCT-3/4 expressing cells. Methods used included qPCR, immunofluorescence and Flow cytometry.
Main results and the role of chance: Extrinsic inducers BMP4 and RA advance hAECs differentiation to large 30–50 mm cells with rounded morphology and zona-pellucida-like structures cells expressing germ cell markers by regulating intrinsic factors such as DAZL and VASA. RA was found to induce PGCs differentiation more efficiently than BMP4 alone, as the expression of VASA was found to be more elevated. However, BMP4 induce epigenetic state that may imply pre-colonized cells expressing germ cell markers, including elevated expression of H3K27me3, H3K4me3 and H3S10phosph in OCT-4 expressing cells. Role of chance: Freshly isolated and spontaneously differentiating hAECs are referred to as control group for differentiating cell and BMP4 and RA treated cells, respectively. Experimental methods include examination of unstained cells or DNA free samples. $P < 0.05$ was considered significant.
Limitations, reason for caution: Placentas were obtained only after uncomplicated vaginal deliveries from healthy mothers with written informed consent.
Wider implications of the findings: Epigenetic patterning is believed to be crucial for prevention, at least in part, of some heritable diseases and reproductive failure. hAECs has unique features that make this model attractive for potential stem cell based therapies such as lack of teratomas formation *in vivo*, low antigenicity and anti-inflammatory. A better understanding of epigenome reprogramming may potentially allow us to manipulate *in vitro* endogenous cells of diseased tissues as well as stem cells to properly differentiate into germ cells.
Study funding/competing interest(s): Funding by University(ies) – Technion-Israel Institute of Technology.
Trial registration number: 0045-09-EMC.
Keywords: primordial germ cells, BMP4, retinoic acid (RA), histone modifications

P-787 Cultured cells from the human oocyte cumulus niche are efficient feeders to propagate pluripotent stem cells

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Study question: Do cells cultured from the human oocyte CCs niche (hCC) support the human induced pluripotent stem (hiPS) cells propagation and pluripotency?
Summary answer: The hiPS cells cultured on hCC (hCC-iPS) maintained their pluripotency potential and exhibited much better self-renewal performance in terms of proliferation rate compared with the classically used fibroblast (hFF) feeders (hFF-iPS).
What is known already: It is known that the maintenance of undifferentiated state requires culturing human pluripotent stem cells (PSC) on a variety of human feeder cells including fetal and adult fibroblast, endometrial cells, adult marrow cells, and placental fibroblast. However, despite the development of diverse culture systems, no standardized method of co-culture has emerged.
Study design, size, duration: Human cumulus cells were collected from patients referred to our centre for intra-cytoplasmic sperm injection (ICSI) for male infertility. The hiPS cells were obtained by reprogramming the human fibroblast foreskin hFF cell line using lentiviral vectors to express human *OCT4, SOX2, NANOG* and *LIN28*. The propagation of hiPS cells was compared between hiPS cultured on hCC and hFF feeders respectively.
Participants/materials, setting, methods: Cumulus cells were dissociated mechanically from the MII oocyte on collection day and adapted to growth on a human collagen substrate for a long period in a xeno-free defined medium. Affymetrix Human Genome U133 Plus 2.0 DNA chips were performed to analyze the transcriptome of hCC and hiPS cells. Levels of expression of pluripotency and differentiation markers were examined by RT-qPCR, immunofluoresence and flow cytometry. Chromosomal abnormalities were analyzed using array comparative genomic hybridization (aCGH).
Main results and the role of chance: We have optimized isolation and culture protocols for long-term maintenance of hCC in xeno-free conditions. This new feeder, characterized at molecular and cellular levels, able to support growth of hiPS cells during long term passaging. Flow cytometric analysis showed that hiPS-hCC retained expression of surface markers (CD24, SSEA4, TRA-1-81 and TRA-1-60), demonstrating successful preservation of stemness. Moreover, hiPS-hCC also improved the competence to differentiate into the three germ layers *in vitro* (embryoid bodies) as well as *in vivo* (teratoma formation). The self-renewal rate of hiPS-hCC was higher than of hiPS-hFF determined by measurement of colonial diameters and cell cycle analysis, suggesting that hCC has a growth-promoting effect. Additionally, hiPS-hCC did not exhibit detectable sub-chromosomal aberrations, confirming their genetic stability. A comparative gene expression study of hCC and hFF feeders revealed significant differences ($p < 0.05$) in expression of cellular matrix components and an up regulation in hCC of genes known to be important players in cell proliferation such as interleukin 6 gene (IL6).
Limitations, reason for caution: More investigations are required to study the role of the cytokines secreted by hCC that are potentially responsible for successful maintenance and higher proliferation of the hiPS cells.
Wider implications of the findings: This culture system can be suggested for autologous hCC as co-culture of human early embryos to boost embryonic development. Moreover, hCC can be used as a model to identify secreted and adhesion molecules that are required for the self-renewal of PSC.
Study funding/competing interest(s): Funding by commercial/corporate company(ies) – Ferring pharmaceutical company supported the study but had no influence on the study design and was not involved in the analysis of the results. There were no competing interests.
Trial registration number: NA.
Keywords: human cumulus cells, pluripotent stem cells, IVF, regenerative medicine

P-788 Reconstitution of an artificial human testis using a 3 dimensional (3D) culture device

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Study question: can albumin phosphate calcium 3D scaffold be replaced with seminiferous extra cellular matrix as a suitable culture device for reconstitution of an artificial testis?
Summary answer: Human testicular derived mesenchymal stromal cells (ht-MSCs) were cultured on a homemade albumin phosphate calcium 3D scaffold. The sections of the scaffolds have shown the successful growth of htMSCs within the spaces inside the scaffold resemble to seminiferous tubes. However, the function of the device needs to be improved.
What is known already: Several reports have shown the generation of stem cells from animal and human testis in 2D culture system. Recent data in human studies have shown that these cells are from stromal origin and have a multipotent capacity instead of pluripotency. One of the reasons of this difference might be due to lack of specific factors to induce pluripotency in human spermatogonial stem cells niche*in vitro*.
Study design, size, duration: A novel scaffold has been designed to improve the 3D culture of the htMSCs *in vitro*. First, htMSCs been cultured in different 2D culture conditions and then monolayer cultures and embryoid bodies (EBs) were passaged onto the scaffolds and cultured for another week. Scaffolds been sectioned, stained.

Participants/materials, setting, methods: Two TESE samples were used after fully concern by patients and minced mechanically and treated by three steps enzymatic digestion to isolate single cells. Single cells have been cultured with HES-KOSR medium with/without feeder layer for week and then passaged and cultured onto a 3D scaffold for 7, 14 days.
Main results and the role of chance: From both TESE samples htMSCs were derived and cultured using HES-KOSR medium onto the mitotically inactivated mouse embryonic fibroblast (MEFs) feeder layer and without MEF feeder layer. Without MEFs cells formed embryoid bodies (EBs), but those onto feeder layer have formed clusters after almost a week. Both clusters and EBs have been passaged onto a new albumin phosphate calcium 3D scaffold with HES-KOSR medium. For each TESE sample, three scaffolds have been used. Scaffolds have been sectioned and stained with hematoxolin and eosin (H & E) and compared with human testis sections. Not lot but some cells have been cultured and growth within the free spaces of the scaffold, comparable to seminiferous tubules sections.
Limitations, reason for caution: It was not feasible to localize the cells within the scaffold similar to their arrangement inside the testis. It might be possible to resolve by using specific antibodies within the scaffold.
Wider implications of the findings: Our findings prepared a 3D culture of htMSCs which would lead itself to design an artificial testis for the future male infertility treatment.
Study funding/competing interest(s): Funding by University(ies) – yazd shahid sadoughi university for medical sciences.
Trial registration number: NA.
Keywords: TESE, stem cells, germ cells, human testis derived mesenchymal stromal cells, tissue engineering

P-789 Derivation conditions affect pluripotency and differentiation propensity of mouse embryonic stem cells

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Study question: Do the state of pluripotency and differentiation propensity of mouse embryonic stem cells (mESCs) vary when they are derived in different culture conditions?
Summary answer: mESCs derived in '2i' or in '3i' show higher pluripotency markers and lower lineage-specific markers expression compared to those derived in LIF-serum. LIF-serum cells tend to differentiate more easily into endoderm whereas '2i' and '3i' cells into the ectoderm and mesoderm respectively than into other lineages during EBs formation.
What is known already: Naive mESCs derived in LIF-serum condition show heterogeneous expression of pluripotency markers, whereas ground-state mESCs derived in '2i' (PD0325901 and Chir99021, FGF and GSK3b signaling inhibitors respectively) are more homogeneous. Nanog is heterogeneous even in 2i-derived mESCs. Inhibiting TGFb signaling with SB431542 during mESCs-derivation promotes the ground-state pluripotency of stem cells. Previous studies have compared different conditions/strains during mESCs derivation. However, it is not clear yet whether all these cells are functionally similar.
Study design, size, duration: mESCs were derived from embryos of '129Ola/Hsd' mice in the following three conditions, using 1000 units/ml LIF in all conditions.

i DMEM based medium with 20% serum + LIF (LIF-serum line)
ii N2B27 medium with '2i' + LIF ('2i' line)
iii N2B27 medium with '3i' ('2i' + SB431542) + LIF ('3i' line)

Participants/materials, setting, methods: Pluripotency markers (Oct4/Sox2/Nanog) expression was examined in undifferentiated mESCs by RT-qPCR. Cells were differentiated spontaneously into embryoid-bodies (EBs) in their respective basal medium after removing LIF and/or inhibitors. Ectodermal-FGF5, mesodermal-T and endodermal-Sox17 markers were examined on control cells collected on D0 and EBs collected on D4, D7 and D10.
Main results and the role of chance: '2i'- and '3i'-derived undifferentiated mESCs showed significantly higher expression of pluripotency-markers and significantly lower expression of lineage-specific-markers compared to LIF-serum-derived pluripotent mESCs ($p < 0.05$). D4-EBs demonstrated more biased differentiation of LIF-serum cells towards the endoderm, whereas '2i' cells towards ectoderm. However, '3i' cells showed differentiation into both ectoderm and mesoderm. On D7, '2i'-EBs exhibited significant up-regulation of all three germ-layer-markers but LIF-serum EBs showed increased expression of endo- and ectodermal markers only. In contrast, '3i' EBs displayed significantly higher expression of meso- and ectodermal markers. On D10, EBs in all three conditions demonstrated the expression of all three lineage-specific-markers. Interestingly, overall comparison of EBs revealed superior degree of differentiation of LIF-serum cells towards endoderm, of '2i' cells towards ectoderm and of '3i' cells towards mesoderm.
Limitations, reason for caution: LIF-serum derived lines were differentiated in serum-containing medium without LIF, whereas '2i' and '3i' lines were differentiated in N2B27 medium without small molecules and cytokines. It will be interesting to examine if these cells still behave similarly when exactly identical differentiation conditions are used.
Wider implications of the findings: Our results show that derivation conditions affect pluripotency and differentiation propensity of mESCs. The ultimate application of pluripotent stem cells in the field of regenerative medicine is to differentiate them towards a particular lineage of interest. Therefore, these findings display a significant role in selecting the appropriate derivation conditions depending upon the final use of cells.
Study funding/competing interest(s): Funding by University(ies) – SG is funded by Special Research Fund (BOF-Ghent University) (Grant number: 01D04212). No competing interests declared.
Trial registration number: NA.
Keywords: embryonic stem cells, embryoid bodies, lineage, pluripotency, differentiation potential

P-790 Comparison of differentiation potential and oocyte regeneration from ovarian surface epithelium-derived ovarian stem cells between young and aged female mice

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Study question: This study examined whether pluripotent and germ cell marker expression and differentiation potential of ovarian surface epithelium (OSE)-derived ovarian stem cells (OSCs) differ with female age in mice
Summary answer: Aged female mice have OSCs in OSE, but their differentiation potential including oocyte regeneration, expression of germ cell marker and number of OSCs were significantly decreased compared to those of young mice
What is known already: Many studies have proposed that OSCs derived from OSE layer of adult mammalian ovaries can produce oocytes. Few studies have reported that ovaries of aged mammalian females including mice and women possess rare premeiotic germ cells that can generate oocytes
Study design, size, duration: Controlled experimental study. Twenty female mice for each age group.
Participants/materials, setting, methods: C57BL/6 female mice of 2 age groups (6–8 and 28–31 weeks) were super ovulated by injection with 5 IU equine chorionic gonadotropin (eCG). Both ovaries were removed after 48 hours and scrapped to obtain OSE. Gene expressions of pluripotent (Oct-4, Sox-2, Nanog) and germ cell markers (c-Kit, GDF-9, and VASA) were evaluated in the intact ovary, scraped OSE, and three weeks postcultured OSE by RT-PCR. After 3 weeks culture of OSE, the number of positively stained Oct-4 by immunohistochemistry and oocyte production were examined
Main results and the role of chance: Expressions of germ cell markers in the intact ovary were significantly decreased in aged females, whereas expressions of pluripotent markers were not detected, regardless of age. Scraped OSE expression of all pluripotent and germ cell markers, except for c-Kit, was similar between both age groups. Three week post-cultured OSE had significantly decreased expression of GDF-9 and VASA, but not c-Kit, in old mice, as compared to young mice; however there was no difference in the expression of other genes. The number of positively stained Oct-4 by immunohistochemistry in postcultured OSE was 2.5 times higher in young mice than aged mice. Oocyte-like structure was spontaneously produced in postcultured OSE. However, while that of young mice revealed a prominent nucleus, zona pellucida-like structure and cytoplasmic organelles, these features were not observed in old mice.

Limitations, reason for caution: This study was for mice. Therefore, further study should be processed for women to prove a clinical effectiveness of the result of the present study.
Wider implications of the findings: These results implicated that advancing female age resulted in decreased potential of differentiation into oocytes or stemness activity of germ cells in OSEs, but they also can produce oocyte-like structure from OSE-derived OSCs. Therefore, if an appropriate milieu for inducing regeneration of oocytes from OSCs in aged female is elucidated, it is expected that this study may contribute to the development of a new strategy for the production of oocytes in the treatment of female age-related infertility and POF.
Study funding/competing interest(s): Funding by national/international organization(s) – This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2013063).
Trial registration number: NA.
Keywords: ovarian stem cells, ovarian surface epithelium, oocyte regeneration, female aging

P-791 Ovarian stem cells in perspective treatment of infertility: a preliminary isolation approach

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Study question: Ovarian stem cells (OSC) have been detected in mice and postulated in human ovaries[1]. They typically expose Ddx-4 (*DEAD box polypeptide 4*), a stemness marker uniquely expressed by germ line cells as both OSCs and spermatogones. Thus, we tried to isolate OSCs from woman ovaries by immunoselection using anti-Ddx-4 antidodies.
Summary answer: Although at low percentage, OSCs are located within the cortex of the human ovary and their isolation is possible by sorting with appropriate combined separation methodology. Once isolated, OSCs appear viable and can be further characterized and/or cultured for additional studies.
What is known already: Despite the general opinion that postnatal mammalian ovaries of most species do not contain renewable germinal OSCs[2], previous work by Tilly and co-workers showed the existence of OSCs in human ovaries since these cells were separated by Ddx-4 positive selection[3]. However, these finding were confuted for the major cytoplasmic expression of Ddx-4. The antigen, indeed, is expressed both on cell membrane and cytoplasm by OSCs, whereas mature oocytes contain only its cytoplasmic tale.
Study design, size, duration: The study was aimed to verify the occurrence and tentatively separate the OSCs from a small number of human ovaries by using magnetic immunoselection and sorting with anti-Ddx-4 reagents and subsequent typing by flowcytometry with specific monoclonal antibodies (MoAb) to specific cell lineage markers.
Participants/materials, setting, methods: Ovarian cortex fragments from three women undergoing ovariectomy in premenopausal age, were digested by collagenase, filtered, suspended in presence of rabbit anti-Ddx-4 antibody and then passed through a column including anti-rabbit IgG conjugated magnetic microbeads. The eluted cells were typed by flowcytometry and confocal microscopy to assess the Ddx-4 localization.
Main results and the role of chance: Within the full cell suspension, we gated the putative OSC population in relation to the cell size and found that the double positive population including $OCT4A^+$/$Ddx\text{-}4^+$ cells, was poorly expressed, whereas a definite enrichment was observed after the immunomagnetic sorting. In fact, the enriched $Ddx\text{-}4^+$ cell population was increased up to 24% from the original 2% value in the initial cortical suspension, with remarkable fluorescence intensity suggesting the high molecular expression of Ddx-4 molecule. By immunofluorescence under confocal microscopy, these cells appeared of small size with a large nucleus, few cytoplasm and highly FITC-fluorescent deposits on the cell membrane reflecting the consistent expression of Ddx-4 molecules. This result supports the suitability of the separation of OSCs by this simple methodology using the immunomagnetic cell sorting.
Limitations, reason for caution: Major skepticism derives from using Ddx-4 as cell surface marker for the OSC sorting since its location in cytoplasm is still detectable in mature oocytes. However, only OSCs express the membrane associated Ddx-4 and further work investigating other membrane markers as OCT-4A and SSEA-4 may improve the OSC separation methods.
Wider implications of the findings: The future applications of these findings to the infertility field will cover different and unrelated conditions ranging from the endocrine defective ovarian reserve to the iatrogenic infertility associated to chemotherapy in cancer patients. On the other hand, besides the infertility treatment, OSCs can be further investigated in parallel studies aimed at restoring the woman endocrine physiology in postmeno-pausal age. However, this approach primarily requires animal experimental models to prove their efficacy in treating endocrine dysfunctions.
Study funding/competing interest(s): Funding by national/international organization(s) – Department of Biomedical Sciences and Human Oncology, University of Bari 'Aldo Moro' IMO, University of Bari, Section of Gynecology and Obstetrics, and Division of Ginecology, Reproductive and IVF Unit, Conversano, BA, Italy.
Trial registration number: Registration in progress at the Ethical Committee of the University of Bari.
Keywords: Infertility, ovarian stem cells, Ddx-4

P-792 Comparison of attachment layers for the priming human embryonic stem cells towards epiblast-like cells

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Study question: How can we adjust the conditions described in the protocol for primordial germ cell formation of mouse embryonic stem cells (mESCs), in order to achieve an equivalent priming of human embryonic stem cells (hESCs) towards epiblast-like cells?
Summary answer: Naïve hESCs did not survive seeding on ornithine and laminin (O/L) as a coating layer, regardless whether the seeding occurred in their nascent naïve medium or in epiblast-like cell (Epi-LC) medium. In contrast, normal colony growth was observed on matrigel and fibronectin.
What is known already: Mouse primordial germ cell like cells (PGC-LC) can be successfully generated *in vitro* from naïve mESC. For this, mESC are first primed towards PGC formation by culture in N2B27 medium with bFGF, Activin A and 1% KSR. This priming step ideally takes 2 days and is done on a coating of O/L. Human PGC-LC have been generated from hESC only at low frequency by spontaneous differentiation.
Study design, size, duration: Three independent naïve hESC lines were seeded onto the following attachment layers: O/L, matrigel, fibronectin and mouse embryonic fibroblasts (MEFs). One group was directly seeded in Epi-LC-medium, another was first seeded in their nascent naïve medium and cultured for one day (habituation), before the medium was changed to Epi-LC-medium.
Participants/materials, setting, methods: All cells were seeded on wells of a 24-well plate, and experiments were performed in three biological replicates. Morphology and cell survival was compared between the different attachment layers after two days of priming in Epi-LC-medium, with and without initial habituation for one day in the nascent naïve medium.
Main results and the role of chance: We observed that seeding of naïve hESCs on an O/L coating and in Epi-LC priming medium, as described for mESC, did not result in viable hESC or Epi-LC colonies. Next we added a habituation period to the protocol in which naïve hESCs are seeded and cultured for one day in their nascent naïve medium, before addition of Epi-LC medium. This approach showed no improvement in colony growth or survival. Both approaches were then tested on matrigel and fibronectin coatings, which allowed good colony growth up to three days in Epi-LC medium. Although signs of differentiation were noticeable after two days, no apparent morphological differences were seen between the approach with or without habituation on both matrigel and fibronectin.
Limitations, reason for caution: It remains to be investigated how well the expression profile of the hESCs after priming in Epi-LC medium, corresponds to that of mouse Epi-LCs, either in matrigel or in fibronectin.
Wider implications of the findings: Good colony growth and survival of naïve hESCs on matrigel and fibronectin, as opposed to O/L, could be the first

requirement to successfully convert the PGC differentiation protocol, described for mouse, to human PGC formation *in vitro*. Future generation of human stem cell-derived gametes will lead to major advances in human germ cell biology and fertility.

Study funding/competing interest(s): Funding by national/international organization(s) – Jasin Taelman is holder of a research fund supported by agency for Innovation through Science and Technology (IWT) (Project number: 131673). The authors declare they have no conflict of interest.

Trial registration number: NA.

Keywords: PGC, EpiLC, hESC, germ cell differentiation

P-793 Spermatogonial stem cells (SSCs): a route to preserving fertility

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Study question: To develop a rapid, reliable and reproducible method for human spermatogonial stem cell (SSC) culture and cryopreservation.

Summary answer: Findings indicate that the technique of establishing a niche-like environment *in vitro* is successful in maintaining long-term cultures of human SSCs, and by combining immunofluorescence (IF) labeling and confocal imaging I have shown that vitrification of human spermatogonial stem cell is successful in maintaining viability.

What is known already: With advances in paediatric oncology, survival rates are high. Following chemo-radiation treatment sperm production is compromised by depletion of SSCs, leading to sub- or infertility. Pre-pubertal boys with cancer do not have a means of preserving their fertility. Human SSC culture remains in its infancy although testicular biopsies are being cryopreserved from pre-pubescent boys in some centres. Independent of time stored, SSC cryopreservation experiments demonstrate a post-thaw SSC viability of 40–60%.

Study design, size, duration: Human SSCs were cultured on 0.2% gelatin-coated plates and in co-culture with testicular somatic cells, incubated at 37°C and 5% CO_2. Images were captured at intervals whilst in culture. Colonies were vitrified and control SSC colonies remained in culture prior to fixation. All experiments were performed in triplicate.

Participants/materials, setting, methods: SSCs isolated from testicular biopsies, taken from azoospermic men were co-cultured with testicular somatic cells. Colonies were fixed in 4% PFA and IF labeled using antibodies against GFRa1 and PLZF. SSCs were cryopreserved using vitrification. Proliferation and cell death was assessed using Ki67 and TUNEL IF labeling and confocal-microscopy.

Main results and the role of chance: The co-culture method was superior, giving rise to SSC chains, clusters and colonies. To date, I have maintained these cultures for a period of 20 weeks. Vitrified human SSC colonies were viable and continued to proliferate upon warming. SSC colonies were fixed, and IF labeling combined with confocal imaging demonstrated comparable viability to the non-vitrified SSC colonies.

Limitations, reason for caution: Future work will concentrate on ensuring genetic integrity is maintained in cultured and vitrified SSC colonies.

Wider implications of the findings: This finding is significant because cryostorage is an essential component of the treatment pathway. Undoubtedly a clinically acceptable cryopreservation method is essential in germ cell cryopreservation; therefore I do feel this is an important practical step forward in the male germline stem cell field. The progress made in harvesting, propagating and vitrifying human SSCs *in vitro* is an important step towards the future development of a fertility preservation service for male childhood cancer survivors.

Study funding/competing interest(s): Funding by national/international organization(s) – The Urology Foundation Research Fellowship. The Shears Northern Research Fellowship - The Royal College of Surgeons of England.

Trial registration number: REC reference: 13/NE/0159, IRAS project ID: 118390.

Keywords: spermatogonial stem cells, fertility preservation, cryopreservation

P-794 Is skewed X chromosome inactivation in human embryonic stem cells driven by a culture advantage?

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Study question: What are the dynamics of X chromosome inactivation (XCI) in a large cohort of female human embryonic stem cell (hESC) lines during long-term culture and differentiation?

Summary answer: We found a stable, though aberrant XCI pattern during long-term undifferentiated culture and after differentiation to somatic and trophoblast lineages: all lines displayed stage III XCI, with major loss of XCI marks, and a completely non-random XCI pattern in lines informative for the microsatellite markers under study.

What is known already: Female hESC cultures have previously been shown to display variable XCI patterns. Unlike mouse embryonic stem cells, most hESC already display XCI at the undifferentiated state. Moreover, a predominant occurrence of non-random XCI patterns has been reported; but the extent and origin of this non-random pattern in hESC remains unresolved.

Study design, size, duration: XCI patterns of 22 female hESC lines were analyzed at different passages during long-term culture, and after differentiation into somatic osteoprogenitor-like cells and the trophoblast lineage. The parental origin of the inactivated X chromosome was identified if DNA from the embryo donors was available.

Participants/materials, setting, methods: We used methylation-sensitive DNA restriction and PCR, and massive parallel bisulphite sequencing for microsatellite markers to study DNA methylation. Histone modifications were analysed by immunostaining. Expression of *XIST* was investigated using real time PCR, while cDNA mini sequencing was used to determine mono- or biallelic gene expression.

Main results and the role of chance: Methylation analysis revealed XCI in all investigated lines. One line was non-informative for the studied markers; all other lines displayed a completely skewed, non-random inactivation pattern, from early passage on and in undifferentiated cells as well as after differentiation. We observed a transition from a random to a non-random pattern in only one line. From ten lines for which donor DNA was available, six lines displayed inactivation of the male donor allele; four of the female donor allele. Massive parallel bisulphite sequencing revealed partially methylated profiles, suggesting erosion of methylation. While in some early passage lines we could still observe repressing histone marks and *XIST* expression, these were very rapidly lost after limited culture time. Strikingly, loss of histone marks occurred in a colony-specific manner.

Limitations, reason for caution: We investigated the methylation status of the X chromosome using three microsatellite markers. Further refinement of the analysis at multiple loci spread over the whole chromosome could offer a more in-depth view of the XCI patterns in hESC.

Wider implications of the findings: The extent of the observed non-random XCI patterns is striking. We excluded a parent-of-origin-related mechanism and also its occurrence as passenger aberration is unlikely because of the number of cell lines involved and the presence at early passages. The transition found in one line rules out an embryonic origin of the skewed XCI, therefore we hypothesize that this XCI skewing originates from a culture advantage conferred by a specific XCI pattern.

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Trial registration number: NA.

Keywords: hESC, X chromosome inactivation

P-795 Human embryonic stem cells derived from embryos donated by male infertility couples showed abnormal germ cells differentiation

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Study question: Whether spermatogenic abnormality will transmit to next generation through IVF.
Summary answer: Our results suggest that male infertility brought out by oligozoospermia may be inherited by IVF.
What is known already: Although many couples suffered oligozoospermia have benefited from IVF, possible defects in germ-specific gene in IVF babies are not yet repaired, thus there are risks of transmitting abnormal genes to offsprings. However, to date there has no clear evidence to testify the risks.
Study design, size, duration: Three normal human embryonic stem cells (hESCs) and two abnormal hESCs with male parents attacking by oligozoospermia were employed. All hESCs were differentiated into post-meiotic spematids by direct induction. The differentiation behaviors of germ cells were observed, analyzed and compared during the 14-day induction course.
Participants/materials, setting, methods: Normal CCRM15, abnormal CCRM16 and CCRM27 were established in our own laboratory. Normal H1 (46, XY) and H9 (46, XX) come from WiCell. The morphology of hESCs and hESCs-derived embryoid bodies (EBs) was compared. The expression pattern of germ cell specific genes was valuated by quantiative-PCR and immustaining.
Main results and the role of chance: The morphology of hES lines, and growth of EBs derived from different hES lines did not showed obvious difference. SOX2 (pluripotency and primordial germ cell (PGC) marker) expression level was high in hESCs and then declined gradually except for CCRM27. The peak expression of SCP3 (pluripotency and PGC marker) appeared later in H9.The expression of PRMM1 and PRDM1 (post-mmeiotic markers) in CCRM27 and CCRM16 were downregulated throughout differentiation stages. TNP1 and TNP2 (post-meiotic markers) showed increased expression in CCRM27 during the whole differentiation course, but expressed critically low in CCRM16 in later differentiation period. Further immustaining results were in accordance with the mRNA expression pattern. Our study indicated that hESCs with male parents suffered from oligozoospermia showed abnormal germ cell differentiation.
Limitations, reason for caution: Our sample size isn't big enough to make an incontrovertible conclusion, thus more normal (>3) and abnormal (>3) male hES lines should be employed to give a more reliable verdict in future studies. Also, besides hESCs derived from oligozoospermia parents, hESCs derived from asthenozoospermia and oligozoospermia parents also should be included in further investigation.
Wider implications of the findings: Our results implicated that a man suffered male infertility caused by oligozoospermia can has babies through IVF, but his boy babies may still be infertility. To completely recover the reproductive function, of IVF generation, the gene mutantion must be repaired at the blastomere stage.
Study funding/competing interest(s): Funding by University(ies) – Funding by hospital/clinic(s) – First affiliated Hospital of Nanjing Medical University.
Trial registration number: NA.
Keywords: male factor infertility, human embryonic stem cell, assisted reproduction techniques (ARTs), spermatogenic abnormality, genetic disorders

P-796 Establishment of embryonic stem cells from parthenogenetic haploid embryos and generation of cloned oocytes in mice

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Study question: The aim of this study was to establish parthenogenetic haploid embryonic stem cells (PHESCs) from mouse parthenogenetic haploid embryos. In addition, we attempted to reconstitute unfertilized mouse oocytes by nuclear transfer with PHESCs.
Summary answer: ES cell-like cells with a maternal haploid chromosome set derived from PH embryos were able to establish and maintain them for a long-term period. Further, cloned oocytes reconstituted with nucleus of PHESCs were able to fertilize and developed to blastocyst stage.
What is known already: The embryonic stem (ES) cells with one set of maternal genome derived from parthenogenetic haploid (PH) embryos are amenable for the genetic analysis and the generation of genetically modified animals. Further, these cells could provide the valuable cell source for the reproductive and the regenerative medicine. However, available information about the haploid ES cells is very limited.
Study design, size, duration: BDF1 mice were used for production of PH embryos. ES-like cells with a haploid genome were established from the inner cell mass (ICM) of the PH blastocyst and determined their biological characteristics. Further, PHESCs in G0/G1 phase were injected into enucleated M-II oocytes to generate cloned oocytes.
Participants/materials, setting, methods: PH embryos were produced by removal of male pronucleus from fertilized oocytes, and were cultured until to blastocyst stage. Expression of ES cell markers in mouse PHESCs was examined immunohistochemically. The DNA content of PHESCs was measured by flow cytometry, and karyotype analysis was also performed. For generation of cloned oocytes, PHESC at G0/G1 phase was injected into enucleated mouse M-II oocyte. The cloned oocytes were fertilized by ICSI and cultured in KSOM.
Main results and the role of chance: After the culture for 4 days, 18.9% of PH embryos were reached to blastocyst stage, and ICM outgrowth was observed in approximately 60% of these. The ICM cells were dispersed, seeded over feeder cells, and spherical colonies were observed. In these cells, colony formation and proliferation were maintained up to 4 months. By immunohistchemical staining, these cells were positive for ES cell markers, such as Oct4, Nanog, Sox2, and Rex1. Further, flow cytometric analysis and karyotyping revealed that these cells had a haploid set of 20 chromosomes (19 autosomes and the X chromosome). After ICSI to cloned oocytes reconstituted by nuclear transfer with PHESCs, 58.6% of these cleaved to 2-cells and 6.2% formed blastocyst.
Limitations, reason for caution: This study was conducted using a mouse model. This finding does not directly represent human reproductive and regenerative medicine.
Wider implications of the findings: Our results may provide novel insights into the haploid embryonic stem cell biology and ES-like cells derived from PH embryos will be a powerful tool for reproductive and regenerative medicine, and genetic analysis.
Study funding/competing interest(s): Funding by hospital/clinic(s) – There are no conflicts of interest to declare.
Trial registration number: NA.
Keywords: parthenogenesis, haploid, embryonic stem cell, nuclear transfer

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